RACCOLTA
PUBBLICAZIONI SCIENTIFICHE PNEI
PSICONEUROENDOCRINOIMMUNOLOGIA

2019

INTERNATIONAL INSTITUTE OF PNEI

INDICE

- **Modulation of the anticancer immunity by natural agents: inhibition of T regulatory lymphocyte generation by arabinoxylan in patients with locally limited or metastatic solid tumors.** pag. 3
  Paolo Lissoni [1,*], Giusy Messina [1], Fernando Brivio [2], Luca Fumagalli [2], Luigi Vigoré [3], Franco Rovelli [3], Luisa Maruelli [4], Mauro Miceli [4], Paolo Marchiori [4], Giorgio Porro [1], Michael Held [5], Giuseppe di Fede [6], Toshi Uchiyamada [7].

- **A Randomized Study of Chemotherapy Versus Biochemotherapy with Chemotherapy plus Aloe arborescens in Patients with Metastatic Cancer.** pag. 8
  Paolo Lissoni [1], Franco Rovelli [1], Fernando Brivio [2], Romano Zago [3], Massimo Colciago [4], Giuseppina Messina [1], Adelio Mora [1] & Giorgio Porro [1].

- **A phase II study of anastrozole plus the pineal anticancer hormone melatonin in the metastatic breast cancer women with poor clinical status.** pag. 13
  Paolo Lissoni [1*], Giuseppe Di Fede [1], Antonio Battista [2], Giusy Messina [1], Remo Egardi [1], Fernando Brivio [3], Franco Rovelli [1], Massimo Colciago [4], Giuseppe Brera [5].

- **Biotherapy with the pineal hormone melatonin plus aloe and myrrh tincture in untreatable metastatic cancer patients as an essence therapy of cancer.** pag. 16
  P. Lissoni [1], F. Rovelli [1], G. Messina [2], F. Brivio [3], B. Boniardi [1], G. Porro [1], L. Vigoré [4], G. Di Fede [1], P. Marchiori [1], G. Brera [5].

- **A PsychoNeuroEndocrineImmune (PNEI) Approach to Enhance the Efficacy of Radiochemotherapy in Glioblastoma** pag. 21
  Lissoni P[1*], Messina G[1], Porro G[1], Porta E[2], Nosetto L[2], Mancuso M[2], Di Fede G[2]

- **The Psychoneuroimmune Pathogenesis of Cancer: Therapeutic Strategy to Normalize Cancer-Related Brain Unbalance Between Hyperfunction of Opioid System and Hypofunction of Cannabinoid-Pineal Axis by Antitumor Pineal Indoles, and the Mu-Opioid Antagonist Naltrexone in Untreatable Advanced Cancer Patients** pag. 25
  Paolo Lissoni*, Franco Rovelli, Giusy Messina, VezikaCenaj, Giuseppe Di Bella, Giorgio Porro, Franco Fraschini, Giuseppe Di Fede

- **A Study on the Influence of Spirituality on the Efficacy of Antitumor Therapies with Natural Anticancer Agents in Untreatable Metastatic Cancer Patients** pag. 30
  Messina G[1], Rovelli F[1], Brivio F[1], Lissoni P[1], Fumagalli L[2*], Compare A[3]

- **A Study on the Endocrine Function of Pineal Gland with Regard To Immune Alterations in Cancer Patients** pag. 35
  Paolo Lissoni*, Vichy Cenaj, Franco Rovelli, Giusy Messina, Giorgio Porro, Fernando Brivio and Giuseppe Di Fede

INDICE

- **Five Year-Survival with High-Dose Melatonin and Other Antitumor Pineal Hormones in Advanced Cancer Patients Eligible for the Only Palliative Therapy** pag. 39
  Paolo Lissoni*, Franco Rovelli, Fernando Brivio, Giusy Messina, Arianna Lissoni, Sonja Pensato and Giuseppe Di Fede

- **The modulation of the endocannabinoid system in the treatment of cancer and other systemic human diseases** pag. 46
  Paolo Lissoni*, Giusy Messina, Giorgio Porro, Roberto Trampetti, Arianna Lissoni, Franco Rovelli, Vezika Cenay, Enrica Porta and Giuseppe Di Fede

- **A phase-2 study of high-dose pineal antitumor hormone melatonin as an adjuvant therapy in triple negative breast cancer** pag. 50
  Paolo Lissoni*, Franco Rovelli, Giusy Messina, Vezika Cenaj, Giorgio Porro, Giuseppe Di Fede

- **The Antitumor Endocrine Molecules of Human Body** pag. 53
  Lissoni P*, Cusmai R, Messina G, Porro G, Brivio F, Pelizzoni F, Monzon A, Roselli MG and Di Fede G

Cancer Therapy Vol 6, 1011-1016, 2008

Modulation of the anticancer immunity by natural agents: inhibition of T regulatory lymphocyte generation by arabinoxylan in patients with locally limited or metastatic solid tumors

Research Article

Paolo Lissoni[1,*], Giusy Messina[1], Fernando Brivio[2], Luca Fumagalli[2], Luigi Vigoré[3], Franco Rovelli[3], Luisa Maruelli[4], Mauro Miceli[4], Paolo Marchiori[4], Giorgio Porro[1], Michael Held[5], Giuseppe di Fede[6], Toshi Uchiyamada[7]

[1]Division of Radiation Oncology, San Gerardo Hospital, Milan, Italy
[2]Division of Surgery, San Gerardo Hospital, Milan, Italy
[3]Laboratory of Immunomicrobiology, San Gerardo Hospital, Milan, Italy
[4]Natur-Spiritual, Milan, Italy
[5]Biological Medicine Center, Rome, Italy
[6]Institute of Biological Medicine, Milan, Italy
[7]Daiwa Pharmaceuticals, Tokyo, Japan

***Correspondence:** Dr. Paolo Lissoni, Divisione di Radioterapia Oncologica, Ospedale S.Gerardo, 20052 Monza, Milano, Italy; Fax: +390392332284, e-mail: p.lissoni@hsgerardo.org

Key words: Anticancer immunity, arabinoxylan, immunostimulation, T regulatory lymphocytes

Abbreviations: interleukin 10, (IL-10); interleukin 6, (IL-6); interleukin-2, (IL-2); interleukn 12, (IL-12); NK cells, ($CD16^+CD56^+$); T cytotoxic lymphocytes, ($CD8^+$); T helper lymphocytes, (TH), ($CD4^+$); T lymphocites, ($CD3^+$); Transforming growth factor beta, (TGF-β) T-regulatory lymphocytes, (T-reg), ($CD4^+CD25^+$)

Received: 30 September 2008; Revised: 1 November 2008
Accepted: 17 November 2008; electronically published: December 2008

Summary

In the last years, several immunomodulating antitumor agents have demonstrated in the nature, particularly from Aloe plant and rice bran. However, the major problem concerning the natural antitumor agents is to define their immune mechanisms of action in relation to the more recent advances in tumor immunobiology. At present, the main cause responsible for the lack of an effective antitumor response in advanced cancer patients is belived to be represented by the generation of a subtype of T helper lymphocytes ($CD4^+$) with suppressive activity on anticancer immunity, the so-called T regulatory lymphocytes (T reg), which may be clinically identified as $CD4^+CD25^+$ cells. On this basis, a study was planned to evaluate the effect of rice bran extract arabinoxylan on T reg cell count and percentage in solid tumor patients in relation to the various lymphocyte subpopulations. The study included 22 evaluable cancer patients, 16 of whom had an untreatable metastatic solid tumor. Arabinoxylan was given orally at a dose of 2000 mg/day for the first month, followed by a dose of 1000 mg/day for the next month. In each patient we evaluated by monoclonal antibodies the absolute number of lymphocytes, T lymphocytes ($CD3^+$), T helper (TH) lymphocytes ($CD4^+$), T cytotoxic lymphocytes ($CD8^+$), NK cells ($CD16^+CD56^+$), T reg lymphocytes ($CD4^+CD25^+$) and TH/T reg ratio before and after 2 months of therapy. No substantial change occurred on therapy in the mean number of lymphocytes, $CD3^+$, $CD8^+$ and NK cells. On the other hand, the mean number of TH cells increased, whereas that of T reg cell decreased on treatment, even though none of these differences was statistically significant. On the contrary, TH/T reg mean ratio significantly enhanced after arabinoxylan therapy. In addition to its previously demonstrated stimulatory action on NK function, this study shows that arabinoxylan may inhibit the production of T reg cells, which are responsible for cancer-related immunosuppression, with a following improvement in the anticancer immunity. If further studies will confirm these results, arabinoxylan could be successfully associated with chemotherapy to induce not only a cytotoxic destruction of cancer cells, but also an improvement in the immune status.

I. Introduction

The recent advances in the definition of the mechanisms responsible for tumor progression have suggested the possibility to control cancer growth not only trough chemotherapy-induced cancer cell destruction, but also by stimulating the anticancer immunity. In addiction to the exisence of endogenous antitumor molecules, several agents capable of stimulating the anticancer immunity have alsso isolated from plants. However, the immunomodulatory effects of most natural immunomodulating agents need to be better investigated in an attempt to establish their mechanisms of action in relation to the most recent discoveries concerning the physiopathology of the anticancer immunity. At present, Aloe extracts (Lissoni et al, 1998) and arabinoxylan extract from rice bran (Ghoneum and Jewett, 2000) would represent some of the potential natural agents which could be utilized in the complementary therapy of human neoplasms. Today, it is known that the antitumor immune response is the end-result of several interactions involving cytokines and immune cells, provided by stimulatory or suppressive effects on the anticancer immunity (Atzpodien and Kirchner, 1990; Rosenberg, 1992). Therefore, the lack of an effective anticancer immune response in most cancer patients with advanced disease would simply depend on the prevalence of immunosuppressive mechansisms with respect to the immunostimulatory ones (Atzpodien and Kirchner, 1990). The anticancer immunity is mainly activated by T helper-type 1 lymphocytes by releasing IL-2 (Whittington and Faulds, 1993), and by dentritic cells, which act as antigen-presenting cells producing IL-12 (Banks et al, 1995), T cytotoxic lymphocytes and NK-LAK system, which are involved in the induction of the antigen-dependent and antigen-independent cytotoxicity, respectively (Atzpodien and Kirchner, 1990). Therefore, IL-2 and IL-12 would represent the main anticancer cytokines in humans. On the contrary, the suppression of the anticancer immune response is mediated by several cytokines, namely IL-10 (Moore et al, 1993), IL-6 (Matsuda and Hirano, 1990) and TGF-β (Shevach, 2002). Recently, however, it has been demonstrated that the various endogenous suppressive factors would exert their inhibitory immune effect through a common end-mechanism, consisting of the generation of a subtype of T helper lymphocytes ($CD4^+$cells), provided by a fundamental suppressive activity on the anticancer immunity, the so-called T regulatory lymphocyte (T reg) (Dieckmann et al, 2001), which at present seems to constitute the main mechanism responsible for cancer-related immunosuppressive status. T reg cells may be identified by the simultaneous expression of the alpha-chain of IL-2 receptor (CD25) and CD4 antigen (Dieckmann et al, 2001). Then, T reg cells may be clinically recognized as $CD4^+CD25^+$ lymphocytes. Therefore, each eventual natural immunomodulating agent would have to be investigated in relation to its possible effect on T reg generation since, at least from a theoretical point of view, each natural agent capable of counteracting T reg activity could positively influence the prognosis of the neoplastic disease by improving the efficacy of the anticancer immune response. Moreover, our previous preliminary studies have suggested that the percentage of T reg cells with respect to the total number of T helper cells, as expressed as CD4/CD4CD25 ratio, may represent an optimal synthetic immune index to investigate the functional status of the anticancer immunity in the single cancer patient, by representing the synthesis of the actions of the great number of immunostimulating and immunosuppressive factors involved in the modulation of the anticancer immunity (Dieckmann et al, 2001). Within the great number of natural agents derived from plants and potentially usefull to be employed in the complementary therapy of cancer, arabinoxylan would seem to represent one of the potential natural agent, because of its efficacy in improving the clinical status of cancer patients (Ghoneum and Jewett, 2000; Ghoneum and Gollapudi, 2005; Markus et al, 2006; Ghoneum et al, 2007). The immunomodulating properties of this nautral substances extracted from plants have been confirmed by experimental studies, but unfortunately most experiments have been limited to the investigations of they effects on non-specific immune parameters for the anticancer immunity, such as NK cell cytotoxicity. In contrast, since reg cells play a fundamental role in suppressing the generation of the anticancer immunty, each potential antitumor immunomodulatory natural substances, would have to be investigated also in relation to their eventual influence on T reg cell system. On the basis of the recent discoveries in tumor immunobiology (Dieckmann et al, 2001; Shevach, 2002), a study was planned to investigate the possible influence of arabinoxylan on both absolute number of T reg cells and their ratio with respect to the total $CD4^+$ T cells in a group of solid tumor patients, affected by locally limited or metastatic disease.

II. Materials and methods

The study included 24 consecutive patients, 18 of whom had a metastatic solid tumor, which did not respond to the conventional anticancer chemotherapies and for whom no other effective standard treatment was available, while the remaining 6 patients had been surgically treated for a locally limited neoplasm. Patients were followed at Biological Medical Institute of Milan and the protocol was approved by the Director of the Institute. Eligibility criteria were, as follows:histologically proven locally limited or metastatic solid tumor, no double tumor, no chronic therapy with corticosteroids because of their immunosuppressive effects and no concomitant treatment with other immunomodulating agents,such as interferons,interleukins and monoclonal antibodies. At the time of the start of arabinoxylan therapy, patients with untreatable metastatic cancer were under treatment with the only supportive care, consisting of anti-inflammatory agents for pain, anti-dopaminergic drugs for nausea and vomiting and with the pineal hormone melatonin for the therapy of the neoplastic cachexia (Banks et al, 1995). Patients were considered as fully evaluable when they had received arabinoxylan therapy for at least 2 consecutive months. Arabinoxylan was given orally at a dose of 1000 mg twice/day for the first month, followed by a dose of 1000 mg/day for the next month. Arabinoxylan was supplied by DAIWA Pharmaceutical (Tokyo, Japan). It was derived from rice bran treated enzymatically with an extract of the shiitake mushrooms. It is a polysaccharide containing β-1,4-xylopironase hemicellulose, commercially available and known as Biobran. For the immune investigation, venous blood samples were collected in the morning after an overnight fast before the onset

of arabinoxylan therapy and after 2 consecutive months of treatment. In each blood sample, we evaluated the absolute number of total lymphocytes, T lymphocytes ($CD3^+$), T helper (TH) lymphocytes ($CD4^+$), T cytotoxic lymphocytes ($CD8^+$), NK cells ($CD16^+$ $CD56^+$ and T regulatory (T reg) lymphocytes ($CD4^+$ $CD25^+$). The different lymphocyte subsets were measured with a flow cytometric assay by using specific monoclonal antibodies supplied by Becton-Dickinson (Milan, Italy). Moreover, because of the importance not only of their absolute number, but also of their percentage with respect to the other lymphocyte subsets, namely to that of $CD4^+$ cells, CD4/CD4CD25 ratio, corresponding to TH/T reg ratio, was also determined before and after therapy. Normal values (95% confidence limits) of T reg number and TH/T reg ratio observed in our laboratory were below 240/mm^3 and above 4.0, respectively. Data were reported as mean +/- SE and statistically analyzed by the Student's t test, the analysis of variance and the chi-square test, as appropriate.

III. Results

Evaluable patients were 22/24, while the remaining 2 patients, both affected by untreatable disseminated liver metastases due to colorectal cancer, rapidly died for disease progression before concluding the two planned months of arabinoxylan therapy. The clinical characteristics of the evaluable patients are reported in **Table 1**. **Figure 1** illustrates changes in the mean number of total lymphocytes, T lymphocytes, T cytotoxic lymphocytes and NK cells occurring after 2 months of arabinoxylan therapy. No substantial variation was found in the mean number of lymphocytes, T lymphocytes, T cytotoxic lymphocytes and NK cells under arabinoxylan treatment. In contrast, as illustrated in **Figure 2,** TH and T reg mean numbers increased and decreased, respectively, after arabinoxylan therapy, without, however statistically significant differences with respect to the values seen prior to therapy. On the contrary, a statistically significant increase in TH/T reg mean ratio was achieved after arabinoxylan therapy ($p<0.025$). The increase in TH/T reg ratio under arabinoxylan therapy was more pronounced in patients with an abnormally low ratio prior to therapy with respect to that occurring in those with normal pre-treatment ratio, however without statistically significant differences (2.3 +/- 0.4 *vs* 1.7 +/- 0.5). In more detail,

Table 1. Clinical characteristics of 22 evaluable cancer patients treated by arabinoxylan.

Characteristics	**n**
M/F	14/8
Median Age (years)	62 (24-82)
Medi an performance status (Karnofsky's score)	90 (70-100)
Tumor histotypes:	
colorectal cancer	6
lung cancer	4
prostate cancer	4
breast cancer	3
renal cell cancer	2
pancreatic cancer	2
soft tissue sarcoma	1
Disease extension:	
Locally limited disease	6
Metastatic disease	16
bone	2
lung	5
liver	3
lung + liver	3
brain	2
peritoneum	1

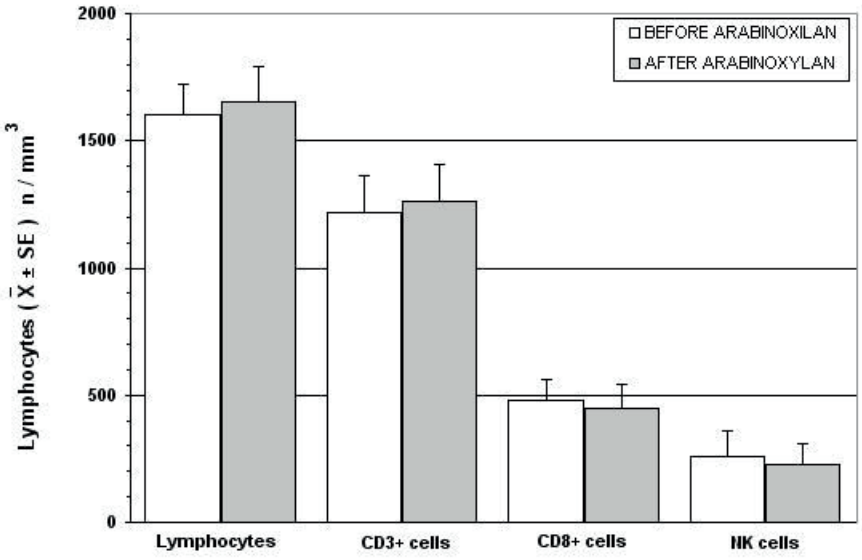

Figure 1. Changes in the number of lymphocytes, Tlymphocytes (CD3), T cytotoxic lymphocytes (CD8) and NK cells (CD16 CD56) after 2 months of arabinoxylan therapy.

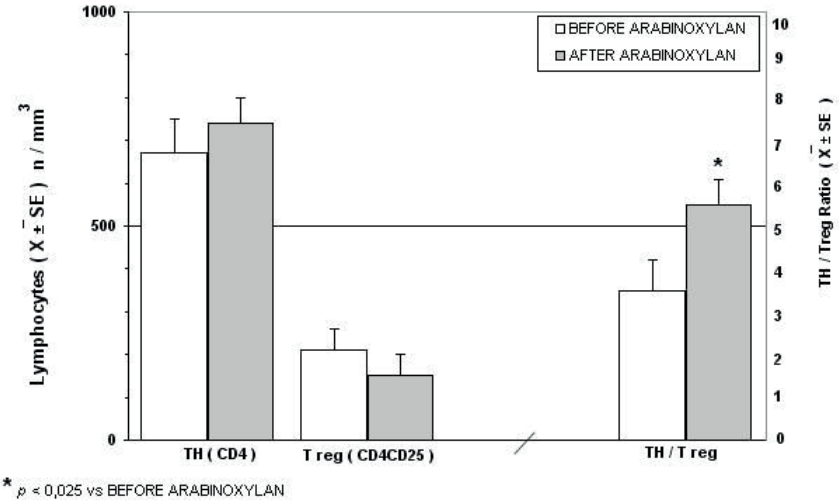

Figure 2. Changes in the mean number of T helper (TH) lymphocytes (CD4) and T regulatory lymphocytes (cd4 cd24) and in TH/T reg mean ratio.

before arabinoxylan therapy,an abnormally low TH/T reg ratio was present in 12/22 (55%) evaluable patients. Arabinoxylan treatment induced a normalization of TH/T reg ratio in 5/12 (42%) patients with an abnormally low ratio prior to therapy. The percentage of arabinoxylan-induced TH/T reg normalization obtained in lymphocytopenic patients was not significantly different from that achieved in patients with normal pre-treatment lymphocyte count (3/7(43%) vs 2/5(40%)). No toxicity was observed under arabinoxylan treatment, which was well tolerated in all patients. Asthenia was present in 8/22 (36%) evaluable patients. An evident relief of asthenia, as assessed by a specific patient report, was obtained under arabinoxylan therapy in 5/8 (63%) patients.

IV. Discussion

Previous experimental studies had already demonstrated some immunomodulating properties of arabinoxylan, in particular consisting of stimulation of NK cytotoxic function (Ghoneum, 1998), whereas NK cell number did not seem to be influenced by arabinoxylan administration. However, it has to be remarked that NK cells were belived to be fundamental in the antitumor immunity until some years ago, before the discovery of the essential role played by the antitumor cytokines, such as IL-2 and IL-12 (Whittington and Faulds, 1993) and dendritic cells, because of their function as antigen-presenting cells (Banks et al, 1995). In fact, it has to be considered that the cytotoxic activity of NK cells is effective only against artificial laboratory cancer cell lines, whose biological malignant properties are different from those presented by fresh human tumor cells (Whittington and Faulds, 1993). In addition, NK cells have been proven to be also able to destroy fresh human cancer cells only after the activation of their cytotoxic function by IL-2 (Atzpodien and Kirchner, 1990). From this point of view, arabinoxylan had been already proven to amplify the stimulatory effect of IL-2 on NK-mediated antitumor cytotoxicity (Ghoneum and Jewett, 2000). In contrast, no study has been performed up to now to evaluate the possible influence of arabinoxylan not only on the mechanisms responsible for the generation of an effective anticancer immune response, but also on those involved in the suppression of anticancer immunity. The results of this preliminary study, carried out to evaluate the influence of arabinoxylan on T reg cells, which represent the most important cells involved in the suppression of the antitumor cytotoxic immune response, demonstrates that arabinoxylan may counteract T reg cell generation by reducing their number and percentage with respect to the total amounts of $CD4^+$ cells and circulating lymphocytes. Since NK cell function is inhibited by T reg activation (Shevach, 2002), the previously demonstrated arabinoxylan-induced stimulation of NK cell cytotoxic function might depend at least in part on its capacity of counteracting T reg generation (Dieckmann et al, 2001). Moreover, this study would suggest that the inhibitory action of arabinoxylan on T reg generation is more pronounced in patients with an abnormally high percentage of T reg cells prior to therapy, with a following pre-treatment abnormally low TH/T reg ratio before therapy, whereas its effect was less evident in patients with a pre-treatment value of TH/T reg ratio within the normal range. Therefore, the influence of arabinoxylan on T reg generation would consist of a modulatory action rather than an inhibitory activity. This finding could explain a potential favourable immunomodulatory effect of arabinoxylan also in patients with autoimmune diseases (Ghoneum, 1998), who in contrast to cancer patients would tend to present abnormally low amounts of T reg cells. In any case, the importance of the inhibition of T reg generation in the induction of an effective anticancer immune response has been recently confirmed by the evidence that the block of T reg activity by specific monoclonal antibodies may induce objective tumor regressions in humans (Yang et al, 2007). Obviously, the major problem is the exact identification of he T reg cell population. Even though T reg cells may express other immune markers, namely FOX-p2 cytoplasmatic antigen, most clinicians are in agreement to identify the CD4+CD25+ cells as T reg

lymphocytes (12). In any case, further studies, by evaluating other immune markers, will be required to better identify T reg cells population, namely FOX-p3, even though recently some Authors have shown that FOX-p3 expression by T reg cells is associated with a lower suppressive activity (Dieckmann et al, 2001; Shevach, 2002). Moreover, it has to be remarked that several patients included in the present study were concomitantly under palliative therapy with the anti-cachectic pineal hormone melatonin (Brzezinski, 1997), which may also play immunomodulating effects (Maestroni, 1993). Therefore, further randomized studies with arabinoxylan alone versus arabinoxylan plus melatonin will be required to better define the immunomodulating action of arabinoxylan. If further clinical and experimental studies will confirm the inhibitory action of arabinoxylan on T reg cell system, it could be included in cytokine-based immunotherapies to enhance their efficacy by counteracting T reg cell generation.

References

Atzpodien J, Kirchner H (**1990**) Cancer, cytokines and cytotoxic cells:interleukin-2 in the immunotherapy of human neoplasms. **Klin Wochenschr** 14, 1-10.

Banks RE, Patel PM, Selby PJ (**1995**) Interleukin-12:a novel clinical player in cytokine therapy. **Br J cancer** 71, 655-659.

Brzezinski A (**1997**) Melatonin in humans. **N Engl J Med** 336, 185-195.

Dieckmann D, Plottner H, Berchtold S, Berger T, Schuler G (**2001**) Ex vivo isolation and characterization of CD4+CD25+ T cells with regulatory properties from human blood. **J Exp Med** 193, 1303-1310.

Ghoneum M (**1998**) Enhancement of human natural killer cell activity by modified arabinoxylane fro rice bran(MGN-3). **Int J Immunother** 14, 89-99.

Ghoneum M, Gollapudi S (**2005**) Synergistic of arabinoxilan rice bran (MGN-3/Biobran in S. Cerevisiae-induced apoptosis of monolayer breast cancer MFC-7 cells. **Anticancer Res** 25(6B), 4187-96.

Ghoneum M, Brown J, Gollapudi S (**2007**) Yeast therapy for the treatment of cancer and its enhancement by MGN-3/Biobran, an arabinoxylan rice bran. Cellular Signaling and Apoptosis Research (Ed. Alex R. Demasi) Cap IV: 185-200.

Ghoneum M, Jewett A (**2000**) Production of tumor necrosis factor-alpha and interferon-gamma from human peripheral blood lymphocytes by MGN-3, a modified arabinoxylan from rice bran, and its synergy with interleukin-2 *in vitro*. **Cancer Detect Prevent** 24, 314-324.

Lissoni P, Giani L, Zerbini S, Trabattoni P, Rovelli F (**1998**) Biotherapy with the pineal immunomodulating hormone melatonin versus melatonin plus Aloe vera in untreatable advanced solid neoplasms. **Nat Immun** 16, 27-33.

Maestroni JGM (**1993**) The immunoneuroendocrine role of melatonin. **J Pineal Res** 14, 1-10.

Markus J, Miller A, Smith M, Orengo I (**2006**) Metastatic hemangiopericytoma of the skin treated with wide local excision and MGN-3. **Dermatol Surg** 32, 145-147.

Matsuda T, Hirano T (**1990**) Interleukin-6 (IL-6). **Biotherapy** 2, 363-371.

Moore KW, O'Garra A, De Waal-Malefyt R (**1993**) Interleukin-10. **Ann Rev Immunol** 11, 165-174.

Rosenberg SA (**1992**) The immunotherapy and gene therapy of cancer. **J Clin Oncol** 10, 181-191.

Shevach EM (**2002**) CD4+CD25+ suppressor T cells:more questions than answers. **Nat Rev Immunol** 2, 389-400.

Whittington R, Faulds D (**1993**) Interleukin-2. **Drugs** 46, 446-514.

Yang JC, Hughes M, Kammula U, Royal R, Sherry RM, Topalian SL, Suri KB, Levy C, Allen T, Mavroukakis, Lowy I, White DE, Rosenberg SA (**2007**) Ipilimubab (anti-CTLA4 antibody)causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. **J Immunother** 30, 825-830.

Paolo Lissoni

in vivo *23*: 171-176 (2009)

A Randomized Study of Chemotherapy *Versus* Biochemotherapy with Chemotherapy plus *Aloe arborescens* in Patients with Metastatic Cancer

PAOLO LISSONI[1], FRANCO ROVELLI[1], FERNANDO BRIVIO[2], ROMANO ZAGO[3], MASSIMO COLCIAGO[4], GIUSEPPINA MESSINA[1], ADELIO MORA[1] and GIORGIO PORRO[1]

[1]Division of Radiation Oncology, [2]Division of Surgery, St. Gerardo Hospital, Monza, Milan; [3]Aloe Foundation, Isernia; [4]I.N.R.C.A Laboratory of Analysis, Lecco, Italy

Abstract. *Background: The recent advances in the analysis of tumor immunobiology suggest the possibility of biologically manipulating the efficacy and toxicity of cancer chemotherapy by endogenous or exogenous immunomodulating substances. Aloe is one of the of the most important plants exhibiting anticancer activity and its antineoplastic property is due to at least three different mechanisms, based on antiproliferative, immunostimulatory and antioxidant effects. The anti-proliferative action is determined by anthracenic and antraquinonic molecules, while the immunostimulating activity is mainly due to acemannan. Patients and Methods: A study was planned to include 240 patients with metastatic solid tumor who were randomized to receive chemotherapy with or without Aloe. According to tumor histotype and clinical status, lung cancer patients were treated with cisplatin and etoposide or weekly vinorelbine, colorectal cancer patients received oxaliplatin plus 5-fluorouracil (5-FU), gastric cancer patients were treated with weekly 5-FU and pancreatic cancer patients received weekly gemcitabine. Aloe was given orally at 10 ml thrice/daily. Results: The percentage of both objective tumor regressions and disease control was significantly higher in patients concomitantly treated with Aloe than with chemotherapy alone, as well as the percent of 3-year survival patients. Conclusion: This study seems to suggest that Aloe may be successfully associated with chemotherapy to increase its efficacy in terms of both tumor regression rate and survival time.*

The recent formulation of chemo-biochemotherapeutic regimens could represent a very simple but promising strategy in the treatment of human neoplasms (1-3). The chemo-biochemotherapeutic combinations have been developed to associate the cytotoxic action of cancer chemotherapy with molecules capable of modulating the antitumor biological response and to counteract the suppressive effect of cancer chemotherapy on host immunobiological responses, which plays a fundamental role in the control of tumor progression and dissemination (4-7). Hence, the rationale of the association between cancer chemotherapy and biological response modifier agents consists of the prevention of chemotherapy-induced damage of host anticancer immunobiological reaction. A great variety of natural molecules with immunostimulatory activity have been isolated from plants commonly used in traditional medicine in an empirical manner, in particular from Aloe, *Cannabis indica* and myrrh (8-10). The immunobiological information available up to now may justify the clinical use of these three plants in the palliative therapy of human neoplasms, at least to improve the efficacy and tolerability of the common standard anticancer therapies, including chemotherapy and radiotherapy. Despite differences in the chemical structure of their molecules, the anticancer activity of aloe, cannabis and myrrh is based on very similar mechanisms, consisting of antiproliferative, immunostimulatory, anti-inflammatory and antioxidant effects (8-10). In cannabis and myrrh, both the antiproliferative and immunoinflammatory-modulating effects are attributed to the same molecules, tethrahydrocannabinol and cannabinol for cannabis (11) and the sesquiterpene T-cadinol for myrrh (12). On the contrary, the antiproliferative and the immunomodulating effects of aloe are mediated by separate molecules. More specifically, the antitumor and antiproliferative effects of aloe are mainly exerted by aloenin-like substances, namely aloe-emodine, whose oncostatic action has been shown to be particularly evident against neuroendocrine cancer cell lines (13). On the other hand, the immunostimulatory properties of aloe are mainly dependent on acemannan and glycomannan (14), whose stimulatory action on anticancer immunity is mediated, at least in part, by the inhibibition of interleukin (IL)-l0 secretion, with a resulting increase in the production of IL-2, which plays a fundamental role in the generation of the anticancer immunity

Correspondence to: Dr. Paolo Lissoni, Division of Radiation Oncology, S. Gerardo Hospital, 20052 Monza, Milano, Italy. Fax: +39 0392332284, e-mail: p.lissoni@hsgerardo.org

Key Words: Aloe, biochemotherapy, natural cancer therapy.

0258-851X/2009 $2.00+.40

(15). The anticancer properties of aloe have been confirmed by several experimental *in vitro* and *in vivo* studies (16, 17), revealing that the anticancer activity of aloe does not depend only on its immunomodulatory effect, as believed until recently, but also on a direct inhibition of cancer cell proliferation through aloenin-like molecules.

This finding is not surprising, since aloenin and other similar molecules may be classified within the group of anthracenic and anthraquinonic substances, whose antiproliferative cytotoxic effects are well known. A considerable number of clinical investigations with aloe extracts have been performed, however, these have yelded controversial results. Aloe therapy has been particularly investigated in the treatment of psoriasis, hyperlipidemia and diabetes mellitus (18-21) and it may exert anticholesterolemic and antidiabetic effects (18). Moreover, it stimulates wound repairing, however, no efficacy has been observed in the treatment of radiotherapy-induced skin damage (21).

Finally, aloe has been used for the tratment of human neoplasm (22), although only preliminary data are available. Despite all of this work, most studies are very limited from a methodological point of view, due to the low number of patients and lack of randomization. Therefore, the present study was planned in an attempt to investigate the influence of a concomitant aloe administration on the efficacy and tolerability of chemotherapy in patients with advanced cancer and poor clinical status.

Patients and Methods

Patients. The study included 240 consecutive patients with metastatic solid tumor, who were treated with chemotherapy with or without aloe treatment. The study was performed by using the variety *Aloe arborescens*. Eligibility criteria were as follows: histologically proven metastatic solid tumor; histological diagnosis of lung cancer or gastrointestinal tract tumor; measurable lesions, no previous chemotherapy for the metastatic disease; no possibility to tolerate the most aggressive polychemotherapies because of low performance status (PS), age and/or concomitant important medical illnesses other than cancer; no brain metastasis and no double tumor. The metastatic disease was established by CT scan and/or NMR or PET. Moreover the diagnosis of poor clinical status was established on the basis of low PS and/or concomitant relevant medical diseases other than cancer. The experimental protocol was explained to each patient, and written consent was obtained.

Treatments. According to tumor histotype, sites of metastases and type of chemotherapy, patients were randomized to receive chemotherapy alone or chemotherapy plus aloe. Chemotherapy consisted of cisplatin (CDDP) plus etoposide (VP16) or weekly vinorelbine (VNR) for non-small cell lung cancer (NSCLC) in patients with good or poor clinical status respectively; CDDP plus VP-16 for small cell lung cancer (SCLC); low-dose oxaliplatin (OXA) plus 5-fluorouracil (5-FU) for colorectal cancer; weekly 5-FU for gastric cancer, and weekly gemcitabine (GEM) for pancreatic adenocarcinoma.

Table I. *Clinical characteristics of 240 evaluable patients withs metastatic solid tumors treated with chemotherapy (CT) alone or CT plus aloe.*

Characteristics	CT	CT + Aloe
No.	121	119
Male/female	67/54	65/54
Median age (years)	64 (61-77)	65 (58-79)
Median performance status (Karnofsky's score)	80 (60-100)	80 (60-100)
Dominant metastasis sites:		
Soft tissues	7	6
Bone	20	16
Lung	26	25
Liver	37	35
Liver + lung	24	25
Liver + peritoneum	7	12

CDDP and VP-16 were given *i.v.* at 20 mg/m^2 and at 100 mg/m^2 for 3 consecutive days every 21 days, corresponding to one complete chemotherapeutic cycle. OXA was given *i.v.* at 85 mg/m^2 on days 1 and 8, in association with 5-FU and folates (FA) at a dose of 500 mg/m^2 and 10 mg/m^2 respectively, at days 18 and l5, by repeating the cycle every 28 days. VNR was given weekly at 25 mg/m^2. Weekly 5-FU consisted of 375 mg/m^2, in association with FA at a dose of 10 mg/m^2.

Finally, GEM was given weekly at 1,000 mg/m^2. *Aloe arborescens* was given orally at a dose of 10 ml thrice daily of a mixture consisting of 300 g of Aloe fresh leaves in 500 g of honey plus 40 ml of 40% alcohol, every day without interruption, either during or after chemotherapy, until the progression of disease, starting 6 days prior to the onset of chemotherapy. Aloe mixture was supplied by Deca (Isernia, Italy). The clinical response and toxicity were assessed according to WHO criteria. PS was evaluated by Karnowsky's score. The clinical responses were radiologically evaluated after at least three cycles of chemotherapy by repeating the same radiological investigation used prior to the onset of chemotherapy, including CT scan, NMR and PET. Patients were monitored weekly by routine laboratory tests. Lymphocyte counts were determined by hemochromocytometric analysis. The evaluation of subjective symptoms, such as fatigue and asthenia, was assessed by an individual report.

Statistical analysis. The results were statistically analyzed by the chi-square test, Student's *t*-test and analysis of variance, as appropriate.

The survival curves were plotted by the Kaplan-Meier method and statistically evaluated by the log-rank test. The differences were considered to be statistically significant when p-values were <0.05.

Results

The clinical characteristics of patients are reported in Table I. As shown, the two groups of patients treated with chemotherapy alone, or chemotherapy plus aloe were well comparable for the main prognostic variables, including

Table II. *Clinical response (WHO criteria) in 240 metastatic solid tumor patients treated with chemotherapy (CT) or CT plus Aloe.*

Histotypes	CT							CT + ALOE						
	n	CR	PR	CR+PR	SD	DC	PD	n	CR	PR	CR+PR	SD	DC	PD
Small cell lung cancer														
CDDP/VP16	22	2	6	8 (36%)	7	15 (68%)	7	23	6	8	14 (61%)*	4	18 (78%)**	5
Non-small cell lung cancer	36	1	6	7 (19%)	11	18 (50%)	18	38	4	8	12 (32%)	14	26 (68%)	12
Weekly VNR	17	0	3	3 (18%)	4	7 (41%)	10	17	2	3	5 (29%)	6	11 (65%)	6
CDDP/VP	19	1	3	4 (21%)	7	11 (58%)	8	21	2	5	7 (33%)	8	15 (71%)	6
Colorectal cancer														
OXA/5-FU/FA	21	1	5	6 (29%)	8	14 (67%)	7	21	2	6	8 (38%)	7	15 (71%)	6
Gastric cancer														
Weekly 5-FU/FA	22	0	0	0	9	5 (28%)	13	19	0	3	3 (16%)	7	10 (59%)	9
Pancreatic adenocarcinoma														
Weekly GEM	20	0	2	2 (7%)	10	8 (50%)	8	18	0	3	3 (17%)	8	11 (73%)	7
Overall	121	4	19	23 (19%)	37	60 (50%)	61	119	12*	28	40 (34%)**	40	80 (67%)**	39

CDDP: Cisplatin; VP16: etoposide; VNR: vinorelbine; OXA: oxaliplatin; 5-FU: 5-fluorouracil; FA: folinic acid; GEM: gemcitabine; CR: complete response; PR: partial response; SD: stable disease; DC: disease control (CR+PR+SD); PD: progressive disease. *p<(0.025 *vs*. CT; **p<0.01 *vs*. CT

histotype, sites of metastasis, PS and age. The observed clinical response in the two groups of patients are reported in Table II.

By considering the overall tumor histotypes, the percentages of complete responses (CR) and partial responses (PR) achieved in patients concomitantly treated with aloe were significantly higher than in those who received chemotherapy alone (40/119 (34%) *vs*. 23/121 (19%), p<0.01). A CR occurred in 12/119 (10%) patients concomitantly treated with aloe and in only 4/121 (3%) patients treated with chemotherapy alone. This difference was statistically significant (p<0.01). Stable disease (SD) was achieved in 37/121 (31%) patients treated with chemotherapy alone and in 40/119 (34%) patients who received a concomitant aloe administration. The disease control (DC=CR+PR+SD) obtained in patients concomitantly treated with aloe showed a significantly higher percentage than that found in patients who received chemotherapy alone (80/119 (67%) *vs*. 60/121 (50%), p<0.0l).

As far as the clinical response in relation to tumor histotype is concerned, the objective tumor response rate (CR+PR) achieved in the group of SCLC patients concomitantly treated with aloe was significantly higher than that found in the group of chemotherapy alone (14/23 (61%) *vs*. 8/22 (36%), p<0.05). Moreover, the percentage of CR was also significantly higher in SCLC patients concomitantly treated with aloe (6/23 (26%) *vs*. 2/22 (9%), p<0.05). Similarly, the objective tumor response (CR+PR) observed in the remaining tumor histotypes, including colorectal cancer, gastric cancer and pancreatic adenocarcinoma, was consistently higher in patients concomitantly treated with aloe, without statistically significant differences.

Figure 1 illustrates the 3-year survival curves achieved in patients treated with chemotherapy alone or chemotherapy plus aloe. As shown, the percentage of 3-year survival obtained in patients concomitantly treated with aloe was significantly higher than that found in the group of chemotherapy alone (p<0.0l). As far as the survival in relation to tumor histotype are concerned, the percentage of 3-year survival achieved in both SCLC and NSCLC patients concomitantly treated with aloe was significantly higher than that obtained in those treated with chemotherapy alone (p<0.05). The survival was also longer in all other tumor histotypes treated with chemotherapy plus aloe, without statistically significant differences. Aloe was well tolerated in all patients and no metabolic undesirable effects were observed. In addition, no aloe-related toxicity occurred, including vomiting and diarrhoea.

From an immunobiological point of view, mean numbers of lymphocytes decreased and increased after chemotherapy in patients treated with chemotherapy alone or chemotherapy plus aloe, respectively, even though none of these differences were statistically significant. However, as illustrated in Figure 2, the mean lymphocyte mean number observed after therapy in patients concomitantly treated with aloe was significantly higher than that observed in the group treated with chemotherapy alone (p<0.05), while no difference was seen before the onset of treatment.

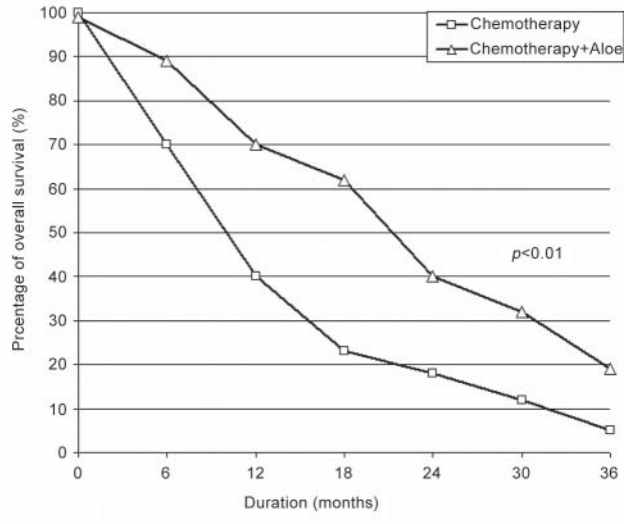

Figure 1. *3-Year survival curves observed in 240 patients with metastatic solid tumor treated with chemotherapy alone or chemotherapy plus aloe.*

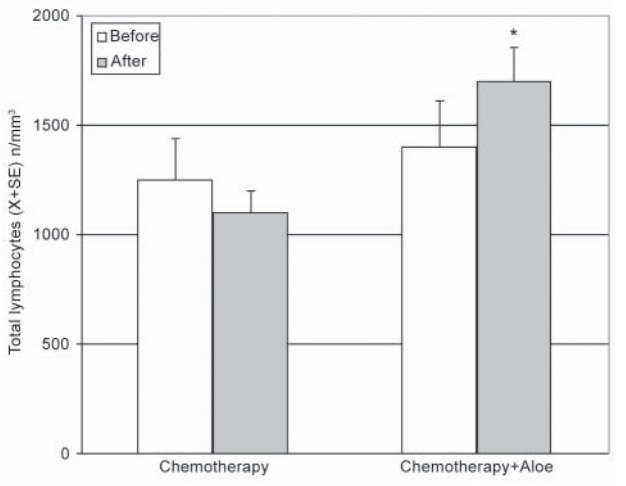

Figure 2. *Mean number of lymphocytes observed before and after the chemotherapeutic treatment in 240 patients with metastatic solid tumor treated with chemotherapy alone or chemotherapy plus aloe. *p<0.05 vs. Chemotherapy.*

Finally, chemotherapy was substantially better tolerated in patients concomitantly treated with aloe. In particular, the occurrence of asthenia and/or fatigue was significantly less frequent in patients concomitantly treated with aloe than in those who received chemotherapy alone (31/119 (26%) *vs.* 56/121 (46%), $p<0.01$). Similarly, VNR-induced constipation was significantly less frequent in the aloe group than in patients treated with VNR alone (3/17 (18%) *vs.* 12/17 (71%), $p<0.0l$). In addition, OXA-induced neurotoxicity, with paresthesic disturbances, was also less frequent in patients who received aloe with respect to those treated with chemotherapy alone (6/21 (29%) *vs.* 9/21(43%)), without statistically significant differences. No other important difference in the occurrence of side-effects was found.

Discussion

The results of this study confirm previous preliminary clinical investigations which had already shown the efficacy of aloe extracts in the palliative therapy of patients with untreatable metastatic cancer, either to improve their quality of life, or to prolong the survival time (22). In addition to these previous results, this study demonstrates the efficacy of aloe in association with cancer chemotherapy, at least in patients with poor clinical status because of low PS or important medical diseases, in whom the therapeutic activity of chemotherapy alone is generally low.

Thus, aloe extracts may exert not only a direct oncostatic effect, but also enhance the efficacy of chemotherapy in terms of both tumor regression rate and survival time as well as reducing some toxicities. Moreover, aloe-induced prolonged survival time was constantly associated with a better quality of life, at least in terms of relief of asthenia and fatigue. Aloe-induced increase in chemotherapy cytotoxic efficacy appear to be particularly evident in SCLC, because of its neuroendocrine nature. This evidence is not surprising, since experimental studies had already shown that the oncostatic properties of aloe substances are more pronounced against neuroendocrine cancer cell lines (13). In any case, aloe-induced increase in chemotherapy anticancer efficacy would depend not only on molecules provided by antiproliferative action, but also on the activity of immunomodulating substances, such as acemannan (8, 14). A particularly interesting combination could be represented by the association between VNR and aloe in the treatment of NSCLC, since aloe seemed either to increase VNR cytotoxic potency, or to correct the most frequent side-effect of VNR, that of severe constipation. The biochemotherapeutic combination of VNR plus aloe could thus constitute a very well tolerated and active therapy for NSCLC patients, including those with poor clinical status. Obviously, the low number of patients for the single tumor histotype does not allow definitive conclusions to be drawn in the treatment of the various solid tumor histotypes by aloe and chemotherapy combination therapy. The relatively low percentage of responses shown by this study for a single histotype with respect to that reported in the literature could depend on the poor clinical status of patients. In any case, further studies will be required to better investigate the real impact of a concomitant aloe therapy on the life of chemotherapy-treated patients with advanced cancer by using more appropriate scales for the quality of life. Moreover, since the study was not blinded, multiple bias may occur. Hence, double-blind randomized studies will be necessary to

confirm these promising results. Finally, further studies should be performed to establish whether aloe extracts may also enhance the efficacy of chemotherapy in patients with good clinical status. Future clinical studies with single aloe molecules, such as aloe-emodine and acemannan for their immunomodulating and antiproliferative properties, respectively, could allow further benefits in the treatment of human neoplasms. Several recent studies (23-27) have contributed to better define the mechanism of the anticancer activity of aloe. However, the exact mechanism of its immunomodulatory antitumor effect has still to be established in detail. Hence, successive studies, by evaluating the most important immune biomarkers, namely IL-2, IL-12, IL-6, IL-10, TGF-β and T regulator lymphocytes, will be essential to establish the influence of aloe on the anticancer cytokine network.

References

1 Atzpodien J and Kirchner H: Cancer, cytokines and cytotoxic cells: interleukin-2 in the immunotherapy of human neoplasms. Klin Wochenschr *68*: 1-11, 1990.

2 Cerea G, Vaghi M, Villa S, Bucovec R, Mengo S, Gardani G, Tancini G and Lissoni P: Biomodulation of cancer chemotherapy for metastatic colorectal cancer. Anticancer Res *23*: 1951-1954, 2003.

3 Lissoni P, Brivio F, Fumagalli L, Messina G, Ghezzi V, Frontini L, Giani L, Vaghi M, Ardizzoia A and Gardani G: Efficacy of cancer chemotherapy in relation to the pre-treatment number of lymphocytes in patients with metastatic solid tumors. Int J Biol Markers *19*: l35-140, 2004

4 Rosenberg SA: The immunotherapy and gene therapy of cancer. J Clin Oncol *10*: 180-199, 1992.

5 Whittington R and Faulds D: Interleukin-2. Drugs *46*: 446-5l4, l993.

6 Lissoni P: Prognostic markers in interleukin-2 therapy. Cancer Biother *11*: 285-287, 1996.

7 Riesco A: Five-year cancer cure: relation to total amount of peripheral lymphocytes and neutrophils. Cancer *25*: 135-140, 1970.

8 Winters WD, Benavides R and Clause VJ: Effects of aloe extracts on human normal and tumour cells *in vitro*. Econ Botany *35*: 89-95, l98l.

9 Grotenhermen F: Pharmacology of cannabinoids. Neuroendocrinol Lett *25*: 14-23, 2004.

10 Qureshi S, Al-Harbi MM, Ahmed M, Raza M, Giancreco AB and Shah AH: Evaluation of the genotoxic, cytotoxic and antitumor properties of *Commiphora molmol* using normal and Ehrlich ascites carcinoma cell-bearing Swiss albino mice. Cancer Chemother Pharmacol *33*: 130-138, l993.

11 Blazquez C, Casanova ML, Planas A, Del Pulgar TG, Villanuéva C, Fernandez-Acenero MJ, Aragones J, Huffman JW, Jorcano JL and Guzman M: Inhibition of tumor angiogenesis by cannabinoids. FASEB J *17*: 529-531, 2003.

12 Claeson P, Zygmunt P and Hogestatt ED: Calcium antagonistic properties of the sesquiterpene T-cadinol. Pharmacol Toxicol *69*: 173-177, 1991.

13 Capasso F, Borrelli F, Capasso R, Di Carlo G, Izzo AA, Pinto L, Mascolo N, Castaldo S and Longo R: Aloe and its therapeutic use. Phytother Res *12*: 124-127, 1998.

14 Davis RH, Parker WL, Sampson RT and Murdoch DP: Isolation of a stimulatory system in an aloe extract. J Am Pediatr Med Assoc *81*: 473-478, 1991.

15 Grimm EA, Mazumder A, Zhang HZ and Rosenberg SA: Lymphokine-activated killer cell phenomenon. J Exp Med *155*: 1823-1841, l982.

16 Soeda M: Extract of Cape aloes inhibited sarcoma 180 and Ehrlich ascites tumours. J Med Soc Jpn *16*: 365-369, l969.

17 t'Hart LA, Van EPH, Van Dijk H, Zaat R and De Silva KT: Two functionally and chemically distinct immunomodulatory compounds in the gel of *Aloe vera*. J Ethnopharmacol *23*: 61-71, 1988.

18 Vogler BK: *Aloe vera* a systematic review of its clinical effectiveness. Br J Gen Pract *49*: 823-828, 1999.

19 Marshall JM: *Aloe vera* gel. What is the evidence? Pharm J *24*: 360-362, 1990.

20 Yongchaiyudha S, Rungpitarangsi V, Bunyapraphatsara N and Chokechaijaroenporn O: Anti-diabetic activity of *Aloe vera*-juice. Phytomedicine *3*: 241-243, 1996.

21 Williams MS, Burk M and Loprinzi CL: Phase III double-blind evaluation of an *Aloe vera* gel as a prophylactic agent for radiation-induced skin toxicity. Int J Radiat Oncol Biol Phys *36*: 345-349, 1996.

22 Lissoni P, Giani L, Zerbini S, Trabattoni P and Rovelli F: Biotherapy with the pineal immunomodulating hormone melatonin *versus* melatonin plus *Aloe vera* in untreatable advanced solid neoplasms. Nat Imm *l6*: 27-33, 1998.

23 Guo J, Xiao B, Liu Q, Gong Z and Le Y: Suppression of C-myc expression associates with anti-proliferation of aloe-emodin on gastric cancer cells. Cancer Invest *26(4)*: 369-374, 2008.

24 Cui Xr, Takahashi H, Shimamura T, Koyanagi J, Komada F and Saito S: Preparation of 1,8-di-*O*-alkylaloe-emodins and 15-amino-, 15-thiocyano-, and 15-selenocyanochrysophanol derivatives from aloe-emodin and studying their cytotoxic effects. Chem Pharm Bull (Tokyo) *56(4)*: 497-503, 2008.

25 Kametani S, Oikawa T, Kojima-Yuasa A, Kennedy DO, Norikura T, Honzawa M and Matsui-Yuasa I: Mechanism of growth inhibitory effect of Cape aloe extract in ehrlich ascites tumor cells. J Nutr Sci Vitaminol (Tokyo) *53(6)*: 540-546, 2007.

26 Guo JM, Xiao BX, Liu Q, Zhang S, Liu DH and Gong ZH: Anticancer effect of aloe-emodin on cervical cancer cells involves G2/M arrest and induction of differentiation. Acta Pharmacol Sin *28(12)*: 1991-1995, 2007.

27 Akev N, Turkay G, Can A, Gurel A, Yildiz F, Yardibi H, Ekiz EE and Uzun H: Tumour-preventive effect of *Aloe vera* leaf pulp lectin (Aloctin I) on Ehrlich ascites tumours in mice. Phytother Res *21(11)*: 1070-1075, 2007.

Received July 16, 2008
Revised September 15, 2008
Accepted October 13, 2008

Cancer Therapy Vol 7, 302-304, 2009

A phase II study of anastrozole plus the pineal anticancer hormone melatonin in the metastatic breast cancer women with poor clinical status

Research Article

Paolo Lissoni [1]*, Giuseppe Di Fede [1], Antonio Battista [2], Giusy Messina [1], Remo Egardi [1], Fernando Brivio [3], Franco Rovelli [1], Massimo Colciago [4], Giuseppe Brera [5]

[1]Institute of Biological Medicine, Milan
[2] Azienda Sanitaria locale 2, Avellino;
[3] Surgery Division, Bassini Hospital, Cinisello,Milan
[4] I.N.R.C.A, Casatenovo, Lecco, Italy
[5]Ambrosian University, Milan, Italy

***Correspondence:** Dr. Paolo Lissoni, Divisione di Radioterapia Oncologica, Ospedale S.Gerardo, 20052 Monza, Milano, Italy; Fax: +390392332284, E-mail: p.lissoni@hsgerardo.org
Key words: Anastrozole, breast cancer, melatonin, pineal gland
Abbreviations: melatonin, MLT; estrogen receptor, ER;

**Received: 9 March 2009; Revised 1 April 2009;
Accepted: 13 April 2009; electronically published: 8 April 2009**

Summary

The recent advances in the psychoneuroendocrinology have suggested the possibility to modulate tumor hormone dependency through a neuroendocrine approach. In particular, it has been proven that the pineal neurohormone melatonin (MLT) may stimulate estrogen receptor (ER) expression in breast cancer cells and inhibit the aromatase activity. On this basis, a study was planned to evacuate the efficacy of a concomitant treatment with the aromatase inhibitor anastrozole plus MLT in metastatic breast cancer.The study included 14 metastatic breast cancer women of poor clinical conditions with ER positive or unknown. Both anastrozole and MLT were given orally at a dose of 1 mg at noon and of 20 mg in the evening, respectively. The clinical response consisted of complete response in 2 and partial response in 6 patients.Then, an objective tumor regression was achieved in 8/14 (57%) patients, with a median duration of 26 months. No neoplastic cachexia occurred on treatment. This preliminary study shows that a neuroendocrine strategy with anastrozole plus the pineal hormone MLT may represent a new effective and well tolerated regimen in the treatment of metastatic breast cancer women, including those with poor clinical status, with therapeutic results apparently superior to those reported in the literature with the only aromatase inhibitor. Then, these results would justify further randomized studies of aromatase inhibitors with or without a concomitant administration of MLT, in an attempt to establish whether the pineal hormone may enhance the efficacy of the aromatase inibibitors in the treatment of human advanced breast cancer.

I. Introduction

Recent experimental studies have demonstrated that the hormone dependency is at least in part under a psychoneuroendocrine regulation (Cos et al, 2008;Grant et al, 2009). In particular, it has been shown that the pineal hormone melatonin (MLT), whose anticancer properties have been well demonstrated (Bartsch et al, 1981; Maestroni, 1993; Reiter et al, 2002), may in vitro stimulate estrogen receptor (ER) expression on breast cancer cell lines (Molis et al,1995). Therefore, the hormone dependency of breast cancer cells would not depend only on intrinsic characteristics of cancer cells themselves, but also on host neuroendocrine regulation of tumor cell proliferation and differentiation (Bartsch et al, 2000). Moreover, cancer progression has been proven to be associated with pineal alterations, consisting of a progressive decline in MLT nocturnal production. (Maestroni, 1993). Therefore the advanced cancer would require a substitutive endocrine therapy with MLT (Bartsch et al, 1981; Maestroni, 1993). Previous preliminary clinical studies had already suggested that the

concomitant administration of the pineal hormone MLT may apparently increase the efficacy of tamoxifen therapy in the treatment of metastatic breast cancer (Lissoni et al, 1995). Moreover, experimental studies have shown that the activity of aromatase enzyme, which is responsible for the peripheral production of estrogens from testosterone (Bagatell et al, 1994), is under a light/dark circadian rhythm (Bhatnagar et al, 1992). Because of the fundamental role of the pineal hormone MLT in the regulation of the daily photoperiod (Bartsch et al, 1981), it is possible to hypothesize that MLT may be involved in the control of the aromatase activity. In fact, recent studies have demonstrated an inhibitory action of MLT on the aromatase activity (Cos et al, 2005). This finding could reserve a prosiming application in the treatment of both early and advanced breast cancer. This statement is justified by the fact that the aromatase inhibitors represent a new class of agents in the endocrine treatment of breast cancer Plourde et al, 1994), with a potential efficacy superior to that achieved by the previous hormonal therapies with anti-estrogens, such as tamoxifene, even though tumor response rate obtained by the aromatase inhibitors are generally not greater than 40%. On this basis, a phase II study was planned in an attempt to evaluate the efficacy of a neuroendocrinotherapeutic regimen consisting of a concomitant administration of the aromatase inhibitor anastrozole and the pineal hormone MLT in metastatic breast cancer women with poor clinical conditions.

II. Materials and methods

The study included 14 consecutive metastatic breast cancer women (median age: 72 years, range 51-82), who were followed at Biological Medicine Institute in Milan, or at Health Local Unit 2 of Avellino, from Feb. 2002 to Sept. 2003. Eligibility criteria were, as follows: histologically proven metastatic breast cancer, measurable lesions, ER positive or unknown, no ability to tolerate chemotherapy because of age, low performance status (PS), important clinical illnesses other than cancer and/or heavy chemotherapeutic pre-treatments, no previous endocrine therapies for the metastatic disease, no double tumor and life expectancy less than 1 year. Previous heavy chemotherapeutic treatment consisting of at least 3 chemotherapeutic lines was made in 11/14 (79 %) patients. Dominant metastasis sites were, as follows: soft tissues:1; bone:1; lung:7 (neoplastic lymphangitis:2); liver:1; lung + liver:1; bone marrow:3. Time-span since first diagnosis of the primary tumor was 44 months (31-66 months). All patients had an acceptable social conditions. The minimum and median follow-up periods were 60 months and 72 months respectively. In all patients, in the case of disease progression, at least to other endocrine therapeutic lines with other aromatase-inhibitors were planned. The experimental protocol, wich was approved by the Health Direction of Biological Medicine Institute of Milan, was explained to each patient and informed consent was obtained. The treatment consisted of anastrozole at a dose of 1 mg/day orally at noon, plus MLT at 20 mg/day orally in the evening, generally half-hour before sleeping, to correct cancer progression-related decline in MLT night secretion(10). Patients were considered to be evaluable when they were treated for at least 3 consecutive months. The clinical response was evaluated according to WHO criteria. Complete response (CR) was the complete disappearance of all neoplastic lesions for at least 1 month. Partial response (PR) was a reduction greater than 50 % of the sum of all neoplastic lesions, for at least 1 month. Stable disesase (SD) was no increase or decrease greater tha 25 % of tumor volume. Progressive Disease (PD) was an increase in tumor volume greater than 25 % or the appearance of new neoplastic lesions. PS was assessed according to Karnofsky's score, consisting of the evaluation of the quality of life in relation to patient activity and bed-rest period. ER was positive in 10 and unknown in the remaining 4 patients. The median PS was 80% (range 70-100). Data were statistically evaluated by the chi-square test and the Student's t test, as appropriate.

Cases	Age	PS	ER	Metastasis sites	Response	Clinical Duration (months)
1	81	80	?	Lung lymphangitis	PR	23
2	76	90	+	Lung	PR	42
3	72	70	+	Lung lymphangitis	CR	38
4	66	100	+	Bone marrow	SD	23
5	82	90	?	Bome marrow	PR	16
6	64	80	+	Lung, bone	PD	-
7	51	100	?	Bone marrow	SD	10
8	77	90	+	Liver	SD	27
9	59	80	+	Lung, bone	SD	13
10	81	90	?	Bone	SD	9
11	62	80	+	Lung	RP	39
12	65	80	+	Lung,bone	PD	-
13	72	90	+	Liver,lung	PR	27
14	75	90	+	Soft tissues	CR	42+

Table 1: Clinical characteristics of metastatic breast cancer women and their clinical response (WHO criteria) to a neuroendocrine regimen consisting of anastrozole plus the pineal hormone melatonin.

III. Results

All patients were fully evaluable for the clinical response. The clinical characteristics of patients and their individual clinical response to the treatment are reported in **Table 1.** As reported, a complete response (CR) was achieved in 2/14 (14%) (soft tissues:1; lung lymphangitis:1). A partial response (PR) was obtained in other 6/14 (43%) (bone:1; lung:3; liver:1; bone marrow:1). Then, an objective tumor response (CR + PR) was reached in 8/14 (57%) patients. The median duration of response was 26 months(range 9-42 months). A stable disease (SD) was seen in other 4/14 (29%), with a median duration of 25 months (range 10-27). Therefore, a disease-control (DC:CR + PR + SD) was achieved in 12/14 (86%) patients, whereas the remaining 2/14 (14%) patients had a progressive disease (PD). No significant difference in tumor response rate was observed between patients with positive or unknown ER (6/10(60%) vs 2/4(50%)). An overall survival at 1 year and at 3 year was achieved in 11/14 (79 %) and in 5/14 (36 %) patients, respectively. Moreover, 3/14 (21%) patients were still alive at 5 years. The treatment was well tolerated in all patients. Moreover, most patients experienced a relief of asthenia under the treatment and in no patient the neoplastic cachexia occurred. Finally, an evident increase in PS mean values was achieved under treatment, even though it did not reach the statistical significance (86 ±5 vs 93 ± 4, mean ± SE).

IV. Discussion

The results of this preliminary phase II study, by showing a percentage of 1-year survival greater than 70% in patients with live expectancy less than 1 year, would suggest that a neuroendocrine regimen consisting of the aromatase inhibitor anastrozole plus the pineal neurohormone MLT may represent a new effective therapeutic strategy in the treatment of metastatic breast cancer women, also in patients with poor clinical conditions, who would not be able to tolerate the most aggressive therapies. The concomitant administration of the pineal hormone would seem to enhance the efficacy of the aromatase inhibitor in terms of objective tumor regressions with respect to the results commonly reported in the literature with the only aromatase inhibitor (Plourde et al, 1994), which are generally lower than 40%. The time to progression would seem to be apparently increased by the concomitant treatment with MLT.This finding is not surprising, since MLT could enhance the therapeutic anticancer acitivity of the aromatase inhibitors by either exerting direct antiproliferative antitumor effects (Bartsch et al, 1981; Maestroni, 1993; Reiter et al, 2002), or further inhibiting the aromatase activity by acting on gene and oncogene expression (Molis et al,1995; Cos et al, 2005). In addiction, MLT appeared to stimulate ER expression of breast cancer lines, by transforming ER negative into ER positive breast cancer, as observed in experimental conditions (Danforth et al, 1983). Since the prognosis of ER positive breast cancers is clearly better than that of ER negative ones, MLT could per se improve the clinical couse of mammary tumors. Finally, because of its interesting therapeutic efficacy as a supportive care (Reiter et al, 2002), MLT would be responsible for the evident improvement in the relief of asthenia and in preventing the occurrence of the neoplastic cachexia. On the other hand, because of the inhibitory effect of MLT (Grant et al, 2009; Reiter et al, 2002) on cancer cell proliferation, the anticancer activity of this polyendocrine regimen would be due not only to an indirect effect, depending on a diminished estrogen production following aromatase enzyme inhibition, but also on a direct inhibition of cancer cell growth, due to MLT itself. Therefore, the results of this preliminary study may justify further clinical randomized investigations with the only aromatase inhibitor versus the concomitant treatment with MLT, in an attempt to confirm the ability of the pineal hormone to enhance the antitumor properties of the aromatase inhibitors in the treatment of metastatic breast cancer women with poor clinical conditions.

References

Bagatell CJ, Dahl KD, Bremner WJ (**1994**). The direct pituitary effect of testosterone to inhibit gonadotrophin secretion in men is partially mediated by aromatization to estradiol. **J Androl** 15:15-21.

Bartsch C, Bartsch H (**1981**) effect of melatonin on experimental tumors under different photoperiods and times of administration. **J Neural Transm** 52:269-279.

Bartsch H, Buchberger A, Franz H,Bartsch C,Maidonis I,Mecke D, Bayer E (**2000**) Effect of melatonin and pineal extracts on human ovarian and mammary tumor cells in a chemosensitivity assay.**Life Sci** 67:2953-2960.

Bhatnagar AS, Muller P, Schenkel L, Trunet PF, Beh I, Schieweck K. (1992). Inhibition of oestrogen biosynthesis and its consequences on gonadotrophin secretion in the male. **J of Steroid Biochemistry and Molecular Biology**;41:1021-1027

Cos S, Martinez-Campa C,Mediavilla MD, Sanchez-Barcelo E (**2005**). Melatonin modulates aromatase activity in MCF-7 human breast cancer cells. **J Pineal Res** 7:136-142.

Cos S, Gonzales A, Martinez-Campa C, Mediavilla MD, Alonzo-Gonzales C, Sanchez-Barcelo EJ (**2008**): Melatonin as a selective estrogen enzyme modulator. **Curr Cancer Drug Targets**. Dec 8(8): 691-702.

Danforth DN, Tamarkin L, Lipmann LE: (**1983**) Melatonin increase oestrogen receptor binding activity of human breast cancer cells. **Nature,** 305**:**323-325.

Grant S.G, Melan MA, Latimer JJ, Witt-Enderby PA (**2009**) : Melatonin and Breast cancer : cellular mechanism, clinical studies and future perspective. **Expert Rev Mol Med** 11:e5

Lissoni P, Barni S, Meregalli S, Fossati V, Cazzaniga M, Esposti D, Tancini G (**1995**) Modulation of cancer endocrine therapy by melatonin:a phase II study of tamoxifen plus melatonin in metastatic breast cancer patients progressing under tamoxifen alone.**Br J Cancer** 71:854-856.

Maestroni GJM (**1993**) The immunoneuroendocrine role of melatonin. **J Pineal Res** 14:1-10.

Molis TM, Spriggs LL,Jupiter Y,Hill SM (**1995**) Melatonin modulation of estrogen-regulated proteins, growth factors,and proto-oncogenes in human breast cancer. **J Pineal Res** 18:93-103.

Plourde PV, Dyroff M, Dukes MD (**1994**) Arimidex: a potent and selective fourth generation aromatase inhibitor.**Breast Cancer Res Treat** 30:103-111.

Reiter RJ, Tan DX, Sainz RM, Mayo JC, Lopez-Burillo S. (**2002**) Melatonin:reducing the toxicity and increasing the efficacy of drugs. **Pharm Pharmacol** 54:1299-1321

Cancer Therapy Vol 7, 397-401, 2009

Biotherapy with the pineal hormone melatonin plus aloe and myrrh tincture in untreatable metastatic cancer patients as an essence therapy of cancer

Research Article

P. Lissoni[1]*, F. Rovelli[1],G. Messina[2], F. Brivio[3], B. Boniardi[1], G. Porro[1], L.Vigore[4], G. Di Fede[1], P. Marchiori[1], G. Brera[5]

[1]Institute of Biological Medicine, Milan;

[2]Psychiatric Division, Policlinico Hospital, Milan;

[3]Division of Surgery, Bassini Hospital, Cinisello,Milan;

[4]Laboratory of Immunomicrobiology,San Gerardo Hospital, Monza, Milan;

[5]Ambrosian University, Milan, Italy.

***Correspondence**: Dr. Paolo Lissoni, Divisione di Radioterapia Oncologica, Ospedale S. Gerardo, 20052 Monza, Milano, Italia. Fax: +390392332284, e-mail: p.lissoni@hsgerardo.org

Key words: Aloe Vera, Melatonin, Mirrh, and Anticancer Immunity

Abbreviations: Melatonin (MLT), complete response (CR), partial response (PR), stable disease (SD), disease control (DC), progressive disease (PD), T helper lymphocytes (TH, $CD4^+$), T regulatory lymphocytes (T reg, $CD4^+$ $CD25^+$)

Received: 30 July 2009; Revised: 18 October 2009
Accepted: 20 October 2009; electronically published: December 2009

Summary

Background: The recent advances in understanding the immunobiological interactions responsible for cancer progression have allowed us to define the mechanisms of action of some plants, whose antitumor properties were already known by the popular Medicine, in particular Aloe and Myrrha, whose mixture was already therapeutically utilized more than 2000 years ago by the Essence medicine. Moreover, some endogenous natural substances, namely the main hormone produced by the pineal gland melatonin (MLT) may also play anticancer activity. On this basis, a study was performed with a biological regimen consisting of MLT, Aloe and Myrrha in untreatable metastatic cancer patients with life expectancy lower than 1 year. Methods: The study included 35 patients. MLT was given orally at 20 mg/day in the evening and a mixed Aloe and Myrrha tincture was administered at a dose of 5 ml/thrice daily. Results: The clinical response consisted of complete response (CR) in 1, partial response (PR) in 2, stable disease (SD) in 19 patients, whereas the remaining 13 patients had a progressive disease (PD). Thus, a disease control (CR + PR + SD) was achieved in 22/35 (63%)patients. Moreover, a survival longer than 1 year was achieved in 17/35 (49%) patients. Finally, DC was associated with an evident improvement in the immune status, namely consisting of a decrease in the number of T regulatory lymphocytes, which are the main cells responsible for the suppression of the anticancer immunity. Conclusion: This preliminary study shows that a biological anticancer regimen consisting of the pineal hormone MLT in association with Aloe and Myrrha mixture, already known at the times of the Essence medical tradition, may induce a control of the neoplastic disease by stimulating the anticancer immunity, in a relevant percentage metastatic cancer patients, who did not respond to the conventional anticancer treatments and for whom no other standard therapy was available.

I. Introduction

The recent better definition of the biochemical mechanisms responsible for cancer cell proliferation and for immune system-mediated tumor cell destruction has allowed the possibility to establish the biochemical actions of several plants already known by the popular Medicine to be provided by empiristic potential anticancer properties, namely Aloe, Myrrha, Cannabis Indica, Turmeric and Hyssopus (Davis et al, 1991; Capasso et al,

1998; Vogler et al, 1999; Claeson et al, 1991; Qureshi et al, 1993; Blazquez et al, 2003; Grotenhermen et al, 2004; Aggarwall et al, 2003; Lodha et al, 2000). In more detail, the anticancer activity of Aloe is due to several therapeutically active molecules capable of inhibiting cancer cell proliferation, such as aloenine, aloesine and aloe-hemodin, or stimulating the anticancer immunity, such as acemannane and glycomannane (Davis et al, 1991; Capasso et al, 1998; Vogler et al, 1999). On the same way, the antitumor therapeutic properties of Myrrha extracts have been proven to exert both anticancer antiproliferative and immunostimulating effects, which are mediated by T-cadinol and muzumboic acid, respectively (Claeson et al, 1991; Qureshi et al, 1993). The therapeutic biological properties of a mixture of Aloe and Myrrha were well known by the Essence medical tradition at Qumran, near to the Death Sea, as reported by John's Gospel (John's Gospel), referring that men connected to the Essence community, such as Nycodemus and Joseph of Arimathea, prepared a mixture of Aloe and Myrrha for the burial of Christ. Together with the Ellenic medical sciences, the Essence medicine represented the most advanced medical tradition in the ancient world. With respect to the Ellenic medicine, which was founded by Hippocrates, the Essence medical science was more symbolic and spiritual, by considering the treatment of the human diseases as a simultaneous chemical, psychic and spiritual regeneration of man, mediated by humans, but originating from God. The Essence philosophy interpreted the Universe, the human History and the individual life of men and women as the expression of a war between two opposite principles, the Light and Dark, and the single human disease was considered to be the consequence of the prevalence of the principle of Darkness, as the unconscious aspect of the human life, on the principle of Light, which in contrast is the expression of the spiritual consciousness. The philosophic and spiritual characteristics of the Essence medical tradition were further amplified by the Islamic Medicine, by affirming the existence in the Nature of a therapeutic remedy for the overall human illnesses, as the manifestation of Love and harmonies of God. The fundamental importance of the light/dark circadian rhythm in regulating the living organisms, including humans, has been recently confirmed by the investigation on the physiology of the pineal gland, which has appeared to regulate the most important biological functions and systems, such as cell proliferation, DNA expression and immune reactions in relation to the light/dark rhythm through the circadian secretion of its most known hormone melatonin (MLT), with high production during the darkness and low secretion during the light period of the day (Iguchi et al, 1982; Attanasio et al, 1985; Jankovic et al, 1997; Brzezinski et al, 1997). As well as Aloe and Myrrha, MLT also has been proven to play an anticancer action and the antitumor properties of MLT have been confirmed by several experimental and clinical studies (Bartsch et al, 1981; Regelson et al, 1987; Lissoni et al, 2002; Sze et al, 1993). The anticancer action of MLT is due to both direct antiproliferative effects and stimulation of IL-2- dependent anticancer immunity (Maestroni 1993; Lissoni et al, 2008). Because of its dependency on the Light/Dark universal rhythm, whose importance was already known by the Essence tradition, the knowledgements of the functions of the pineal gland, including its anticancer fundamental role, may be considered as the last contribution of the Essence science to the treatment of the human diseases, namely cancer, since the Essence medicine was the first to discover the therapeutic properties of the mixture of Aloe and Myrrha. Moreover, preliminary data would suggest the possibility to amplify the anticancer action of MLT by Aloe extracts (Lissoni 2002). On these bases and in agreement with the well experimentally documented anticancer activity of its overall compounds (Davis et al, 1991; Claeson et al, 1991; Bartsch et al, 1981), in this preliminary study we have evaluated the clinical efficacy of a biological regimen, consisting of Aloe, Myrrha and the pineal hormone MLT, which could be symbolically defined as an Essence therapy, in the treatment of metastatic cancer patients, who failed to respond to the conventional antitumor therapies, including chemotherapy, endocrine therapy and anti-angiogenic treatment, or who were unable to tolerate the conventional therapies and for whom no other standard treatment was available. The objective of the study was to establish whether the association of other natural anticancer agents such as Aloe and Myrrh might further enhance the antitumor efficacy of MLT in the treatment of human neoplasm, with respect to the historical ones achieved with MLT alone.

II. Materials and methods

The study included 35 consecutive metastatic cancer patients, who were followed at the Institute of Biological Medicine of Milan. The therapeutic protocol was explained to each patient and informed consent was obtained. Eligibility criteria were, as follows: histologically proven metastatic solid tumor, measurable lesions, no double tumor, lack of response top the conventional anticancer therapies or poor clinical conditions unable to subtain a chemotherapeutic approach, a life expectancy less than one year, no chronic concomitant therapy with corticosteroids because of their immunosuppressive effects and a minimum follow-up of 12 months. The clinical characteristics of patients are reported in **Table 1**. The treatment consisted of MLT at 20 mg/day orally during the dark period of the day according to its light/dark circadian rhythm (Iguchi et al, 1982; Attanasio et al, 1985; Jankovic et al, 1997; Brzezinski et al, 1997), plus a mixture of Aloe Vera and Myrrha tincture, containing 60% of Aloe and 40% of Myrrha, which was administered orally at a dose of 5 ml thrice/day at 8- hour intervals. The treatment was continued until the progression of disease. Both MLT (Melaton-Med) and mixed Aloe and Myrrha tincture (Mirral) were supplied by Natur-Spiritual (Milan, Italy). The clinical response was evaluated according to WHO criteria. The treatment was also evaluated in relation to its possible immunomodulating effects on the anticancer immunity, by measuring the absolute number of the most important anticancer lymphocyte subset and that of the main immunosuppressive lymphocyte subpopulation, consisting of T helper lymphocyte (TH) and T regulatory lymphocyte (T reg), respectively (Shevach et al, 2002). Lymphocyte subsets were measured by a flow cytometric assay and monoclonal antibodies supplied by Becton-Dickinson (Milan, Italy). TH and T reg lymphocytes were identified as $CD4^+$ cells and $CD4^+$ $CD25^+$ cells, respectively. CD4/CD4CD25 cell ratio was also established. Normal values of CD4/CD4CD25 ratio observed in our laboratory (95% confidence limits) was

greater than 4.0. The immune analysis was made before the onset of treatment and after three months of therapy. Finally, patients were also clinically evaluated from a psychological point of view by the Rorschach test (Rorschach et al, 1921) and spiritually investigated by a specific patient spiritual questionnaire, previously reported in literature (Lissoni et al, 2008). Moreover, patients, who asked a psychospiritual therapeutic approach, were followed through a specific psychospiritual herapeutic method, consisting of an educational program carried out to stimulate the concomitant rediscovery of the perception of pleasure and the spiritual sensitivity. In more detail, according to previous studies (Lissoni et al, 2008), patients were stimulate to become conscious that both pleasure repression and self-punishment may suppress the anticancer immunity and promote cancer cell dissemination. Data were reported as mean ± SE and statistically analyzed by the chi-square test, the Student's t test and the analysis of variance, as appropriate. Moreover, the 1-year survival curves were plotted according to Kaplan-Meier method and statistically analyzed by the log-rank test.

Table 1: Clinical characteristics of 35 untreatable metastatic cancer patients.

Characteristics	**N**
Male / Female	19/16
Median age (year s)	63 (52-81)
Median Performance status (Karnofsky's score)	90 (70-100)
Tumor histotypes:	
Lung cancer	10
Nonsmall cell lung cancer	7
Small cell lung cancer	3
Colorectal cancer	5
Pancreatic cancer	4
Ovarian cancer	4
Prostate cancer	4
Gastric cancer	3
Biliary tract cancer	3
Malignant melanoma cancer	2
Dominant metastasis sites:	
Soft tissues	2
Bone	3
Lung	10
Liver	7
Lung + liver	5
Peritoneum	6
Brain	2
Previous Chemotherapies	31 / 35

Table 2: Clinical results in response to Melatonin plus Aloe and Myrrh in relation to tumor hitotypes.

Tumor Histotype	**N**	**CR**	**PR**	**CR+PR**	**SD**	**DC** (CR+PR+SD)	**PD**
Overall patients	35	1	2	3 (9%)	19 (54%)	22 (63%)	13 (37%)
Nonsmall cell lung cancer	7	0	0	0	5	5	2
Small cell lung cancer	3	0	0	0	2	2	1
Colorectal cancer	5	0	0	0	4	4	1
Pancreatic cancer	4	0	1	1	1	2	2
Ovarian cancer	4	0	0	0	2	2	2
Prostate cancer	4	0	0	0	2	2	2
Gastric cancer	3	0	0	0	2	2	1
Biliary tract cancer	3	0	1	1	1	2	1
Malignant melanoma cancer	2	1	1	1	0	1	1

III. Results

As shown in **Table 2**, an objective tumor regression was achieved in 3/35 (9%) patients, consisting of a complete response (CR) in one patient with node metastases due to malignant melanoma and 2 partial responses (PR), the former in a patient with liver metastases due to pancreatic adenocarcinoma and the latter in a patient with biliary tract cancer-induced liver involvement. The median duration of the response was 11 months (Aggarwall et al, 2003; Lodha et al, 2000; John's Gospel; Iguchi et al, 1982; Attanasio et al, 1985; Jankovic et al, 1997; Brzezinski et al, 1997; Bartsch et al, 1981). A stable disease (SD) was observed in 19/35 (54%) patients (non-small cell lung cancer: 5; small cell lung cancer: 2; colorectal cancer: 4; gastric cancer: 2; pancreatic cancer: 1; biliary tract cancer: 1; prostate cancer: 2; ovarian carcinoma: 2). Then, a disease control (DC), consisting of CR, PR and SD, was achieved in 22/35 (63%) patients. On the contrary, the remaining 13/35 (37%) patients had a progressive disease (PD). The median duration of DC was 8 months (Qureshi et al, 1993; Blazquez et al, 2003; Grotenhermen et al, 2004; Aggarwall et al, 2003; Lodha et al, 2000; John's Gospel; Iguchi et al, 1982; Attanasio et al, 1985; Jankovic et al, 1997; Brzezinski et al, 1997; Bartsch et al, 1981; Regelson et al, 1987). A survival longer than 1 year was achieved in 17/35 (49%) patients and the percentage of 1-year survival observed in patients with DC was significantly higher with respect to that found in those who had a PD (15/22(68%) vs 2/13(15%), $P < 0.01$). As far as the ratio was found in 21/35 (60%) patients. The mean numbers of TH and T-reg lymphocytes increased and decreased on therapy, respectively, without however statistically significant differences with respect to the pre-treatment values (TH: 592 ± 46 vs 544 ± 38/mm^3; T reg: 226 ± 28 vs 277 ± 22/mm^3). On the same way, CD4$^+$ /CD4$^+$ CD25$^+$ mean ratio increased on therapy, without however significant differences (3.1 ± 0.4 vs 2.8 ± 0.3). On the contrary, by evaluating the immune variations in relation to the clinical response, a significant decrease in T-reg mean number and a significant increase in CD4+ /CD4$^+$ CD25$^+$ mean ratio were observed in patients with DC (T-reg: 189 ± 14 vs 268 ± 217/mm^3, $p<0.05$; CD4$^+$ /CD4$^+$ CD25$^+$: 5.9 ± 0.3 vs 2.2 ± 0.4, $p < 0.01$), whereas T-reg mean count enhanced (309 ± 28 vs 284 ± 25/mm^3) and CD4+ /CD4$^+$ CD25$^+$ mean ratio diminished (2.6 ± 0.5 vs 2.9 ± 0.3) in patients with PD, even though none of these differences was statistically significant. TH means number enhanced (686 ± 38 vs 584 ± 41/mm^3) in patients with DC and decreased (576 ± 46 vs 598 ± 37/mm^3) in patients with PD, without however significant differences. A lack of both spiritual sensitivity and pleasure feeling at the Rorschach test was observed in 21/35 (60%) patients. Moreover, the percentage of DC obtained in patients expressing pleasure and spiritual sensitivity at the Rorschach test was significantly greater with respect to that achieved in patients with suppression of both pleasure and spirituality (12 /14(86%) vs 10/21(48%), $p<0.05$). On the same way, the mean values of the spiritual score were significantly higher in patients who achieved a DC than in those who had a PD (72 ± 4 vs 53 ± 3, $p<0.025$). The treatment was well tolerated in all patients. A mild transient diarrhoea, due to the laxative action of aloine, occurred in only 4/35 (11%) patients. Moreover, a clear improvement in the well being was reported in 14/22 (64%) patients with DC and in only 3/13 (23%) patients with PD. This difference was statistically significant ($P < 0.05$). Finally, in none of the patient the neoplastic cachexia occurred.

IV. Discussion

This preliminary biotherapeutic study shows that a biological strategy consisting of the pineal hormone MLT, Aloe and Myrrha, each of who has been proven to play antitumor activity (Davis et al, 1991; Claeson et al, 1991; Bartsch et al, 1981), may induce a control of the neoplastic growth in a relevant percentage of metastatic cancer patients, for whom no other standard antitumor therapy was available. Moreover, this study demonstrates that the control of the neoplastic disease achieved by this biological strategy may influence the clinical course of the neoplastic disease, a prolonged survival with respect to that observed in patients, who had no benefit from the treatment. In particular, by comparing these results with those historically obtained with MLT alone (Lissoni 2002; Maestroni 1993) it seems that Aloe and Myrrh association further amplify the anticancer action of MLT (Lissoni 2002; Maestroni 1993). Therefore these preliminary data would justify successive randomized trials with MLT alone vs. MLT plus Aloe and Myrrh to confirm the greater efficacy of a polytherapy with several biological natural agents, with respect to single agent. In addition, this study would suggest that the therapeutic efficacy of this natural biological regimen is mainly mediated by the immune system by piloting in an antitumor way the host immunobiological reaction and in particular it seems to be able to counteract advanced cancer-related abnormally enhanced function of T-reg cell system, which would represent the main cause responsible for the lack of an effective anticancer immune reaction in the disseminated neoplastic disease (Shevach 2002). Finally, this study would seem to suggest that the efficacy of an anticancer immunobiological regimen, consisting of MLT, Aloe and Myrrha, may be influenced by both psychological and spiritual status of patients and in particular the evidence of a suppression of both pleasure and spiritual feeling may predict a reduced efficacy of the treatment in terms of control of the neoplastic growth. Generally, the Oncologists subdivide the medical treatments of cancer into curative and palliative therapies, by commonly considering as antitumor curative drugs the only chemotherapeutic agents. From this point of view, a clinical approach with natural biological anticancer agents, which is generally considered as a complementary medicine, cannot be simply defined as palliative treatment, because of its capacity of counteracting cancer cell proliferation also in patients for whom there was no other standard anticancer therapy. Further promising results in terms of control of the neoplastic progression could be achieved by considering that MLT is not the only anticancer hormone produced by the pineal gland (Bartsch

et al, 1981; Regelson et al, 1987; Lissoni et al, 2002; Sze et al, 1993). In fact, at least another pineal hormone, the 5 -methoxytryptamine, may play an anticancer action, with in vitro antiproliferative effects superior to those of MLT itself (Sze et al, 1993). Retinoids play also anticancer effects through cytodifferentiating and anti-angiogenic activities. In addition, at least five other plants could be successfully employed in the treatment of human neoplasms (Blazquez et al, 2003; Grotenhermen et al, 2004; Aggarwall et al, 2003; Lodha et al, 2000), including Hyssopus, Cannabis Indica, Turmeric and Incense may play anticancer effects. Moreover, Hyssopus, whose potential anticancer activity would be due to diosmine, could be particularly useful in the treatment of lung cancer patients, because of its very potent expectorating activity (Lodha et al, 2000). Cannabis Indica contains several cannabinoid agents provided by direct anticancer antiproliferative and anti-angiogenic actions (Blazquez et al, 2003; Grotenhermen et al, 2004). Finally, according to preliminary studies (unpublished data), curcumin, the main active anticancer molecule produced by turmeric (Aggarwall et al, 2003), would be particularly useful in the treatment of cancer of pancreas. Therefore, further studies will be required to establish which may be the best biological natural anticancer combination, by considering the therapeutic and the supportive care effects, the toxicity and the social coast of the various potential both endogenous and exogenous natural antitumor substances.

References

Davis RH, Parker WL, Sampson RT, Murdoch DP. Isolation of a stimulatory system in an Aloe extract. J Am Pediatr Med Assoc 1991; 81:473-8.

Capasso F, Borrelli F, Capasso R, Di Carlo G, Izzo AA, Pinto L et al. Aloe and its therapeutic use. Phytother Res 1998; 12:124-7.

Vogler BK. Aloe Vera: a systematic review of its clinical effectiveness. B J Gen Pract 1999; 49:823-8.

Claeson P, Zygmunt P, Hogestatt ED. Calcium antagonistic properties of the sesquiterpene T- cadinol. Pharmacol Toxicol 1991; 69:173-7.

Qureshi S, Al-Harbi MM, Ahmed M, Raza M, Giangreco AB, Shah AH. Evaluation of the genotixic, cytotoxic and antitumor properties of Commiphora molmol using normal and Erlich ascites carcinoma cell-bearing Swiss albino mice. Cancer Chemother Pharmacol 1993; 33130-8.

Blazquez C, Casanova ML, Planas A, Del Pulgar TG, Villanueva C, Fernandez-Acenero MJ et al. Inhibition of tumor angiogenesis by cannabinoids. FASEB J 2003; 17:529-31.

Grotenhermen F. Pharmacology of cannabinoids. Neuroendocrinol Lett 2004; 25:14-23.

Aggarwall BB, Kumar A, Bharti AC. Anticancer potential of curcumin. Preclinical and clinical studies. Anticancer Res 2003; 23:363-98.

Lodha R, Bagga A. Tradictional Indian system of Medicine. Ann Acad Med Singapore 2000; 29:37-41.

John's Gospel 19,38-40.

Iguchi H, Kato KI, Ibayashi H. Age-dependent reduction in serum melatonin concentrations in healthy subjects. J Clin Endocrinol Metab 1982; 55:27-9.

Attanasio A, Borrelli P, Gupta D. Circadian rhythms in serum melatonin from infancy to adolescence. J Clin Endocrinol Metab 1985; 61:388-90.

Jankovic BD. Neuroimmunomodulation. Ann NY Acad Sci 1994; 741:3-38.

Brzezinski A. Melatonin in humans. N Engl J Med 1997; 336:185-95

Bartsch H, Bartsch C. Effects of melatonin on experimental tumors under different photoperiods and times of administration. J neural Transm 1981; 52:269-79

Regelson W, Pierpaoli W. Melatonin: a rediscovered antitumor hormone? Cancer Invest

1987; 5:379-85

Lissoni P. Is there a role for melatonin in supportive care? Supp Care Cancer 2002; 10:110-6.

Sze S, Ng T, Liu W. Antiproliferative effect of pineal indoles on cultured tumor cell lines. J Pineal Res 1993; 14:27-33.

Maestroni GJM. The immunoneuroendocrine role of melatonin. J Pineal Res 1993; 14:1-10.

Lissoni P, Brivio F, Fumagalli L, Messina G,Vigorè L, Parolini D et al. : Neuroimmunomodulation in Medical Oncology: application of Psychoneuroimmunology with subcutaneous low-dose IL-2 plus the pineal hormone melatonin in patients with untreatable metastatic solid tumors. Anticancer Res 2008; 28:1377-82.

Shevach EM. CD4+CD25+ suppressor T cells: more questions than answers. Nat Rev Immunol 2002; 2:389-400.

Rorschach H. Psychodiagnosytics. Ed HA Huber, Bern, Stuttgart, Toronto. Verlag, 1921.

Lissoni P, Messina G, Parolini D, Balestra A, Brivio F, Fumagalli L et al.:A spiritual approach in the treatment of cancer. In Vivo 2008; 22:557-82.

Dr. Paolo Lissoni

JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Radiation Oncology

Research article

A PsychoNeuroEndocrineImmune (PNEI) Approach to Enhance the Efficacy of Radiochemotherapy in Glioblastoma

Lissoni P[1*], Messina G[1], Porro G[1], Porta E[2], Nosetto L[2], Mancuso M[2], Di Fede G[2]

[1]*International Institute of PsychoNeuroEndocrineImmunology (PNEI), Milan, Italy*

[2]*Institute of Biological Medicine, Milan, Italy*

**Corresponding author: Dr. Paolo Lissoni, Corso Plebisciti 19 - 20122 Milan, Italy, Tel:+39 – 331.5298426 ; Email: p.lissoni@hsgerardo.org*

Received: 03-31-2016

Accepted: 05-20-2016

Published:

Abstract

GBM would represent perhaps the only tumor, whose prognosis had achieved no evident benefits in terms of survival from the main oncological therapies, including chemotherapy, immunotherapy and anti-angiogenic treatments. According to the recent advances in the Psychoneuroendocrinology, an improvement in GBM therapy could arise from the knowledge of the psychoneuroendocrine mechanisms responsible for GBM cancer cell growth, and, at present, it has been proven that GBM cells may express opioid receptors, whose activation stimulate cancer proliferation, whereas melatonin (MLT) and other pineal indole hormones, namely the 5-methoxytryptamine (5-MTT), may suppress GBM growth. In addition, several plants, such as Aloe, Myrrh, Boswellia, Magnolia and Cannabis Indica, have appeared to exert an anticancer activity on several tumor histotypes, including GBM. On these bases, a study was planned by associating a neuroendocrine and phytotherapeutic combination to the standard therapy of GBM with radiotherapy (RT) plus chemiotherapy of temozolomide (TMZ). The study included 30 consecutive patients with histologically proven GBM after radical or palliative surgery. The neuroendocrine regimen consisted of an oral administration of MLT at 100 mg/day in the dark period plus 5-MTT at a dose of 5 mg/day in the light period of the day plus the opioid antagonist naltrexone (NTX) at escalating doses until a maximal dosage of 50 mg/day in the morning. The phytotherapeutic regimen included Aloe, Myrrh, Magnolia and Boswellia. Finally, patients were randomized to received also Cannabis infusion. A disease control, including partial response and stable disease, was achieved in 16/30 (53%) patients, and it was associated with a survival longer than 1 year in 17/30 (57%) patients. At the end, the 3-year survival achieved in patients concomitantly treated by Cannabis was significantly higher that that found in patients, who received no Cannabis therapy. This preliminary study would suggest that a neurorendocrine approach, carried out to biologically counteract GBM growth, in association with the standard therapy with RT plus TMZ may increase the overall survival of GBM patients.

Keywords: Glioblastoma; Melatonin; Naltrexone; Opioid system Pineal gland

Introduction

Brain glioblastoma (GBM) still remains the most untreatable neoplastic disease. Several factors have been taken into consideration to identify possible subtypes of GBM with different prognostic behaviour, but, at present, the main prognostic factors would be represented by age, performance status (PS) and methyl-guanine DNA-methyltransferase (MGMT). The prognosis is worse in aged patients and in those with low PS. In patients 60 year older the overall survival time is generally less than 6-9 months [1,2]. In contrast, patients positive for MGMT expression would have a longer survival and a better response to chemotherapy [3]. Almost all clinical therapeutic studies, performed up to now, have been carried out with the only radiotherapy (RT) and chemotherapy (CT). Only the temozolomide (TMZ) has been substantially used as potentially active chemotherapeutic agent, without taking into consideration the possible existence of endogenous growth factor stimulating GBM cancer cell growth, as well as estrogens for breast cancer and androgens for prostate cancer, and, on the other hand, pos-

sible endogenous inhibitory factors on GBM cancer cell proliferation. In fact, it is known since many years that GBM cells may express mu-opioid receptors and that mu-opioid agents may stimulate GBM cancer cell growth [4].

Therefore, the evidence of tumor mu-opioid receptors expression would be associated with a poor prognosis, because of the stimulatory action of mu-opioid agonists, such as beta-endorphin, on cancer growth. On the contrary, the pineal indole hormones [5] and the cannabinoid agonists from Cannabis Indica [6] have been proven to inhibit GBM cell proliferation. Melatonin (MLT) represents the most investigated pineal hormone provided by a well documented anticancer activity on several tumor histotypes, including GBM [5], but at least another pineal indole hormone, the 5-methoxytryptamine (5-MTT), has appeared to exert in vitro an anticancer action superior to that of MLT itself [7]. In addition, cancer progression has been proven to be associated with a progressive decline in MLT secretion, mainly during the night [8], and most in general with a diminished pineal endocrine function [9]. MLT exerts its effects by acting on specific MLT receptors MT 1 and MT 2 ([10], and it has been demonstrated that tumor expression of MLT receptors are associated with a better prognosis in cancer patients [11]. Pineal deficiency may be corrected by an exogenous administration of the main pineal indole hormones, whereas the stimulatory activity of brain opioid system may be counteracted by the administration of the long-acting opioid antagonist naltrexone (NTX) [12].

On the basis of these data and according to a neuroendocrine strategy, it seems to be justified the employment of pineal hormones and opioid antagonists in the treatment of GBM in association with the standard radiochemotherapeutic regimen or after progression on RT plus CT with TMZ. This preliminary phase 2 study was carried out to evaluate the impact of a neuroendocrine schedule with pineal hormones and opioid antagonists in association with the standard therapy by RT plus CT in the treatment of GBM.

Materials and Methods

The study included 30 consecutive GBM patients, who underwent the standard treatment with RT plus CT with TMZ in association with a neuroendocrine regimen consisting of the oncostatic pineal hormones MLT and 5-MTTplus the mu-opioid antagonist NTX. Eligibility criteria were as follows: histologically proven GBM, measurable lesions, macroscopically radical or palliative surgery, and life expectancy less than 1 year. The experimental protocol was explained to patients, and their consent was obtained. The clinical characteristics of patients are reported in Table 1. The standard treatment consisted of RT 60 Gy in 2-Gy 30 fractions plus TMZ at 75 mg/m^2/day orally during RT, followed by 6 cycles of TMZ at 200 mg/m^2/day for 5 consecutive days every 28 days. The pineal endocrine therapy consisted of an oral administration of MLT at 100 mg/day during the dark phase of the day plus 5-MTT at a dose of 10 mg/day during the light phase of the day, corresponding to the time of their circadian secretion. Moreover, in case of progression, because of the evidence of a dose-dependency in its antitumor activity [13], MLT dose was increased of 100 mg/day every time, until a maximal dosage of 500 mg/day. NTX was given orally at a daily dose of 50 mg, starting with a dose of 20 mg/day in the morning by slowly increasing the dose of 10 mg every month in an attempt to reduce liver toxicity of NTX [12]. The supportive care with natural agents consisted of the oral administration of antitumor plants, including a mixture of Aloe arborescens [14] plus Myrrh [15] (60/ 40% ratio) at a dose of 10 ml thrice/day, Magnolia cortex at 500 mg twice/day [16], and Boswellia [17], also provided by an anti-oedema activity, at 1000 mg twice/day. At the end, according to their free adhesion and compliance, patients were randomized to receive also Cannabis flos (19% tetra-hydro-cannabinol) as an infusion of Cannabis 0.5 mg/ liter of water, by drinking it at 100 ml three times/day. The clinical response was evaluated by WHO criteria. Data were statistically analyzed by the chi-square test, the Student's test and the log-rank test, as appropriate.

M / F	20 / 10
Median age (years)	65 (range 21-75)
Median PS (ECOG)	1 (range 0-3)
Tumor sites	
- Frontal cortex	13
- Temporal cortex	7
- Temporo- parietal cortex	5
- Occipital cortex	2
- Corpus callosum	3

Table 1. Clinical characteristics of 30 GBM patients.

Results

The clinical response (WHO) is shown in Table 2. A macroscopically radical surgery was obtained in no patient. No complete response (CR) was achieved after RT plus CT. A partial response (PR) was obtained in 3/13 (23%) patients treated also by Cannabis and in none of the 17 patients, who received no Cannabis infusion. A stable disease (SD) occurred in 6/17 patients treated without Cannabis and in 7/13 patients under Cannabis treatment. Therefore, the percentage of disease control (DC) (PR + SD) obtained in patients concomitantly treated with Cannabis was significantly higher with respect to that found in patients, who did not receive Cannabis infusion (10/13 (77%) vs 6/17 (35%), $P < 0.05$). The percent of 3-year survival is illustrated in Figure 1. A survival longer than 1 year and than 3 years was achieved in 17/30 (57%) and in

5/30 (17%) patients, respectively. Moreover, the percentage of 3-year survival achieved in patients concomitantly treated by Cannabis was significantly longer than that found in patients who did not receive Cannabis infusion (4/13 (31%) vs 1/17 (6%), P < 0.05).

CLINICAL RESPONSE +						
PATIENTS	n	CR	PR	SD	DC	PD
ALL PATIENTS	30	0	3	13	16	14
CANNABIS	13	0	3	7	10 *	3
NO CANNABIS	17	0	0	6	6	11

+ CR: complete response; PR: partial response; SD:stable disease; DC (CR +PR+SD): disease control; PD: progressive disease.

* P <0.05 vs no Cannabis

Table 2. Clinical response (WHO) to radio-chemotherapy plus neuroendocrin approach plus or without Cannabis infusion in GBM patients.

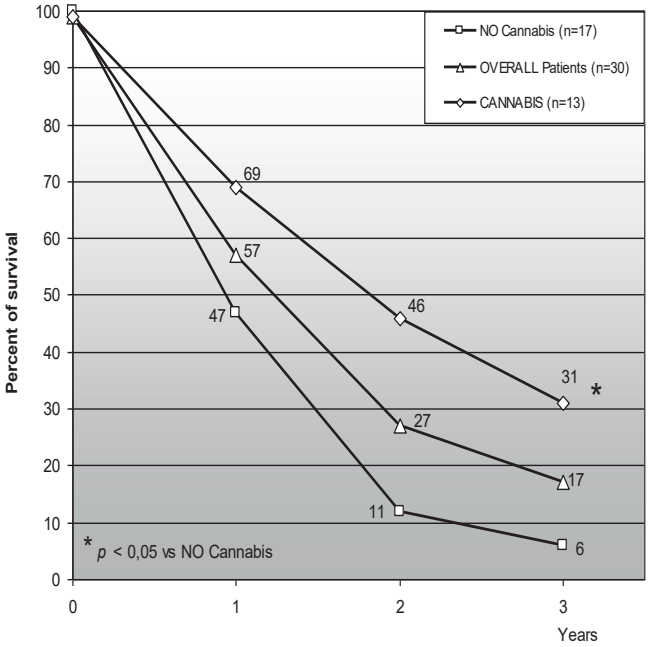

Figure 1. Survival curves in GBM patients on neuroendocrine therapy with or without Cannabis infusion.

The neuroendocrine treatment was well tolerated in all patients. No biological toxicity occurred, and the only side-effect was sleepiness or paradoxical excitation for few days in 5/30 (17%) under high-dose MLT administration. On the contrary, most patients referred an improvement in their mood and a mild relief of asthenia. Finally, no cancer progression-related cachexia occurred.

Discussion

With respect to the expected survival time and by considering that most GBM patients included in the clinical investigation were 60-year older, therefore with an expected survival time generally less than 9 months [1, 2], the results of this preliminary study would show that the survival of GBM patients may be improved by associating to the standard radio-chemotherapy schedule the administration of an oncostatic neuroendocrine regimen, consisting of antitumor pineal hormones plus opioid antagonists in association with plants with well documented anticancer antiproliferative immunomodulating properties. Obviously, randomized clinical studies will be required to confirm the therapeutic efficacy of a concomitant neuroendocrine phytotherapeutic combination in association with the standard radio-chemotherapy in the treatment of GBM. However, the survival achieved by this combination has been clearly superior to that described by previous clinical studies of GBM patients treated with MLT alone after progression under RT [18]. Moreover, this study would suggest that the further association of cannabinoids may prolong the survival time with respect to GBM patients, who did not received Cannabis infusion. This evidence is not surprising, since cannabinoid have been proven to exert direct antiproliferative and anti-angiogenic effects on several tumor histotypes, including brain GBM [6]. The evaluation of mu-opioid [4] and MLT receptor expression [10, 11] on GBM cells could identify possible subgroups of tumors with different prognostic profiles, and in more detail cancer expression of MT receptors could predict a better prognosis [11], whereas that of mu-opioid receptors would be associated with a poor prognosis, because of the stimulatory role of opioids on GBM cancer cell proliferation [4]).Therefore, the identification of MLT and opioid receptor expression on GBM cells could allow to identify possible subgroups of patients, who could obtain more benefits from a neuroendocrine approach with pineal hormones and opioid antagonists.

In conclusion, this study would simply represent only the first suggestion to further explore the therapeutic efficacy of a neuroendocrine strategy in the treatment of GBM, consisting of the administration of the same endogenous hormones provided by an anticancer activity on GBM cell growth, namely the pineal hormones, in association with opioid antagonists to inhibit the brain opioid system, which would play a stimulatory role on GBM development [4].

Further therapeutic results could be achieved by associating the neuroendocrine-approach in GBM therapy to the more recent immunotherapeutic techniques with anti-immune check-point monoclonal antibodies, mainly those against CTLA-4 and PD-1 [19-21].

Acknowledgements

The Authors acknowledge Pneipharma, a Division of Natur (Milan, Italy), for the support given to the study.

References

1. Brandles AA. State of the art treatment for high grade brain tumors. Semin Oncol. 2003, 30 (6 Suppl 19): 4-9.

2. Hentschel SJ, Sawaya R. Optimizing outcomes with maximal surgical resection of malignant gliomas. Cancer Control. 2003, 10(2): 109-114.

3. Gerson SL. MGMT: its role in cancer aetiology and cancer therapeutics. Nat Rev Cancer. 2004, 4(4): 296-307.

4. Westphal M, Li CH. Beta-endorphin: characterization of binding sites specific for the human hormone in human glioblastoma SF 126 cells. Proc Natl Acad Sci USA. 1984, 81: 2921-2923.

5. Reiter RJ. Mechanisms of cancer inhibition by melatonin. J Pineal Res. 2004, 37(3): 213-214.

6. Grotenhermen F. Pharmacology of cannabinoids. Neuroendocrinol Lett. 2004, 25(1-2): 14-23.

7. Sze SF, Ng TB, Liu WK. Antiproliferative effect of pineal indoles on cultured tumor cell lines. J Pineal Res.1993, 14(1): 27-33.

8. Brzezinski A. Melatonin in humans. N Engl J Med.1997, 336(3): 186-195.

9. Maestroni GJM. The immunoneuroendocrine role of melatonin. J Pineal Res.1993, 14(1): 1-10.

10. Danielczyk K, Dzjegiel P. MT 1 melatonin receptors and their role in the oncostatic action of melatonin. Postepy Hig Med Dosw. 2009, 63: 425-434.

11. Nemeth C, Humpeler S, Kallay E, Mesteri I, Svoboda M et al. Decreased expression of the melatonin receptor 1 in human colorectal carcinomas. J Biol Regul Homeost Agents. 2011, 25(4): 531-542.

12. Krabtee BL. Review of naltrexone, a long-acting opioid antagonist. Clin Pharm. 1984, 3(3): 237-281.

13. Lissoni P, Porro G, Messina G, Porta E, Rovelli F et al. Morphine, melatonin, marijuana, magnolia and myrrh as the "five M" schedule in the treatment of cancer pain and the possible dose-dependency of the antitumor and analgesic effects of the pineal hormone melatonin. Anticancer Res. 2014, 34(10): 6033-6034.

14. Guo J, Xiao B, Liu Q, Gong Z, Le Y. Suppression of c-myc expression associated with anti-proliferation of aloe-emodin on gastric cancer cells. Cancer Invest. 2008, 26(4): 369-374.

15. Hanus LO, Rezanka T, Dembitsky VM, Moussaief A. Myrrh-Commiphora chemistry. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2005, 149(1): 3-28.

16. Fried LE, Arbiser J. Honokiol, a multifunctional antiangiogenic and antitumor agent. Antioxid Redox Signal. 2009, 11(5):1139-1148.

17. Tucker AO. Frankincense and Myrrh. Econ Bot. 1986, 40(4): 425-433.

18. Lissoni P, Meregalli S, Nosetto L, Barni S, Tancini G et al. Increased survival time in brain glioblastoma by a radioneuroendocrine strategy with radiotherapy plus melatonin compared to radiotherapy alone. Oncology. 1996, 53(1): 43-46.

19. Pardoll DM. The blockade of immune check points in cancer immunotherapy . Nat Rev Cancer. 2012, 12(4): 252-264.

20. Weber J. Immune check points proteins:a new therapeutic paradigm for cancer pre-clinical background: CTLA-4 and PD-1 blockade. Sem Oncol. 2010, 37(5): 430-439.

21. Ascierto PA, Kalos M, Schaer DA, Callahan MK, Wolchok JD. Biomarkers for immunostimulatory monoclonal antibodies in combination strategy for melanoma and other cancer types. Clin Canc Res. 2013, 19(5):1009-1020.

Journal of Cellular Immunology and Serum Biology
ISSN: 2471-5891
OPEN ACCESS
Research article
Ommega Publishers
DOI: 10.15436/2471-5891.19.2442

The Psychoneuroimmune Pathogenesis of Cancer: Therapeutic Strategy to Normalize Cancer-Related Brain Unbalance Between Hyperfunction of Opioid System and Hypofunction of Cannabinoid-Pineal Axis by Antitumor Pineal Indoles, and the Mu-Opioid Antagonist Naltrexone in Untreatable Advanced Cancer Patients

Paolo Lissoni*, Franco Rovelli, Giusy Messina, VezikaCenaj, Giuseppe Di Bella, Giorgio Porro, Franco Fraschini, Giuseppe Di Fede

Institute of Biological Medicine, Milan, Italy

***Corresponding author:** Paolo Lissoni, Institute of Biological Medicine, Milan, Italy; E-mail: paolo.lissoni@gmx.com

Abstract

Today it is known that *in vivo* the immune reactions cannot be separated from their neuroendocrine regulation, which is mainly mediated by the brain opioid system and by the functional unit constituted by brain cannabinoid system and pineal gland. The opioid system is active in stress and depression conditions, and it mediates the suppression of the anticancer immunity. On the contrary, the pineal-cannabinoid functional system, which is involved in the perception of pleasure and mind spiritual expansion, stimulates the anticancer immunity, by playing a fundamental role in the natural resistance against cancer. Then, cancer progression would be due to an unbalance between hypoactivity of the cannabinoid-pineal system and hyperactivity of the opioid system, which could be corrected by a substitute therapy of the main antitumor pineal hormones, including Melatonin (MLT) and 5-Methoxytryptamine (5-MTT) in association with cannabinoids to normalize the cannabinoid-pineal function, and by the administration of opioid antagonists, such as Naltrexone (NTX) to counteract the opioid hyeractivity. The present study was carried out to evaluate the influence of a concomitant NTX administration in advanced cancer patients, for whom no other conventional anticancer therapy was available, and who had progressed under a complementary therapy with the only pineal hormones. The study included 14 untreatable solid tumor cancer patients. All drugs were given orally every day without interruption according the following schedule: MLT at a dose of 100 mg/day in the dark period of the day, 5-MTT at 10 mg/day in the light period of the day, and NTX at 20 mg in the evening. A control of tumor growth was achieved in 8/14 (57%) patients, and it was associated with an improvement in Lymphocyte-To-Monocyte Ratio (LMR). These preliminary results would suggest that the concomitant block of the opioid system by NTX may allow a control of tumor growth superior to that, which may be obtained with the only pineal antitumor hormones, and this effect would be mediated at least in part by an improvement in the immune status, as suggested by the rise in LMR values. Further promising antitumor results could be achieved with the association of cannabinoid agonists, or with Fatty Acid Amide Hydrolase (FAAH) inhibitors to enhance brain cannabinoid content.

Keywords: Cancer; Cannabinoid system; Melatonin; Naltrexone; Opioid system; Pinealgland

Received Date: February 25, 2019 **Accepted Date: April 22, 2019** **Published Date: April 27, 2019**

Citation: Paolo, L., et al. The Psychoneuroimmune Pathogenesis of Cancer: Therapeutic Strategy to Normalize Cancer-Related Brain Unbalance Between Hyperfunction of Opioid System and Hypofunction of Cannabinoid-Pineal Axis by Antitumor Pineal Indoles, and the Mu-Opioid Antagonist Naltrexone in Untreatable Advanced Cancer Patients. (2019) J Cell Immunol Serum Biol 4(1): 14-.

Introduction

The different hypotheses concerning the possible influence of the psychological and spiritual status on cancer onset and development[1-3], have finally found their confirmation on scientific bases after the discovery of the fundamental role of the immunity in tumorcell growth inhibition[4], and the existence of a psychoneuroendocrine regulation of the immune responses, including the anticancer immunity[5-8]. Then, the main responsible for the natural biological resistance against cancer is the immune system, whose function however, is under a neuroendocrine regulation. Despite the great complexity of the NeuroImmunoModulation (NIM), it is possible to identify two major brain interneuronal immune modulatory systems, consisting of the opioid system[5,6], and the endocannabinoid system[7,8] through its functional connections with the pineal gland[9,10]. The opiod system, namely through a mu-opioid receptor, may inhibit the antitumor immunity[5,6], whereas the pineal-cannabinoid system axis stimulates the antitumor immunity[7,8,11]. The opioid system is active in stress, depression, and anxiety conditions, whereas the pineal-cannabinoid axisis operating in the perception of pleasure and spiritual expansion of mind. This evidence may constitute the explanation of the protumoral influence of stress and depression, and on the other side the preventing antitumor effect of pleasure and spirituality on tumor growth[3]. In more detail, the mu-opioid agonists, such as beta-endorphin and morphine, have been proven to play a pro-tumoral action through several mechanisms, including a direct proliferative activity, an angiogenic action, and a suppression of the antitumor immunity by inhibiting the secretion of IL-2 and IL-12, which represent the main anticancer cytokines in humans, respectively from T Helper-1 (TH1) lymphocytes, and by stimulating that of immunosuppressive cytokines, namely TGF-beta and IL-10, from regulatory T lymphocytes (T reg)[5,6]. On the other hand, both pineal and brain cannabinoid system play a natural anticancer activity[9-11]. The pineal gland has appeared to represent the main immunomodulating organ in the human body by modulating the cytokine network[11-14] through the light/dark circadian release of the indole Melatonin (MLT)[11], other less investigated indole, suchas the 5-Methoxytryptamine (5-MTT)[15], and beta-carbolines, namely the pinealine[16], all provided by anticancer activity, even though the mechanisms of action have been clarified for the only MLT. The pineal is the main anticancer organ in humans, and it counteracts tumor growth through several mechanisms, including a direct antiproliferative cytotoxic action, an anti-angiogenic activity, and an antitumor immunostimulatory effect, namely consisting of a direct stimulation of IL-2 and IL-12 secretions[11-14], while the cannabinoid agents would play an anticancer activity namely by a direct inhibition of cancer cell proliferation, whereas their effects on the antitumor immunity are still controversial[7,8]. The pineal gland may modulate the activity of both brain opioid and cannabinoid systems, but the pineal gland would namely consitute an unique fundamental functional axis with the cannabinoid system in mediating the perception of pleasure and the spiritual expansion of consciousness through a reciprocal stimulatory influence, since CB1 cannabinoid agonists may directly stimulate MLT release from the pineal gand[9], and on the other side MLT has been proven to contribute to the inhibition of Fatty Acid Amide Hydrolase (FAAH)[10], the enzyme responsible for cannabinoid degradation[7,8], with a following increase in brain cannabinoid content. Then, the functionless of cannabinoid-pineal system constitutes a fundamental requirement for the status of health. The recent advances in Psycho Neuro Endocrino Immunology (PNEI) researches have shown than cancer progression is associated with a progressive unbalance between brain opioid and endocannabinoid systems, consisting of the association between hyperfunction of the opioid system and hypofunction of the cannabinoid system[7,8,17]. The cannabinoid hypofunction would be at least in part a consequence of the progressive decline in the pineal function with cancer progression, which constitutes the main cancer-related endocrine deficiency[11,18] because of the interactions occurring between pineal and cannabinoid system[9,10]. This unbalance would already explain cancer progression, because of the protumoral role of the opioid system and the anticancer one of the cannabinoid system. The existence of a cancer-related brain opioid system hyperactivity is documented by the evidence that the concomitant administration of the mu-opioid antagonist Naltrexone (NTX) may abolish the promoting effect of stress on cancer development[17]. On the other side, the occurrence of cancer-related brain cannabinoid system hypofunction would be suggested by the evidence of a progressive decline in pleasure perception, the so-called anaedonia, with tumor progression, because of the fundamental role of the cannabinoid system in the perception of pleasure, including appetite and sexual interest[7,8]. The endogenous cannabinoid system may be clinically investigated by measuring the blood or liquoralconcentrations of the two main endogenous cannabinoids, the Arachidonyl-Ethanol-Amide (AEA), the so-called anandamide because of its psychedelic effects, and the 2-Arachidonyl-Glycerol (2-AG), or in a more synthetic manner by the simple detection of the blood levels of FAAH, the main enzyme involved in cannabinoid metabolism and degradation[7,8,19], since it has been shown that high blood levels of FAAH are associated with abnormally low concentrations of both AEA and 2-AG, by reflecting a condition of cannabinoid hypofunction, whereas low FAAH levels allow increased cannabinoid concentrations[20], as an expression of cannabinoid hyperactivity. Moreover, it has been shown that the evidence of an enhanced FAAH synthesis or activity, which allows an endogenous cannabinoid deficiency, may induce a chronic inflammatory status[21], because of the anti-inflammatory activity of the cannabinoid system, mainly due to an inhibition of IL-17 secretion from TH17 lymphocytes[7,8]. Finally, it has been shown that the inflammatory response induced by the increased levels of FAAH may exert a negative prognostic significance in cancer, cardiovascular diseases, and neurodegenerative pathologies[21]. On the contrary, the inhibition of FAAH synthesis, with a following increase in the endogenous content of cannabinoids, has been proven to exert a therapeutic action in several human diseases by counteracting the inflammatory response[19-21]. Therefore, in addition to its importance in the perception of pleasure and consciousness status,the endocannabinoid system would play a fundamental role in maintaining the status of health, including the cardiovascular function and the immuno-inflammatory response. At present, one of the most simple FAAH inhibitors is represented by the same Cannabidiol (CBD), the non-psychotropic agent of Cannabis[7,8,22]. On the contrary, no study has been performed up to now in an attemp to evaluate the influence of the pineal gland and its main hormone

MLT on FAAH synthesis and activity. On the same way, no study has been carried to investigate the interactions occurring between FAAH activity and heart endocrine function, namely consisting of the secretion of atrial natriuretic peptide [ANP] and endothelin-1 [ET-1]. The anti-inflammatory action of ANP[23] and the pro-inflammatory one played by ET-1[24] could be due at least in part to a possible inhibitory effect of ANP and a possible stimulatory action of ET-1, respectively, on FAAH activity. Cancer-related opioid system hyperfunction may be simply blocked by the administration of the mu-opioid antagonist NTX, while the cannabinoid-pineal hypofunction may be corrected by the exogenous administration of pineal indoles and cannabinoid agonists. Therefore, the use of cannabinoids in cancer therapy could deserve not only palliative benefits, but it could also influence cancer progression itself because of the antitumor role of the cannabinoids agents[7,8]. FAAH inhibitors could be also successfully used to correct cancer-related cannabinoid system deficiency. On the contrary, there are controversial results concerning the use of the opioid antagonists, such as NTX, in Oncology, since either low-dose[25] or high-dose NTX[17,26], have been proposed, respectively in an attempt to modulate opioid receptor sensitivity, or to completely block the functionless of the opioid system and its immunosuppressive and protumoral activity. On the basis of the evidence of cancer-related opioid hyperactivity in association with cannabinoid failure, a study was performed to evaluate the impact of a correction of cannabinoid-pineal axis deficiency by pineal indoles and cannabinoid agents in association with a control of opioid system hyperfunction by the mu-opioid antagonist NTX on tumor growth and survival in advanced cancer patients, for whom no other standard anticancer therapies were available.

Patients and Methods

The phase II study included 14 consecutive untreatable advanced solid tumor patients (M/F: 8/6; median age 62 years, range 54-81), for whom no other effective standard anticancer therapy was available, and who had already been under complementary medicine with the two main pineal antitumor hormones, consisting of Melatonin (MLT) and 5-Methoxytryptamine (5-MTT) [14,15], according to previous clinical experimental studies[27], both orally with MLT at a dose of 100 mg/day in the dark period of the day and 5-MTT at a dose of 10 mg during the light period of the day. Eligibility criteria were, as follows: histologically proven solid tumor, measurable lesions, no double tumor, no chronic therapy with opioids to avoid the possibile NTX-induced withdrawal syndrome, no availability olf other standard anticancer treatments, and progression under a previous therapy with the only pineal hormones. Tumor histotypes were, as follow: Glioblastoma (GBM): 7; malignant astrocytoma: 3; colon cancer: 1; gastric cancer: 1; pancreatic adenocarcinoma: 1; lung adenocarcinoma: 1. The clinical response was assessed by the more appropriate radiological examinations, and according to the WHO criteria. Under the previous therapy with the only pineal indoles, a disease control (DC) was obtained in 9/14 (64%) patients, consisting of partial response (PR) in 1 and stable disease (SD) in 8, whereas the remaining 5 patients had a Progressive Disease (PD). After disease progression, patients received the same doses of pineal indoles with the association of the mu-opioidantagonist NTX at an oral dose of 20 mg/day in the evening, by evaluating the clinical response after 3 months of therapy. The experimental study was explained to each patient, and written consent was obtained. Moreover, on the basis of the well demonstrated negative prognostic significance of low values of lymphocyte-to-monocyte ratio (LMR)[29] because of the antitumor immunostimulatory and immunosuppressive role of lymphocytes and monocytes, respectively[30], LMR wase valuated at weekly intervals. Normal values of LMR obtained in our laboratory (95 % confidence limits) was greaterthan 2.1. Moreover, becuse of the possible hepatotoxicity of NTX, transaminase levels were also particularly monitored. Data were statistically analyzed by the chi-square t, and the Student's t test, as appropriate.

Results

No complete response was observed in patients under therapy with pineal indoles plus NTX. A partial response (PR) was achieved in one patient with GBM. Seven other patients had a SD. Then, a DC (PR + SD) was achieved in 8/14 [57%], whereas the other 6 patients had a PD. The percentage of DC was higher in patients, who had already obtained a DC under the previous therapy with the only pineal indoles than in those who had a PD, event hough the difference was not statistically significant because of the low number of cases[6/9 (67%) VS 2/5 (40%)]. As far as the immune response is concerned, abnormally low pretreatment values of LMR were seen in 6/14 (43%) patients. The percentage of DC achieved in patients with low LMR values prior to therapy was lower than that achieved in patients with normal pretreatment LMR values, without, however, significant differences [5/8 (63%) vs 3/6 (50%)]. Moreover, as illustrated in Figure 1, LMR mean values increased in patients who achieved a DC, and decreased in those with PD with respect to the pretreatment values, even though the differences were not statistically significant. However, LMR mean values observed after 3 months of therapy in patients with DC were statistically significantly higher than those found in patients with PD ($P < 0.05$). No toxicity occurred on treatment, and in particular no important transaminase increase was observed under NTX administration.

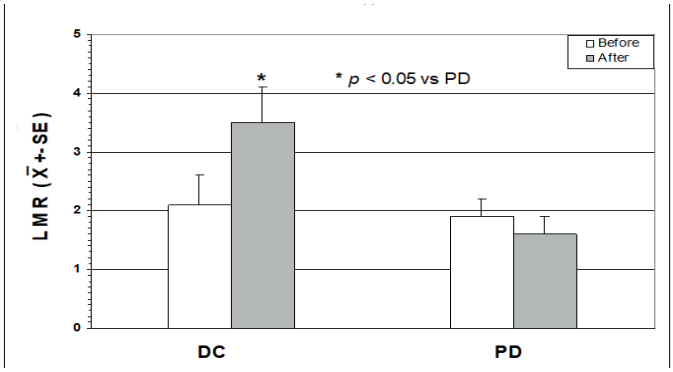

Figure 1: Lymphocyte-to-monocyte (LMR) in patients with disease control (DC) or progressive disease (PD) before and after 3 months of therapy

Discussion

According to a possible psychoneuroendocrine of cancer, which

considers cancer-related immunosuppression of a consequence of an unbalancebe tweenopioid and cannabinoid-pineal systems, which represent the two main brain neuroimmunomodulatory systems, this preliminary phase II study seems to suggest that the association of a block of the opioid system through the administration of a mu-opioidantagonist, suchas NTX, may allow a further control of tumor growth in advanced cancer patients, for whom no other effective standard anticancer therapy was available, and who had already received some benefits from the previous therapy with the only most investigated pineal antitumor hormones, including MLT and 5-MTT, which may reactivate the functionless of cannabinoid-pinealaxis[7-10]. This finding is not surprising, since cancer-related neuroimmune alterations do not consist of the only endogenous cannabinoid system deficiency, but also on a concomitant hyperacitivy of the opioid system[17], which may be counteracted by the administration of the opioid antagonist NTX. This statement is particularly justified in the case of brain tumors, since their expression of opioid receptors has been proven to predict a greater biological malignancy and a worse prognosis[28]. Finally, the improvement in the efficiency of the antitumor immunity, as shown by the increase in LMR, observed in patients, who achieved a DC under NTX therapy, would suggest that NTX may stimulate the anticancer immunity by counteracting opioid system-mediated immunosuppression occurring in cancer. Obvioulsy, further studies will be required to better define the immunomodulating effects of NTX, particularly on regulatory T lymphocytes (T reg), which are the main suppressive regulator of the anticancer immunity, since in experimental conditions it has been shown that NTX may counteract T reg cell generation and activation[29]. However, since LMR represents a synthetic parameter reflecting the relation between antitumor immunostimulatory and protumoral immunosuppresive events, respectively exerted by lymphocyte and macrophage systems[30], LMR increase in patients, who obtained a control of tumor growth on NTX administration, would suggest that NTX-induced block of brain opioid system may contribute to cancer control by also improving the antitumor immunity. Further therapeutic results in terms of control of the clinical course of the neoplastic disease in cancer patients, for whom no other conventional treatment is available, could be achieved by the association with another antitumor pineal hormone of beta-carboline nature, the pinealine[16], as well as by the direct administration of cannabinoid agents, or cannabidiol to inhibit FAAH activity[7,8,22], with a following increase in brain cannabinoid content and function.

References

1. Riley, V. Psychoneuroendocrine influences on immunocompetence and neoplasia. (1981) Science 212(4499): 1100-1109.
Pubmed | Crossref | Others
2. Antoni, M.H. Psychoneuroendocrinology and psychoneuroimmunology of cancer: plausible mechanisms worth pursuing? (2003) Brain Behav Immunol 1(1): 84-91.
Pubmed | Crossref | Others
3. Lissoni, P., Messina, G., Lissoni, A., et al. The psychoneuroendocrine-immunotherapy of cancer: historical evolution and clinical results. (2017) J Res Med Sci 22(1): 45-52.
Pubmed | Crossref | Others
4. Riesco, A. Five-year cancer cure: relation to total amount of peripheral lymphocytes and neutrophils. (1979) Cancer 25(1): 135-140.
Pubmed | Crossref | Others
5. Manfredi, B., Sacerdote, P., Bianchi, M. Evidence for an opioid inhibitory tone on T cell proliferation. (1993) J Neuroimmunol 44(1): 43-46.
Pubmed | Crossref | Others
6. Sacerdote, P., Panerai, A.E. Role of opioids in the modulation of TH1/TH2 responses. (1999) Neuro immunomodulation 6: 422-423.
Pubmed | Crossref | Others
7. Grotehnermen, F. Pharmacology of cannabinoids. (2004) Neuro Endocrinol Lett 25(1-12): 14-23.
Pubmed | Crossref | Others
8. Nagarkatti, P., Pandey, R., Rieder, S.A., et al. Cannabinoids as novel anti-inflammatory drugs. (2009) Future Med Chem 1(7): 1333-1349.
Pubmed | Crossref | Others
9. Lissoni, P., Resentini, M., Mauri, R., et al. Effects of tetrahydrocannabinol on melatonin secretion in man. (1986) Horm Metabol Res 18(1): 77-88.
Pubmed | Crossref | Others
10. Spadoni, G., Bedini, A., Furiassi, L., et al. Identification of bivalent ligands with melatonin receptor agonist and Fatty Acid Amide Hydrolase (FAAH) inhibitory activity that exhibit ocular hypotensive effects in the rabbit. (2018) J Med Chem 61(17): 7902-7916.
Pubmed | Crossref | Others
11. Brzezinski, A. Melatonin in humans. (1997) N Engl J Med 336(3): 186-195.
Pubmed | Crossref | Others
12. Maestroni, G.J. The immunon euroendocrine role of melatonin. (1993) J Pineal Res 14(1): 1-10.
Pubmed | Crossref | Others
13. Conti, A., Maestroni, G.J.M. The clinical immunotherapeutic role of melatonin. (1995) J Pineal Res 19(3): 103-110.
Pubmed | Crossref | Others
14. Lissoni, P. The pineal gland as central regulator of cytokine network. (1999) Neuro Endocrinol Lett 20(6): 343-349.
Pubmed | Crossref | Others
15. Sze, S.F., Ng, B., Liu, W.K. Antiproliferative effect of pineal indoles on cultured tumor cell lines. (1993) J Pineal Res 14(1): 27-33.
Pubmed | Crossref | Others
16. Airaksinen, M.M., Kari, I. Beta-carbolines, psychoactive compounds in the mammalian body. (1981) Med Biol 59(1): 21-34.
Pubmed | Crossref | Others
17. Lewis, J.W., Shavit, Y., Terman, G.V. Apparent involvement of opioid peptides in stress-induced enhancement of tumor growth. (1983) Peptides 4(5): 635-638.
Pubmed | Crossref | Others
18. Bartsch, C., Bartsch, H. Melatonin in cancer patients and in tumor-bearing animals. (1999) Adv Exp Med Biol 467: 247-264.
Pubmed | Crossref | Others
19. Ogawa, S., Kunugi, H. Inhibitors of fatty acid amide hy-

drolase and mono-acyl-glycerol-lipase: new targets for future antidepressant therapy. (2015) Curr Neuro Pharmacol 13(6): 760-775.
Pubmed | Crossref | Others

20. Ahn, K., Johnson, D.C., Cravatt, B.J. Fatty acid amide hydrolase as a potential therapeutic target for the treatment of pain and CNC disorders. (2009) Exp Opin Drugs Disov 4(7): 763-784.
Pubmed | Crossref | Others
21. Godlewski, G., Alapafuja, S.O, Baktai S, et al. Inhibitor of fatty acid amide hydrolase normalizes cardiovascular function in hypertension without adverse metabolic effects. (2010) Cell Chem Biol 17(11): 1256-1266.
Pubmed | Crossref | Others
22. Scharf, E.L. Translating endocannabinoid biology into clinical practice: cannabidiol for stroke prevention. (2017) Cannabis Cannabinoid Res 2(1): 259-264.
Pubmed | Crossref | Others
23. De Vito, P. Atrialnatriuretic peptide: a hold hormone, or a new cytokine? (2014) Peptides 58: 108-116.
Pubmed | Crossref | Others
24. Grant, K., Loizidou, M., Taylor, I. Endothelin-1: a multifunctional molecule in cancer. (2003) Br J Cancer 88(2): 163-166.
Pubmed | Crossref | Others
25. Brown, N., Panksepp, J. Low-dose naltrexone for disease prevention and quality of life. (2009) Med Hypoth 72(3): 333-337.
Pubmed | Crossref | Others
26. Lissoni, P., Messina, G., Porro, G., et al. A psycho-neuroendocrino-immune (PNEI) approach to enhance the efficacy of radiochemotherapy in glioblastoma. (2016) J Radiat Oncol 3: 1-4.
Pubmed | Crossref | Others
27. Lissoni, P., Rovelli, F., Brivio, F., et al. Five-year survival with high-dose melatonin and other antitumor pineal hormones in advanced cancer patients eligible for the only palliative therapy. (2018) Res J Oncol2: 1-7.
Pubmed | Crossref | Others
28. Westphal, M., Li, C.H. Beta-endorphin: characterization of binding sites specific for the human hormone in human glioblastoma SF 126 cells. (1984) Proc Natl Acad Sci USA 81(9): 2921-2923.
Pubmed | Crossref | Others
29. Lissoni, P., Messina, G., Tantarelli, R., et al. The psychoneuroimmunotherapy of humans immune-mediated systemic diseases, including cancer and autoimmune diseases. (2017) J Mol Oncol Res 1(1): 7-13.
Pubmed | Crossref | Others
30. Nishijma, T.F., Muss, H.B., Shachar, S.S., et al. Prognostic-value of lymphocyte-to-monocyte ratio in patients with solid tumors: a systematic review and meta-analysis. (2015) Cancer Treat Rev 41(10): 971-978.
Pubmed | Crossref | Others

Research Open

Cancer Studies and Therapeutics
Volume 2 Issue 2

Research Article

A Study on the Influence of Spirituality on the Efficacy of Antitumor Therapies with Natural Anticancer Agents in Untreatable Metastatic Cancer Patients

Messina G[1], Rovelli F[1], Brivio F[1], Lissoni P[1], Fumagalli L[2]*, Compare A[3]

[1]International Institute of PsychoNeuroEndocrinoImmunology, Milan, Italy
[2]*Surgery Division, Manzoni Hospital, Lecco, Italy
[3]Department of Psychology, University of Studies, Bergamo, Italy

Correspondence to: Fumagalli L, International Institute of PsychoNeuroEndocrinoImmunology, Milan, Italy.

Received: April 28, 2017; **Accepted:** May 17, 2017; **Published:** June 06, 2017;

Abstract

The recent discoveries of the existence of natural anticancer agents either from plants, such as Aloe, Myrrh and Magnolia, or from the human body, namely the pineal hormones, allowed the possibility to elaborate new therapeutic natural combinations as a link between the commonly used palliative and curative cancer therapies, which would have not considered in a separate manner. The present study was carried out to evaluate the influence of the spiritual status on the efficacy of a natural anticancer combination containing pineal anticancer hormones in association with Aloe, Myrrh and Magnolia extracts in a group of 70 untreatable metastatic solid tumor patients with life expectancy less than 1 year. The spiritual sensitivity was evaluated by an appropriate faith test for patients affected by an untreatable disease. The percentages of both objective tumor regressions and disease control obtained in patients with high faith score were significantly higher with respect to those found in patients with low faith score. On the same way, the 3- year percent of survival achieved in patients with high faith score was significantly longer than that found in the other group. This study would suggest the efficacy of an antitumor therapeutic strategies with natural anticancer agents also in metastatic cancer patients form whom no other standard antitumor treatment was available, with a greater efficacy in the presence of a real status of spiritual faith.

Keywords: *spirituality, cancer disease, psychoneuroimmunology*

Introduction

Being cancer a biological war between a human host and an apparently unconscious tumor mass, it is obvious that the prognosis of the neoplastic diseases may depend on both tumor characteristics and the psychobiological identity of cancer patients. Tumor characteristics regard histology, disease extension, biological grading and eventual genetic mutations of cancer cells. At the other side, the individual identity of the single cancer patients involves their consciousness status, psychological behaviour, life style, but also and mainly their endocrine, neuroendocrine and immune status in addition to their clinical conditions [1]. Until some years ago, the human diseases were considered to be due to organicistic or psychosomatic reasons. On the contrary, with the progressive advances in the area of Psychoneuroendocrinoimmunology (PNEI), it was understood that the psychospiritual status of patients may influence the biological body not only through the nervous system, but also through complex nervous, neuroendocrine and endocrine interactions with the immune cells, which after their activation may interact with the endocrine and nervous systems by releasing immunomodulating proteins, the so-called cytokines, which are also able to exert neuroendocrine effects by realising complex feed-back circuits between neuroendocrine and immune systems [2].

As far as the psychological and spiritual point of view is concerned, must be remarked that until few years ago and yet up to now by most researchers, the spirituality has been simply considered only as a part of the psychological status of humans, and only recently some preliminary clinical investigations have suggested that the spirituality is a different condition from both psychology and religion [3]. As far as the relation between psychology and spirituality is concerned, it is possible to affirm that the Psychology represents the analysis of the emotional life, which has its energetic matrix in the sexuality, whereas the Spirituality regards the reality of the different consciousness states. At the other side, the relation between Religion and Spirituality, according to a definition previously reported in the literature [4], the Spirituality is the research of the ultimate meaning of life, while Religion is only a set of beliefs and ritual practices within a well defined religious institution, then it would simply represents one of the possible ways to realize own self spirituality, even though more widely followed with respect to an individual manner to live and feel the spiritual dimension. Then, the individual spirituality may be realized through the same religion or other mysticai experiences, and it Is not a simpie set of emotions, but it constitutes a status of consciousness. Moreover, in agreement with PNEI discoveries [5], both emotions and consciousness states require a well defined psychoneuroendocrine mediation. Then, from a clinical

point of view, the two major problems concern the identification of adequate methods to clinically investigate not only the religious profile of patients, but also their spiritual sensitivity, as well as of possible eventual blood biochemical parameters able to reflect the psychological and spiritual status of patients and its influence on the clinical course of the neoplastic disease. However, most studies carried out up to now have been generally limited to the investigation of the influence of the personal religion rather than the real status of cancer patients. In any case, even though limited to the investigation of the influence of religion on the prognosis of cancer, preliminary clinical results seem to suggest that the religious support may allow an increase in the survival time of advanced cancer patients and to improve their clinical status, even though through still unknown mechanisms [3, 4]. The recent advances in PNEI knowledgements, by demonstrating that the immune responses in vivo are physiologically under a psychoneuroendocrine modulatory control [6,7], which represents the biochemical mediation of the spiritual and psychological status of the patients, may allow the hypothesis that the spiritual status may influence the clinical course of the neoplastic disease and the efficacy of the different antitumor therapies by stimulating the immune system and piloting it in an antitumor way through the activation of well-defined psychoneuroendocrine circuits [8]. Moreover, it has to be considered that until about 20 year ago, almost all scientific investigations in the oncological area were limited to the identification of possible carcinogens in the nature, either endogenous molecules, such as estrogens and androgens, or exogenous substances, capable of inducing the malignant transformation. On the contrary, more recent researches have demonstrated the existence of several antitumor plants containing well characterized anticancer molecules, in particular aloe hemodin from Aloe [9], guggulsterone from Myrrh [10] and honokiol from Magnolia [11], as well as more surprisingly the evidence of anticancer endogenous molecules, which would be responsible for the natural immunobiological resistance against cancer onset and growth, in particular some indole hormones released by the pineal gland, namely melatonin (MLT) [12] and 5-methoxytryptamine (5-MTT) [13], and the great group of beta-carbolines [14], which are mainly produced by pineal gland itself. All those natural anticancer agents has no important toxicity. Therefore, the existence of both endogenous and exogenous anticancer agents with a complete lack of biological toxicity but with well known antitumor properties, would justify their empioyment in the medical Oncology in an attempt to realize a link between the simple palliative and the curative therapies of cancer, since several anticancer natural agents, according to the PNEI knowledgements, may deserve both palliative and antitumor effects on cancer progression at least in terms of survival time. The present study was performed to investigate the influence of the spiritual status of consciousness on the antitumor efficacy of a psychoneuroendocrine regimen with antitumor pineal hormones in association with the most investigated anticancer plants in a group of metastatic solid tumor patients, for whom there is no other standard effective therapy of their tumor, by evaluating the spiritual status through a previously described clinical test to explore the spiritual faith in patients affected by an untreatable disease [15].

Materials and Methods

The study included 70 untreatable metastatic solid tumor patients. Eligibility criteria were, as follows: histologically proven metastatic solid neoplasm, measurable lesions, no availability of standard antitumor therapies because of progression on previous chemotherapies, age or low performance status (PS), and life expectancy less than 1 year. Patients affected by metastatic breast cancer or prostate carcinoma were excluded from the study, because of the availability for those tumors of well tolerated hormonal therapies also by the standard medical Oncology. The faith test for patients affected by an untreatable disease employed in the study was performed by the observation of the clinicians in an attempt to exclude possible unconscious mental manipulations in their answers by the patients, and it consisted of the analysis of five major criteria [15], by assigning 20 points to each single criterion, with a maximum score of 100 points and by defining the presence of a real status of spiritual faith for a minimal score of at least 60 points or more. The five criteria were, as follows: 1) complete self-consciousness by the patients of the severity of their diagnosis and prognosis in terns of life expectancy: the absence of an adequate knowledge of the severe prognosis would transform the faith in a simple illusion; 2) lack of excessive anxiety: the anxiety would represent the opposite mental condition with respect to a real spiritual faith; 3) lack of an exaggerated attribution of value by the patients to the professional capacities of the single clinicians, being their disease as considered as untreatable on the basis of the standard medical therapies;4) lack of an excessive analytic tendency by the patients to understand the chemical mechanisms involved in the efficacy of treatments instead of their significance in terms of reactivation of an effective biological natural anticancer resistance; 5) perception of own neoplastic disease not only as a personal problem, despite pain and other intolerable symptoms, but also as an individual manifestation of a general universal suffering involving all humans. The clinical characteristics of patients are reported in Table 1. Lung cancer, pancreatic adenocarcinoma and colorectal cancer were the neoplasms most frequent in our patients. The PNEI strategy of cancer cure consisted of the oral administration of the two most investigated anticancer pineal hormones, MLT and 5-MTT, in association with a phyto-therapeutic regimen consisting of the administration of extracts of the most investigated antitumor plants, including Aloe arborescens, Myrrh and Magnolia. MLT was given at 100 mg/day during the dark period of the day, while 5-MTT was administered at 5 mg in the early afternoon. Magnolia cortex, with a honokiol content of at least 50%, was given at 500 mg twice/day. Finally, Aloe and Myrrh were given at a dose of 10 ml twice/day of a mixture of 60% Aloe and 40% Myrrh. Patients with brain metastases also received Boswellia at 1000 mg/day in the morning, because of its anti-oedema effect. The clinical response was assessed by the WHO criteria by repeating the radiological examinations at 3-month intervals. Data were statisticaliy analyzed by the chi-square test. The survival curves were calculated by the Kaplan-Meyer method and statisticaliy analyzed by the log-rank test.

Table 1. Clinical characteristics of 70 untreatable metastatic solid tumor patients.

CHARACTERISTICS	
M/F:	37 / 33
Median age	65 years (range 43 — 92)
Median PS (ECOG)	1 (0—3)
RELIGIOUS FAITH	
- Specific religion:	29/ 70 (41%)
- Catholic Christian religion:	23
- Protestant Christian religion:	2
- Oriental Christian religion:	1
- Buddhism:	2
- Islam:	1
- No religion or undefined religion:	41/70 (59%)
TUMOR HISTOTYPE	
- Lung cancer:	18
- Nonsmall celi:	15
- Smail celi:	3
- Pancreatic adenocarcinoma:	14
- Colorectal cancer:	13
- Gastric adenocarcinoma:	5
- Biliary tract cancer:	4
- Hepatocarcinoma:	3
- Bladder carcinoma:	3
- Gynecoiogic tumors:	4
- Ovarian cancer:	3
- Endometrial adenocarcinoma:	1
- Melanoma:	2
- Soft tissue sarcoma:	4
METASTASIS SITES	
- Soft tissues:	18
- Bone:	2
- Lung:	16
- Liver:	18
- Liver + lung:	6
- Peritoneum:	4
- Brain:	6
PREVIOUS CHEMOTHERAPY:	52/70(74%)

Results

The clinical response achieved in our patients is reported in Table 2. A complete response (CR) was obtained in 2/70 (3%) patients, who were affected the former by gastric cancer and the latter by lung adenocarcinoma. A partial response (PR) was achieved in other 9 patients (colon cancer: 2; melanoma: 2; lung cancer:1; pancreatic cancer:1; endometrial adenocarcinoma:1; biadder cancer:1; biliary tract carcinoma: 1). Then, an objective tumor regression was observed in 11/70 (16%) patients. A stable disease (SD) was found in other 41 patients. Therefore, a disease control (CR + PR + SD) was obtained in 52/70 (74%) patients, whereas the remaining 18 patients (26%) had a progressive disease (PD). A faith score of at least 60 points was found in 51/70 (73%) patients. By considering faith score in relation to the other individuai variables, no significant differences between males and females was observed in the percent of values of at least 60 points (28/37 (76%) vs 22/33 (67%). On the same way, no difference in the percent of high faith score occurred in relation to the three most frequent neoplasms (lung: 12/18 (67%); colon: 9/13 (69%); pancreas: 9/14 (64%)). Moreover, more surprisingly there was no significant difference in the percent of faith score of at least 60 between patients who followed a specific religion and those who had no religion or no defined religion (22/29 (76%) vs 29/41 (71%). Finally, by considering the clinical response in relation to the faith score, the percent of objective tumor regressions (CR+PR) achieved in patients with faith score of 60 or more was significantly higher with respect to that found in patients with values iess than 60(11/51(19%) vs 1/19 (5%), $P<0.05$). On the same way, the percent of DC (CR+ PR+SD) achieved in patients with high faith score was significantly higher than that observed in those with low faith score (44/51(86%) vs 8/19 (42%), $P< 0.01$). Table 3 shows the clinical response in relation to the differeni values of faith score. A progressive increase in the percent of DC occurred concomitantly with the increase in faith score values. Finally, the 3-year survival curves observed in our patients are illustrated in Figure 1. The percentage of 3-year survival reached by patients with faith score of at least 60 was significantiy higher than that found in patients with low faith score ($P<0.05$).

Table 2. Clinical response (WHO criteria) in 70 untreatable cancer patients in relation to their faith score.

		CLINICAL RESPONSE +					
Patients	**n**	**CR**	**PR**	**CR+PR**	**SD**	**DC**	**PD**
Overall patients	70	2 (3%)	9	11 (16%)	41	52 (74%)	18 (26%)
Faith score > 60	51	2	8	10 (19%)*	34	44(86%)**	7 (14%)
Faith score < 60	19	0	1	1(5%)	7	8(42%)	11(58%)

+ CR: complete response; PR: partial response; SD: stable disease; DC (CR + PR + SD): disease control; PD: progressive disease

* $P< 0.05$ vs low faith score; ** $P< 0.01$ vs low faith score

Table 3. Clinical response (WHO criteria) in 70 untreatable cancer patients in relation to the different values of faith score.

		CLINICAL RESPONSE					
FAITH SCORE (points)	**n**	**CR**	**PR**	**CR + PR**	**SD**	**DC**	**PD**
20	5	0	0	0	1	1 (20%)	4 (80%)
40	14	0	2	2(14%)	6	8 (57%)	6 (43%)
60	33	0	3	3(9%)	18	21(64%)	12 (36%)
80	15	1	3	4(27%)	9	13 (87%)	2 (13%)
100	3	1	0	1(33%)	2	3 (100%)	0

Discussion

This study, ca rried out in a considerable number of untreatable metastatic cancer patients, would suggest that a neuroendocrine approach with endogenous anticancer molecules, such as the antitumor pineal hormones, and natural antitumor plants, may counteract cancer growth also in patients, who had been considered as untreatable according to the standard anticancer treatments. Moreover, the study shows that the efficacy of therapy is higher in cancer patients with a true spiritual faith, al least in the untreatable ones, even though it cannot be excluded that the reduced therapeutic efficacy observed in patients with low faith score may be simply due to an interruption or a discontinuation of therapy. In any case, even though we are only at the beginning of the possibility to understand the psychochemical mechanisms responsible for mediating the influence of the spiritual faith on the clinical course of the neoplastic diseases, the recent advances in PNEI knowledgements have demonstrated the possibility to modulate the immune system, including the anticancer immunity, by acting on its psychoneuroendocrine regulation [2, 16]. Then, in agreement with the PNEI discoveries, showing a stimulatory effect of both pleasure and spiritual sensitivity and an inhibitory one of stress and depression on the anticancer immunity, it is probable that the increased efficacy of cancer therapies with natural antitumor agents and the prolonged survival time achieved in patients with evidence of spiritual faith may mainly be due to an improvement in the potency of the immune reaction against cancer dissemination [17-19]. Moreover, the study would show that the presence of a real spiritual faith is relatively independent from the adhesion to a specific well defined religion, then it would represent an individual variable rather than to depend on external behaviours, such as the religious practices, by confirming the observations of previous authors, who had considered religion and spirituality as different human conditions [3, 4]. In more detail, since the anticancer action of the pineal hormones and of most antitumor piants is due to both antiproliferative and immunomodulating effects [20], at present, according to the PNEI discoveries, it is possible at present to identify two major functional psychoneuroendocrine systems involved in the mediation of the influence of emotions and spirituality on the anticancer immunity, consisting of the former brain opioid system-pituitary adrenal gland, which is related to stress, pain, anxiety and depression and which plays an inhibitory effect on the anticancer immunity by stimulating T regulatory (T reg) and inhibiting T helper-1 (TH1) lymphocyte functions [21], and the latter brain cannabinergic-mirror neuron-pineal gland functional axis, which on the contrary is related to both pleasure perce1ption and spiritual sensitivity, and which enhances the anticancer immunity by stimulating TH1 and inhibiting T reg activities [22-24]. In any case, both systems would be essential for the survival of the biological species, since the opioid system-pituitary-adrenal gland functional axis would play a fundamental role in the adoptive mechanisms to the environmental and social conditions, while at the other side the cannabinergic system-mirror neuron systems-pineal gland axis would be in relation to the both biological and mind evolution , as suggested by the appearance of cannabinoid receptors in a subsequent time with respect to that of the opioid ones [22], as well as by the evidence of the fundamental role of mirror neurons in the processes of imitation, learning, language, memory and self-consciousness [23] and of the involvement of pineal molecules, such as the beta-carbolines, in mind expansion [25]. If successive studies will confirm the possibility to prolong the survival time and improve the clinical status of metastatic cancer patients, for whom no other standard therapy may be available, by the administration of natural endogenous and exogenous anticancer molecules, the application of the faith score could allow to predict the probability of efficacy of natural treatments themselves, as well as for the commonly used anticancer therapies in relation to the different tumor histotypes and disease extensions.

References

1. Rubinow DR (1987) Brain, behaviour and immunity: an interactive system. *J Natl Cancer Inst Monogr* 10: 79–82.
2. Besedovsky H, Sorkin E, Keller M, Müller J (1975) Changes in blood hormone levels during the immune response. *Proc Soc Exp Biol Med* 150: 466–470. [crossref]
3. Stefanek M, McDonald PG, Hess SA (2005) Religion, spirituality and cancer: current status and methodological challenges. *Psychooncology* 14: 450–463. [crossref]
4. Balducci L, Meyer R (2001) Spirituality and medicine: a proposal. *Cancer Control* 8: 368–376. [crossref]
5. Antoni MH1 (2003) Psychoneuroendocrinology and psychoneuroimmunology of cancer: Plausible mechanisms worth pursuing? *Brain Behav Immun* 17 Suppl 1: S84–91. [crossref]
6. Del Rey A, Besedovsky H, Sorkin E, Dinarello CA (1987) Interleukin-1 and glucocorticoid hormones integrate an immunoregulatory feedback circuit. *Ann N Y Acad Sci* 496: 85–90. [crossref]
7. Manfredi B, Sacerdote P, Bianchi M, Locatelli L, Veljic-Radulovic J, et al. (1993) Evidence for an opioid inhibitory effect on T cell proliferation. *J Neuroimmunol* 44: 43–48. [crossref]
8. Lissoni P (1999) The pineal gland as a central regulator of cytokine network. *Neuro Endocrinol Lett* 20: 343–349. [crossref]
9. Lissoni P, Rovelli F, Brivio F, Zago R, Colciago M, et al. (2009) A randomized study of chemotherapy versus biochemotherapy with chemotherapy plus Aloe arborescens in patients with metastatic cancer. *In Vivo* 23: 171–175. [crossref]
10. Hanus LO, Rezanka T, Dembitsky VM, Moussaieff A (2005) Myrrh--Commiphora chemistry. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 149: 3–27. [crossref]
11. Fried LE, Arbiser L. Honokiol (2009) A multifunctional antiangiogenic and antitumor agent. *Antioxid Redox Signal* 11: 1139–1148.
12. Maestroni GJ (1993) The immunoneuroendocrine role of melatonin. *J Pineal Res* 14: 1–10. [crossref]
13. Sze SF, Ng TB, Liu WK (1993) Antiproliferative effect of pineal indoles on cultured tumor cell lines. *J Pineal Res* 14: 27–33. [crossref]
14. Song Y, Wang J, Teng SF, Kesuma D, Deng Y, et al. (2002) Beta-carbolines as specific inhibitors of cyclin-dependent kinases. *Bioorg Med Chem Lett* 12: 1129–1132. [crossref]
15. Lissoni P, Messina G, Balestra A, Colciago M, Brivio F, Fumagalli L, et al. (2008) Efficacy of cancer chemiotherapy in relation to synchronization of cortisol rhythm, immune status and psychospiritual profile in metastatic non-small cel lung cancer. *In Vivo* 22: 257–262.
16. Riley V (1981) Psychoneuroendocrine influences on immunocompetence and neoplasia. *Science* 212: 1100–1109. [crossref]
17. Buswell RS (1975) Letter: The pineal and neoplasia. *Lancet* 1: 34–35. [crossref]
18. Maestroni GJ, Conti A, Pierpaoli W (1988) Pineal melatonin, its fundamental immunoregulatory role in aging and cancer. *Ann N Y Acad Sci* 521: 140–148. [crossref]
19. Reiter RJ (2004) Mechanisms of cancer inhibition by melatonin. *J Pineal Res* 37: 213–214. [crossref]
20. Brzezinski A1 (1997) Melatonin in humans. *N Engl J Med* 336: 186–195. [crossref]
21. Hassan ATM, Hassan ZM, Moazzeni SM, Mostafaie A, Shahabi 5, et al. (2009) Naloxone can improve the antitumor immunity by reducing the CD+CD25+Foxp3+ regulatory T celis in BALB/c mice. *mt J Immunopharmacol* 9: 1381–1386.
22. Grotenhermen F (2004) Pharmacology of cannabinoids. *Neuro Endocrinol Lett* 25: 14–23. [crossref]
23. Rizzolatti G, Craighero L (2004) The mirror-neuron system. *Annu Rev Neurosci* 27: 169–192. [crossref]

24. Messina G, Lissoni P, Bartolacelli E, Magotti L, Clerici M, et al. (2010) Relationship between Psychoncology and psychoneuro endocrinoimmunology (PNEI).: enhanced T regulatory lymphocyte activity in cancer patients with self-punishment evaluated by Rorschach test. *In Vivo* 24: 75–78.

25. Ishida J, Wang HK, Bastow KF, Hu CQ, Lee KH (1999) Antitumor agents 201. Cytotoxicity of harmine and beta-carboline analogs. *Bioorg Med Chem Lett* 9: 3319–3324. [crossref]

Citation:
Messina G, Rovelli F, Brivio F, Lissoni P, Fumagalli L, Compare A. (2017) A Study on the Influence of Spirituality on the Efficacy of Antitumor Therapies with Natural Anticancer Agents in Untreatable Metastatic Cancer Patients. *Cancer Stud Ther J* Volume 2(2): 1–5

Research Article

iMedPub Journals
www.imedpub.com

Research Journal of Oncology

2017
Vol.1 No.1:101

A Study on the Endocrine Function of Pineal Gland with Regard To Immune Alterations in Cancer Patients

Paolo Lissoni*, Vichy Cenaj, Franco Rovelli, Giusy Messina, Giorgio Porro, Fernando Brivio and Giuseppe Di Fede

Institute of Biological Medicine, Milan, Italy

***Corresponding author:** Paolo Lissoni, Institute of Biological Medicine, Milan, Italy, Tel: +39 02 5830 0445; E-mail: paolo.lissoni@gmx.com

Received date: 09 September 2017; **Accepted date:** 06 October 2017; **Published date:** 10 October 2017

Citation: Lissoni P, Cenaj V, Rovelli F, Messina G, Porro G, et al. (2017) A Study on the Endocrine Function of Pineal Gland With Regard To Immune Alterations in Cancer Patients. Res J Oncol. Vol. 1 No. 1: 101.

Abstract

Several experimental studies have demonstrated that pineal gland plays a key anticancer role through the release of several hormones provided both by cytotoxic and immunostimulatory effects; the best-researched of all is melatonin (MLT). Despite the well documented anticancer role of the pineal gland, few clinical studies have been performed up to now in order to explore the pineal function in cancer patients. However, the results agree with the evidence of a progressive decline in the pineal function during the clinical course of neoplastic disease. Moreover, it is known that cancer progression is associated with a progressive decline in the effectiveness of anticancer immunity, which is mainly activated by lymphocytes and suppressed by monocyte-macrophage system, and which may be clinically investigated by evaluating the simple lymphocyte-to-monocyte ratio (LMR). So far, however, very few data about the possible relation between cancer-related immune and pineal alterations are available. The present study was carried out to evaluate the pineal function in a group of non-metastatic and metastatic cancer patients in relation to the LMR values. The pineal function was investigated by measuring the light and dark urinary excretion of the main MLT metabolite, the 6-sulfatoxymelatonin (6-MTS). A normal light/dark rhythm in the circadian excretion of 6-MTS was present in the non-metastatic patients, whereas a loss of pineal rhythm occurred in the metastatic group. In the same way, a normal light/dark rhythm was present in the patients with normal LMR values, whereas no rhythm was observed in those with abnormally low LMR values. According to the results previously reported by other authors, this study confirms that the metastatic neoplastic disease is characterized by a loss of the light/dark rhythm in the pineal function. Moreover, by showing a relation between the loss of light/dark pineal rhythm and low LMR values, this study would suggest that cancer-related immune alterations may depend at least in part on the altered pineal function, because of the fundamental immunomodulatory role of pineal itself.

Keywords: Cancer; Lymphocyte-to-monocyte ratio; Melatonin; Pineal gland; 6-sulfatoxymelatonin

Introduction

Despite the well demonstrated anticancer role of the pineal gland in experimental conditions [1-3], very few clinical studies have been performed up to now to evaluate the pineal endocrine function in cancer patients [4,5]. In any case, either in animals or in humans, cancer progression has appeared to be constantly associated with a progressive deficiency in the pineal endocrine function, as shown by the progressive decline in the production of the most investigated pineal hormone, the indole hormone melatonin (MLT), which has been proven to play a physiological anticancer activity through either a direct cytotoxic action, or an immunostimulatory effect on the anticancer immunity [6,7]. In more detail, MLT antitumor cytotoxic action is due to several mechanisms, the most important of them are consisting of the induction of tumor cell apoptosis and the inhibition of the action of several tumor growth factors. At the other side, the stimulatory effect of MLT on the anticancer immunity is mainly depending on a stimulation of the main antitumor cytokines in humans, consisting of IL-2 and IL-12. In normal conditions, MLT is mainly secreted during the dark period of the day [8], with a following well defined light/dark circadian rhythm in MLT secretion. MLT, however, is not the only hormone responsible for the anticancer activity of the pineal gland, since at least two other pineal hormones have appeared to exert a direct anticancer cytotoxic action, consisting of the indole 5-methoxytryptamine [9] and the beta-carboline pinoline [10]. Moreover, histological damages of the pineal gland have been demonstrated in patients died from cancer [11]. Therefore, the progressive decline in MLT secretion would not represent the only pineal endocrine deficiency with cancer progression. Therefore, the pineal endocrine deficiency would constitute one of the main biological alterations responsible for cancer

onset and development. The pineal endocrine deficiency could either precede or promote cancer development, or be induced by cancer dissemination. Pineal deficiency may predispose to tumor onset, since it has been demonstrated that a pineal deficiency induced by surgical or pharmacological pinealectomy has been proven to promote cancer development, whereas the administration of pharmacological doses of MLT has appeared to reduce the incidence of both spontaneous and chemically-induced tumors [12]. In fact, all psychological conditions predisposing to cancer, including stress, depression, pleasure repression and changes in light/dark rhythm, are constantly characterized by potential alterations in the pineal endocrine function [8-12]. On the other hand, tumor cells may directly produce the enzyme 2, 3-indole-dioxygenase (IDO) [13], which is able to induce tryptophan depletion and a consequent pineal endocrine deficiency, since all pineal hormones originate from tryptophan itself. In addition to the pineal endocrine deficiency, another fundamental biological alteration stimulating cancer growth is the lack of an effective anticancer immune reaction. Despite its complexity, it is currently known that the anticancer immune response in humans is substantially due to T helper-1 (TH1) lymphocytes through the release of IL-2 and to dendritic cells by the secretion of IL-12 [14,15]. IL-2 plays an anticancer activity by inducing the evolution of NK cells into LAK cells, which are able to destroy fresh human cancer cells irrespectively of their antigenicity [16]; IL-12 instead is able to counter cancer cell proliferation by stimulating cytotoxic T lymphocytes, which exercises an antigen-dependent cytotoxicity, by promoting T lymphocyte differentiation into TH1 cells, by counteracting the generation of regulatory T lymphocytes (T reg) [17], which in contrast suppress the antitumor immunity [18], and by playing an anti-angiogenic activity. MLT stimulates the anticancer immunity through several mechanisms, which may be synthetized into three essential effects: stimulation of IL-2 release from TH1 lymphocytes, stimulation of IL-12secretion by dendritic cells [7] and inhibition of T reg cell generation [19]. Therefore, due to the stimulatory effect of MLT on the anticancer immunity, cancer-progression related immunodeficiency could depend at least in part on the pineal endocrine failure. Up to now, however, there are no data available on the possible relationship between cancer-related pineal deficiency and immune alterations, which may characterize the advanced neoplastic diseases. Until a few years ago, the clinical evaluation of the immune status of cancer patients required several and expensive immune detections, including the measurement of lymphocyte subsets and cytokine blood concentrations. Recent studies, however, have demonstrated that the simple lymphocyte-to-monocyte ratio (LMR) may reflect the interaction between the anticancer immune reaction, which is mainly mediated by lymphocytes, and the chronic inflammatory status, which is mediated by the monocyte-macrophage system and which suppresses the anticancer immunity by allowing the generation of T reg lymphocytes. Therefore, abnormally low LMR values represent a sign of immunosuppression of anticancer immunity. For this reason LMR may be a simple and inexpensive biomarker for the clinical follow-up of the of anticancer immunity status in cancer patients in relation to the response to the various antitumor therapies and to the clinical course of the neoplastic disease. The present study was performed to investigate which relation may exist between the pineal endocrine function, evaluated by detecting MLT secretion, and the immune status, as synthetized by evaluating LMR, in a group of cancer patients with locally limited or metastatic disease.

Patients and Methods

The study included 30 consecutive cancer patients affected by the most common neoplasms, 16 of whom showed a metastatic disease. Eligibility criteria were, as follows: histologically proven solid tumor, measurable lesions, and no therapy with drugs potentially influencing MLT secretion from at least 1 week prior to study, including corticosteroids, opioids, beta-blockers and alpha-2 agonists. The clinical characteristics of patients are reported in **Table 1**. MLT secretion was assessed by measuring the urinary daily excretion of its main metabolite, the 6-sulphatoxy melatonin (6-MTS), by comparing the values observed during the light (8.0 AM-8.0 PM) and the dark (8.0 PM-8.0 AM) period of the day. 6-MTS was detected by a commercially available enzyme immunoassay kits (Melatonin-Sulfate Urine – ELISA, IBL INTERNATIONAL GMBH / Tecan Group Company) and the values were reported as mcg/ml. The circadian rhythm of MLT was considered to be within the normal range when 6-MTS values of the dark urinary sample were at least two times greater than those of the light sample. Finally, LMR values were considered to be normal when they were greater than 2.1 (95% confidence limits). Data were reported as mean ± SE, and statistically analyzed by the chi-square test, and the Student's t test, as appropriate.

Table 1 Clinical characteristics of 30 solid tumor patients.

Characteristics	**n**
Sex (M/F)	13/17
Median age (years)	62 (37-81)
Tumor histotype	
Breast cancer	10
Lung cancer	4
Colorectal cancer	4
Gastric cancer	3
Pancreatic cancer	3
Ovarian cancer	3
Sarcoma	3
Disease extension	
Locally limited disease	14
Metastatic disease (Dominant sites)	16
Soft tissues	3
Bone	2

Lung	2
Liver	3
Peritoneum	3
Brain	3

Results

A normal 6-MTS rhythm, with night values at least greater two times than the light ones, was present in only 14/30 (47%) patients. Moreover, the percentage of patients with normal pineal rhythm observed in the non-metastatic group was significantly higher with respect to that found in the metastatic group (10/14 (71%) vs. 4/16 (25%), P<0.01). Abnormally low values of LMR were seen in 11/30 (37%) patients, and the percentage of patients with low LMR values was significantly higher in the metastatic group than in the non-metastatic one (3/14 (21%) vs. 8/16 (50%), P<0.01). Moreover, the percentage of patients with normal pineal rhythm was significantly higher in the group of patients with normal LMR values than in those with abnormally low LMR values (11/19 (59%) vs. 3/11 (27%), P<0.05). On the other side, the percentage of normal LMR values observed in the group of patients with normal 6-MTS rhythm was significantly higher than that found in patients, who had no rhythm (12/14 (86%) vs. 7/16 (44%), P<0.01). Furthermore, patients with normal 6-MTS rhythm showed significantly higher mean values of LMR than those found in patients, in whom the rhythm was absent (4.7 ± 0.4 vs. 2.3 ± 0.3, P<0.01). Day and night 6-MTS urinary values observed in non-metastatic and in metastatic patients, as well as in patients with normal or abnormally low LMR values are illustrated in **Figure 1**. In patients with locally limited tumor, a light/dark rhythm was still present, with night mean values of 6-MTS significantly higher with respect to those found during the light period of the day (P<0.025). Conversely, no circadian rhythm in 6- MTS production was seen in metastatic patients, since there was no significant difference between day and night 6-MTS mean values. Night mean values of 6-MTS observed in non-metastatic patients were significantly higher than those found in metastatic patients (P<0.05). Likewise, night mean values of 6-MTS observed in patients with normal LMR values were significantly higher than those found in patients with low LMR values (P<0.05), while no significant difference occurred in 6-MTS mean values during the light period of the day.

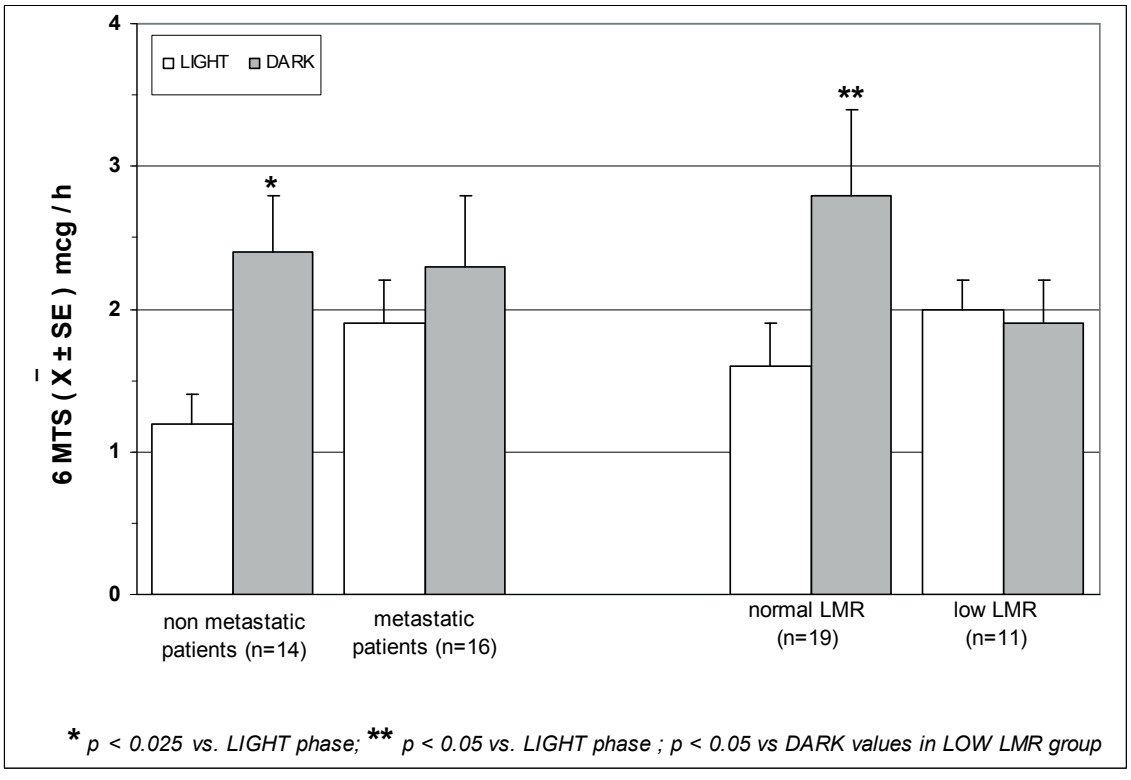

Figure 1 6 MTS light and dark mean values in non-metastatic and metastatic cancer patients and in those with normal or low lymphocyte-to-monocyte ratio (LMR).

Discussion

As in previous preliminary experimental and clinical investigations, this study confirms that cancer progression is associated with a progressive decline in the nocturnal pineal production of MLT, with a consequent progressive loss of the natural resistance against cancer growth, which is mainly mediated by the pineal gland and the immune system. Moreover, this study also confirms that cancer dissemination is constantly characterized by profound immune alterations, as

shown by the evidence of a decline in LMR values in metastatic patients, confirming that cancer progression is associated with a progressive decline in lymphocyte functions and with a concomitant increase in monocyte-macrophage system activation, that mediates the suppression of the anticancer immunity by allowing the generation of T reg cells [20].

Conclusion

Finally, this study shows that there is an association between pineal and immune alterations, because of the evidence of a greater percentage of immune alterations in those patients, who had no light/dark circadian rhythm in the pineal endocrine function. Therefore, cancer progression-related alterations in the antitumor immunity would depend, at least in part, on the progressive decline in the pineal endocrine function. This finding is not surprising considering the stimulatory role of MLT on the generation of an effective anticancer immune reaction through the activation of TH1 lymphocyte functions and the inhibition of the monocyte-macrophage system [21]. Therefore, cancer therapy with MLT could represent a new antitumor treatment, capable of acting at the same time either as an endocrine therapy, or as an immunotherapy by inducing an effective anticancer immune reaction through a modulation of the same mechanisms responsible for the central psycho-neuro endocrine regulation of the immune system, including the anticancer immunity [22]. Obviously, further clinical studies will be required to *in vivo* confirm the great number of immune effects induced by MLT, namely by monitoring changes in lymphocyte subsets and in the blood levels of those cytokines mainly involved in the control of the inflammatory response and the antitumor immunity, including the antitumor cytokines IL-2 and IL-12, and the immunosuppressive ones IL-10 and TGF-beta. If further studies will confirm the capacity of MLT to pilot the cytokine network in an antitumor way, MLT could be successfully used in association with the recent anti-checkpoint inhibitor immunotherapies of cancer.

Acknowledgments

The authors sincerely thank Dr. Sa Monica Raggi (Lab. of Microbiology, San Gerardo Hospital, Monza, Italy) for her support in the translation of the present work.

References

1. Buswell RS (1975) The pineal and neoplasia. Lancet 1: 34-35.
2. Regelson W, Pierpaoli W (1987) Melatonin: a rediscovered antitumor hormone? Cancer Invest 5: 379-385.
3. Reiter RJ (2004) Mechanisms of cancer inhibition by melatonin. J Pineal Res 37: 213-214.
4. Di Bella L, Scalera G, Rossi MT (1979) Perspectives in pineal function. Prog Brain Res 52: 475-477.
5. Bartsch C, Bartsch H (1999) Melatoin in cancer patients and in tumor-bearing animals. Adv Exp Med Biol 467: 247-264.
6. Maestroni JGM (1993) The immunoneuroendocrine role of melatonin. J Pineal Res 14: 1-10.
7. Guerrero JM, Reiter RJ (2002) Melatonin-immune system relationships. Curr Topics Med Chem 2: 167-180.
8. Brzezinski A (1997) Melatonin in humans. N Engl J Med 336: 186-195.
9. Sze SF, Ng TB, Liu WK (1993) Antiproliferative effect of pineal indoles on cultured tumor cell lines. J Pineal Res 14: 27-33.
10. Song Y, Wang J, Teng SF, Kesuma D, Deng Y, et al. (2002) Beta-carbolines as specific inhibitors of cyclin-dependent kinases. Bioorg Med Chem Lett 12: 1129-1132.
11. Hadjiu SI, Porro RS, Lieberman PH (1972) Degeneration of the pineal gland of patients with cancer. Cancer 29: 706-709.
12. Conti A, Maestroni JGM (1995) The clinical immunotherapeutic role of melatonin in Oncology. J Pineal Res 129: 103-110.
13. Mittal D, Gubin MM, Schreiber RD, Smyth MJ (2009) Insights into cancer immunoediting and its three component phases-elimination, equilibrium and escape. Curr Opin Immunol 27: 16-25.
14. Atzpodien J, Kirchner H (1990) Cancer, cytokines and cytotoxic cells: Interleukin-2 in the immunotherapy of human neoplasms. Klin Wochenschr 68: 1-11.
15. Banks RE, Patel PM, Selby PJ (1999) Interleukin-12: A new clinical player in cytokine therapy. Br J Cancer 80: 407-411.
16. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA (1982) Lymphokine-activated killer cell phenomenon. J Exp Med 155: 1823-1841.
17. Prochazkiva J, Pokoma K, Holan V (2012) IL-12 inhibits the TGF-beta-dependent T cell developmental programs and skews the TGF-beta-induced differentiation into a Th1-like direction. Immunobiology 217: 74-82.
18. Zou W (2006) Regulatory T cells, tumor immunity and immunotherapy. Nat Rev Immunol 6: 295-307.
19. Liu H, Wei JE, Xie MR, Wang SE, Zhou RX (2011) Role of CD4+CD25+ regulatory T cells in melatonin-mediated inhibition of murine gastric cancer cell growth in vivo and in vitro. Anat Rec (Hoboken) 294: 781-788.
20. Eo WK, Chang HJ, Kwon SH, Koh SB, Kim YO, et al. (2016) The lymphocyte to monocyte ratio predicts patient survival and aggressiveness of ovarian cancer. J Cancer 7: 289-296.
21. Mantovani A, Allavena P, Sica A, Balkwill F (2008) Cancer-related inflammation. Nature 454: 436-444.
22. Lissoni P (1999) The pineal gland as a central regulator of cytokine network. Neuroendocrinol Lett 20: 103-110.

Research Article

iMedPub Journals
www.imedpub.com

Research Journal of Oncology

2018
Vol.2 No.1:2

Five Year-Survival with High-Dose Melatonin and Other Antitumor Pineal Hormones in Advanced Cancer Patients Eligible for the Only Palliative Therapy

Paolo Lissoni*, Franco Rovelli, Fernando Brivio, Giusy Messina, Arianna Lissoni, Sonja Pensato and Giuseppe Di Fede

Institute of Biological Medicine, Milan, Italy

***Corresponding author:** Paolo Lissoni, Institute of Biological Medicine, Milan, Italy, Tel: +39 02 5830 0445; E-mail: paolo.lissoni@gmx.com

Received date: 09 March 2018; **Accepted date:** 19 March 2018; **Published date:** 26 March 2018

Citation: Lissoni P, Rovelli F, Brivio F, Messina G, Lissoni A, et al. (2018) Five Year-Survivals with High-Dose Melatonin and Other Antitumor Pineal Hormones in Advanced Cancer Patients Eligible for the Only Palliative Therapy. Res J Oncol. Vol.2 No. 1: 2.

Abstract

Cancer progression has appeared at least in part to be due to a deficiency of the mechanisms responsible for the natural antitumor immune response. Moreover, more recent studies have demonstrated that cancer-related immunosuppression does not depend only of alterations of immune cells themselves, but also on an altered neuroendocrine regulation of the antitumor immune response, which is mainly inhibited by the mu-opioid agonists, such as beta-endorphin, and stimulated by the pineal gland through at least three immunostimulating molecules, able to exert a direct antiproliferative anticancer activity without any important biological toxicity, consisting of the indole hormones melatonin (MLT) and the 5-methoxytryptamine (5-MTT), and of the beta-carboline pinealine (PNL). Finally, cancer progression has been shown to be constantly associated with a progressive decline in the endocrine function of the pineal gland, which could be involved in cancer dissemination itself. Then, the simple endocrine oncostatic pineal replacement therapy could counteract cancer growth and enhance the survival time, as suggested by preliminary clinical studies. On the basis, a pineal endocrine regimen was proposed in a group of untreatable advanced solid tumor patients, for whom no other effective standard anticancer therapy was available. The study included 212 patients, suffering from the most common tumor histotypes and eligible for the only best supportive care and with life expectancy less than 1 year. All pineal indoles were given orally at the time corresponding to that of their maximal circadian secretion, every day without interruption until disease progression. MLT was given at pharmacological doses (100 mg/day in the night period), while 5-MTT during the light period and PNL at the onset of the evening were administered at mild-pharmacological doses (5-MTT: 10 mg/day; PNL: 1 mg/day). A disease control (DC) was achieved in 111/212 (52%) patients, with an objective tumor regression in 16/212 (8%), irrespectively of tumor histotype. A 1-year and 5-year percentages of survival were achieved in 46% and 11%, respectively, and there were significantly higher in patients with DC than in the progressed ones. Finally, the evidence of normal pretreatment values of lymphocyte-to-monocyte ratio (LMR) and/or their normalization on therapy have appeared to be associated with most favorable clinical results. No biological toxicity occurred on pineal endocrine oncostatic treatment. This study shows that an endocrine substitutive therapy with the most known antitumor pineal hormones may represent a new non-toxic inexpensive anticancer therapy, which can improve the survival and control cancer growth also in patients for whom no other effective therapy is available, at least to improve their life. By concluding according to their results the palliative therapy of untreatable cancer patients for whom no other standard therapy available could be associated with a concomitant therapy with natural anticancer agents, namely the same pineal hormone.

Keywords: Lymphocyte-to-monocyte ratio; Melatonin; Metoxytryptamine; Pineal gland

Introduction

Each living organisms may generate both pro-tumoral and anti-tumoral events, from whose equilibrium depends the physiological growth of the normal cells until their apoptosis-induced death. The antitumor biological response, which is responsible for the natural resistance against cancer growth, would depend not only on immune factors, but also on the physiological psychoneuroendocrine regulation of the immune system, which may act by either stimulating or suppressing the antitumor immunity, as shown by the great number of researches in the area of the Psycho-neuro-endocrino-immunology (PNEI) [1-3]. In particular, it has been shown that the opioid system may inhibit the anticancer immunity [4] by promoting the generation of regulatory T lymphocytes (T reg), which may suppress the antitumor immune response through the secretion of immunosuppressive cytokines, such as TGF-beta and IL-10 [5], and by inhibiting T helper-1 lymphocyte (TH1) and dendritic cells functions [6], with a following decline

in the production of IL-2 and IL-12, respectively, that represent the main antitumor cytokines in humans [7,8]. On the contrary, the anticancer immunity has been proven to be stimulated by the pineal gland through the release of several indole hormones [9] and beta-carbolines [10], whose activity is connected with the brain cannabinergic system, by constituting a fundamental neuroendocrine functional axis [11]. More in detail, stress-induced promoting effect on cancer onset and development has appeared to be mediated by the opioid system, mainly through the release of mu-opioid agonists, such as the beta-endorphin, since it may be blocked by the concomitant administration of the mu-opoid antagonist naltrexone [4]. On the other hand, pleasure and spiritual expansion of mind may counteract tumor dissemination by activating the pineal-cannabinergic functional axis [12]. As far as the pineal activity is concerned, the main anticancer molecules are consisting of the indoles melatonin (MLT) and 5-methoxytryptamine (5-MTT) [9], and the beta-carboline pinealine [10], which exert their anticancer action by either directly inhibiting cancer cell proliferation, or stimulating the anticancer immunity, namely through the activation of TH1 lymphocytes and dendritic cells, with a following enhanced production of IL-2 and IL-12 [13,14]. The antitumor immunomodulating effects of MLT are mainly due to the stimulation of lymphocyte functions [15], whereas those played by 5-MTT, pinealine, as well as by cannabinoids, would mainly depend on an inhibition of macrophage-mediated immuno-inflammatory response [9,10], which has been proven to suppress the anticancer immunity [16,17]. Therefore, from a neuroimmune point is concerned, cancer growth may be considered as the consequence of an altered balance involving the main structures responsible for the neuroimmunomodulation of the immune responses, consisting of an enhanced brain opioid sistem activity in association with a concomitant diminished function of the pineal-cannabinergic system axis [18]. In fact, the progressive decline in the pineal function, namely consisting of a progressive lack of the nocturnal increase in MLT levels with a consequent disappearance of its physiological light/dark circadian rhythm [19], would represent the main cancer progression-related endocrine deficiency either in animals, or in humans [20,21]. Cancer-related pineal endocrine deficiency woul regard not only MLT, but probably the whole pineal endocrine activity, since pineal histological damages have been described in patients died from cancer [22]. However, despite it is known since more than 50 years that the pineal gland plays a fundamental role in the maintenance of the natural anticancer immunobiological resistance [9-11] and the complete absence of any biological toxicity exerted by the pineal indole and beta-carboline hormones [19], few clinical studies have been performed up to now with MLT alone or MLT in association with other antitumor pineal molecules to evaluate their efficacy in the treatment of advanced cancer patients, who failed to respond to the conventional chemotherapies and target therapies, at least in terms of palliative therapy. In any case, preliminary clinical studies have already shown that high-dose MLT alone may induced a stabilization of the neoplastic disease in a clinically relevant percentage of cancer patients, for whom no other standard anticancer therapy was available, and with life expectancy less than 6 months-1 year [23]. Moreover, it has been shown that the anticancer activity of MLT is a dose-dependent phenomenon, and may be further amplified by the concomitant administration of other antitumor pineal molecules, namely 5-MTT and pinealine [23-25]. However, many others natural anticancer strategies have been elaborated in the last year [26-28]. The present study reports the 5-year survival achieved by the pineal endocrine therapy with high-dose MLT plus 5-MTT plus pinealine in advanced cancer patients, for whom no other standard antitumor therapy was available, and its relation with the clinical response and the immune status by determining the lymphocyte-to-monocyte ratio (LMR), which has been proven to reflect and to synthetize the complex interaction between immunosuppressive and immunostimulatory events involved in the antitumor immunity [29].

Materials and Methods

Patient enrollment

The study included 212 advanced cancer patients, for whom no other standard anticancer therapy was available, then eligible for the only palliative treatment, who had a follow up of at least five years. Eligibility criteria were, as follows: histologically proven solid tumor, measurable lesions, metastatic or advanced neoplastic disease, no availability of conventional anticancer therapy because of lack of response to the previous standard treatments or poor clinical conditions unable to sustain a chemotherapeutic approach, no double tumor, and life expectancy less than 1 year.

Study plan

All pineal hormones were given orally. MLT was administered at 100 mg/day during the dark period of the day, according to its physiological circadian rhythm, generally half-hour before sleeping. 5-MTT was given at 10 mg/day during the light phase of the day, generally at 1.00 P.M. Finally, pinealine was administered at 1 mg/day in the evening, generally 3 hours prior to sleep. Moreover, the supportive care was planned according to a phythotherapeutic approach, by using plants, which have been proven to give some subjective benefits in previous clinical studies [30], namely Aloe, Myrrh, and Magnolia. The treatment with pineal hormones was continued without interruption until disease progression. In the presence of a clear subjective clinical benefit, pineal hormone therapy was still continued despite the progression of the neoplastic disease. The clinical characteristics of patients are reported in **Table 1**. The clinical response was evaluated according to WHO criteria by repeating the radiological investigations, including CT scan and NMR, before the onset of treatments and at 3-month intervals until disease progression. Moreover, the clinical response was correlated with LMR values, which were detected prior to therapy and at 1-month intervals. Normal values of LMR obtained in our laboratory (95% confidence limits) were greater than 2.1. Data were statistically analyzed by the chi-square test, the Student's

t test. Finally, the survival curves were made according to the Kaplan-Meyer method, and statistically assessed by the log-rank test.

Table 1 Characteristics of 212 untreatable advanced cancer patients treated with pineal endocrine therapy (PET).

Characteristics	n
M/F	118/94
Median age (years)	63 (22- 92)
Median performance status 1	(0 – 3)
Previous chemotherapy	178/212 (84%)

Results

Clinical response to therapy

The clinical response and the 5-year percentages of survival observed in the overall patients and in relation to the single tumor histotypes are reported in **Table 2**. A complete response (CR) was achieved in 2/212 (1%) patients (non-small cell lung cancer: 1; gastric cancer: 1). Moreover, a partial response (PR) was obtained in other 14/212 (7%) patients (non-small cell lung cancer (NSCLC): 2; colorectal cancer: 2; pancreatic adenocarcinoma: 1; hepatocarcinoma: 1; biliary tract cancer: 2; ovarian cancer: 2; bladder cancer: 1; triple negative breast cancer (TNBC): 1; melanoma: 2). Then, an objective tumor regression was achieved in 16/212 (8%) patients. A stable disease (SD) was observed in 95/212 (45%). Therefore, a disease control (DC) (CR+PR+SD) was achieved in 111/212 (52%) patients, whereas the remaining 101 patients (48%) had a progressive disease (PD). As shown, the 5-year survival observed in the overall patients and in relation to their clinical response is illustrated in **Figure 1**. The 1-year, 3-year and 5-year survival percentages were 46%, 18%, and 11%, respectively. Moreover, the survival time obtained in patients, who achieved an objective tumor regression (CR+PR), was significantly longer with respect to that found in those, who had no tumor regression ($P<0.01$). Finally, the survival time found in patients with SD was also significantly longer than that observed in patients with PD ($P<0.05$).

Table 2 Clinical response (WHO citeria) and survival time to pineal endocrine therapy (P.E.T.) in 212 untreatable advanced cancer patients, and their relation to tumor histotype.

Patients +	Clinical Response ++							Survival Time (Year)				
	n	CR	PR	CR + PR (%)	SD	DC (%)	PD	1	2	3	4	5
Overall Patients	212	2	14	16 (-8%)	95	111 (-52%)	101	98 (-46%)	56	38	27	23 (-11%)
Tumor Histotype												
Lung cancer	36	1	2	3	16	19	17	17	9	7	5	5
-NSCLC	29	1	2	3	14	17	12	15	7	6	4	4
-SCLC	7	0	0	0	2	2	5	2	2	1	1	1
Colorectal cancer	25	0	2	2	13	15	10	13	8	5	4	4
Pancreatic cancer	22	0	1	1	10	11	11	12	4	3	2	1
Gastric cancer	12	1	0	1	3	4	8	3	3	3	2	1
Biliary tract cancer	11	0	2	2	2	4	7	6	3	2	2	1
Hepatocarcinoma	6	0	1	1	3	4	2	3	2	1	0	0
Ovarian cancer	14	0	2	2	7	9	5	9	6	3	2	2
Bladder cancer	5	0	1	1	3	4	1	3	2	1	1	1
Prostate cancer	4	0	0	0	3	3	1	3	3	2	2	2
TNBC	6	0	1	1	2	3	3	3	2	2	1	1
Soft tissue sarcoma	15	0	0	0	8	8	7	5	3	3	2	2
Melanoma	10	0	2	2	4	6	4	4	2	2	1	1
Glioblastoma	46	0	0	0	21	21	25	19	9	4	3	2

NSCLC: non-small cell lung cancer; SCLC: small cell lung cancer; TNBC: triple negative breast cancer, CR: complete response; PR: partial response; SD: stable disease; DC: disease control; PD: progressive disease

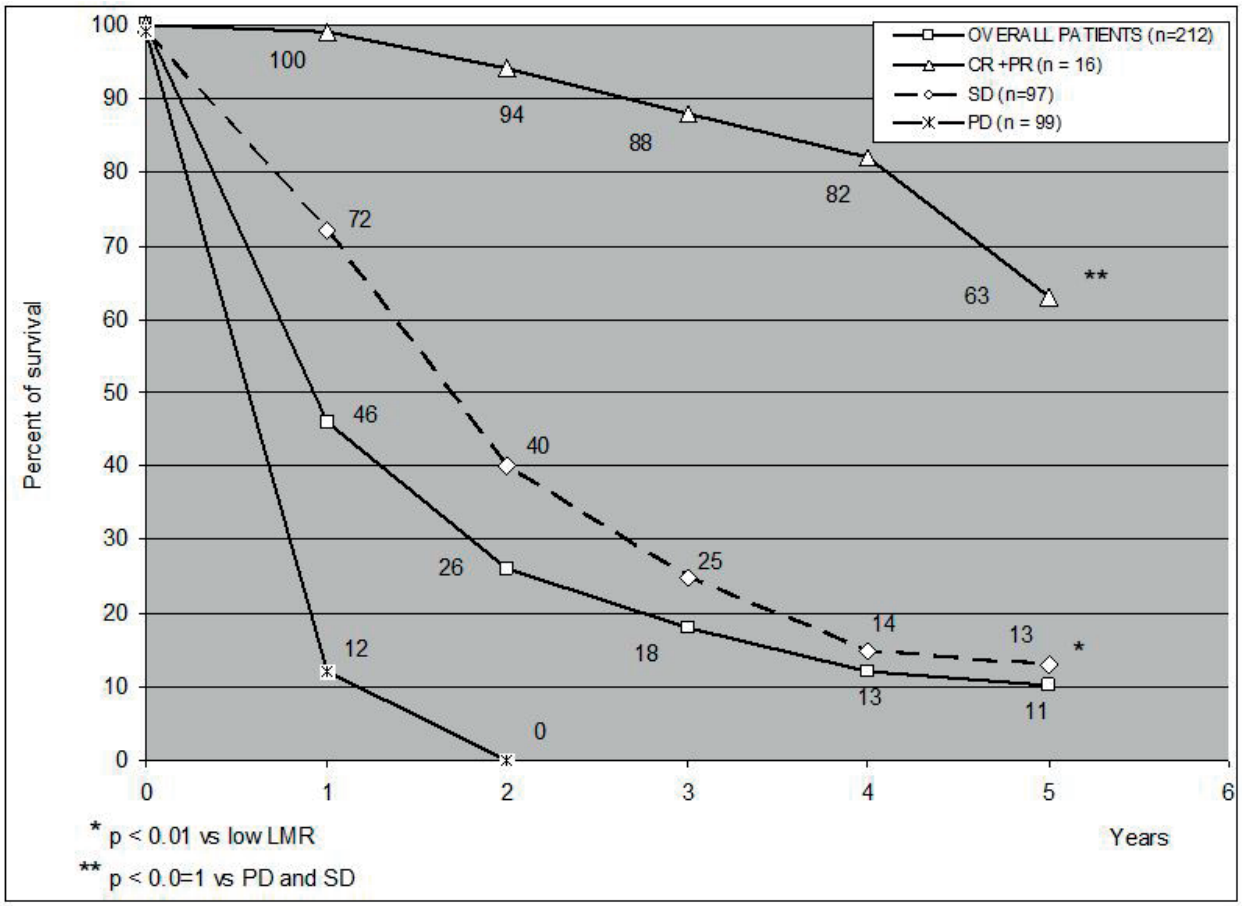

Figure 1 Five-year survival in relation to the clinical response.

Immune effect of therapy

From the point of view of the immunological status is concerned, abnormally low pretreatments values of LMR were seen in 131/212 (62%) patients. The clinical response in relation to LMR pretreatment values are shown in **Table 3**. As reported, both objective tumor regression and DC percentages observed in patients with normal pretreatment values of LMR were significantly higher than those found in patients with abnormally low LMR values prior to therapy (P<0.01 and P<0.05, respectively). In addition, as illustrated in **Figure 2**, the 5-year percentage of survival observed in patients with normal LMR values prior to therapy was significantly longer than that achieved in patients with low pretreatment LMR values (P<0.01). Finally, as far as patients with PD are concerned, 44/101 (44%) patients, who had a PD, continued the pineal therapy despite the progression of their disease, because their improved clinical status. After 6 months and 1 year, only 34/101 (34%) and 2/101 (2%) were still alive. Both patients still alive at 1 year had continued the pineal therapy, whereas no patient, who interrupted the treatment, was alive. Moreover, the percentage of 9-month survival achieved in progressed patients, who continued the pineal therapy, was significantly longer than that found in those, who interrupted the endocrine treatment (14/44 (32%) vs. 0/57, P<0.05). Finally, abnormally low LMR values prior to therapy were seen in 70/101 (69%) patients with PD. The 9-month survival percentage observed in patients with PD but normal pretreatment values of LMR was significantly longer than that found in progressed patients with abnormally low values of LMR prior to therapy (9/31 (29%) vs. 5/70 (7%), P<0.05). The treatment was well tolerated, and most patients experienced a clear subjective benefit in mood, anxiety, sleep quality and asthenia. No biological toxicity occurred under pineal therapy, and some transient undesirable effects, such as headache, increase in anxiety, and sleep disturbances, occurred for few days in only 23/212 (11%) patients, without the need to interrupt the treatment.

Table 3 Clinical response (WHO criteria) in relation to LMR pretreatment values to pineal endocrine therapy (P.E.T.) in 212 untreatable advanced cancer patients.

Lmr Pretreatment Values +	Clinical Response ++						
	n	CR	PR	CR+PR (%)	SD	DC (%)	PD (%)
Normal Values	81	2	9	11 (14%) *	52	63 (78%)**	18 (22%)
Low Values	131	0	5	5 (4%)	43	48 (37%)	83 (63%)

+ LMR: lymphocyte-to-monocyte ratio; normal values more than 2.1; ++ CR: complete response; PR: partial response; SD: stable disease; DC: disease control; PD: progressive disease

*P<0.01 vs. low LMR values; P<0.05 vs. low LMR values

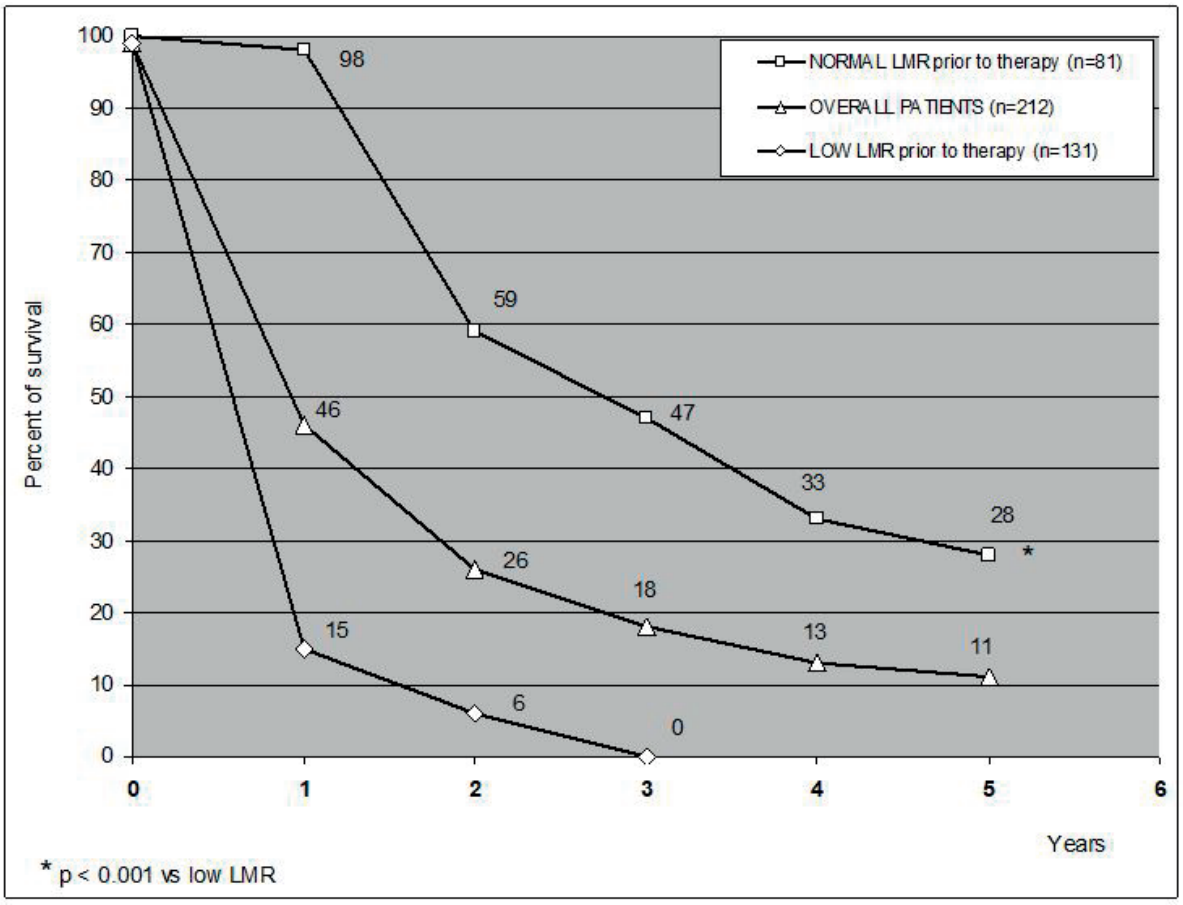

Figure 2 Five-year survival in relation to LMR pretreatment values.

Discussion

According to previous preliminary clinical results [23-25], this study confirms in a greater number of untreatable advanced cancer patients and for a longer period of follow-up that the endocrine therapy with high-dose of MLT in association with the administration of the other two main anticancer molecules of the pineal gland, including 5-MTT and pinealine, may induce some tumor regression and prolong the survival time in patients eligible for the only palliative therapy because of the lack of response to the previous antitumor therapies, and life expectancy lower than 1 year. Moreover, the pineal endocrine therapy-induced prolongation of the survival time has appeared to be greater in patients, who achieved an objective tumor regression or disease stabilization, by suggesting that pineal endocrine-induced control of cancer growth is not a simple epiphenomenon, since it has been proven to predict a longer survival. This finding is not surprising since the only MLT has been already observed to represent the only molecule capable of counteracting the

whole six main mechanisms responsible for cancer dissemination [23], including stress-induced immunosuppression, cancer cell transformation, intercellular joint alterations, stimulation of the neoangiogenic processes, tumor cell production of immunosuppressive factors and tumor expression of FAS-L, which allows the apoptosis of T lymphocytes after their interaction with the cancer cells [30]. In addition, the antitumor activity of MLT may be enhanced by the concomitant association with other pineal anticancer molecules, by justifying the possible evidence of tumor regressions or tumor stabilization also in very advanced cancer patients, for whom no other standard anticancer therapy may be available. Moreover, this study would suggest that the efficacy of a pineal endocrine antitumor therapy is greater in patients with normal pretreatment values of LMR, which may synthetize the whole status of the anticancer immunity in the single cancer patient [29]. The different efficacy of therapy may be influenced by the previous therapies, namely radiotherapy, because of the influence on lymphocyte count. Then, the evidence of abnormally low LMR values would reflect an immunosuppressive status of the anticancer immunity, with a consequent lower efficacy of the various anticancer treatments. Finally, previous studies had already shown a greater efficacy of the anticancer therapies in the presence of a real spiritual faith condition, as assessed by an adequate clinical test [31]. Some recent biomarkers, such as LMR, could be use full to clinically monitor the immune status of cancer patients [32,33]. Then, in the presence of a clinical response consisting of objective tumor regression or neoplastic disease stabilization, of a normal LMR values prior to therapy and an adequate spiritual faith score, it is probable that the pineal endocrine antitumor therapy may contribute to the control of the neoplastic growth and modify the prognosis of an untreatable advanced neoplastic disease also in patients, for whom no other conventional anticancer therapy may be available. On the contrary, tumor histotype does not seem to influence the efficacy of the pineal anticancer therapy in a relevant manner, even though glioblastoma and pancreatic adenocarcinoma would seem to represent the less responsible neoplasms to the treatment. However, by considering their low life expectancy after failure of the various therapies, glioblastoma and cancer of pancreas would be also influenced by the pineal therapy, at least in terms of survival time with respect to the expected one. Obviously, further randomized studies with the only best supportive care (BSC) or with BSC plus the pineal endocrine anticancer therapy will be required to confirm that the administration of the main anticancer molecules produced by the pineal gland may prolong the survival time also in patients with advanced cancer, eligible for the only palliative therapy and with life expectancy less than 1 year, since the survival of untreatable cancer patients, for whom no other standard anticancer therapy is available, is constantly generally less than 1 year or 6 months.

Conclusion

This preliminary study, by showing a possible increase in the survival time in patients with untreatable tumors and life expectancy less than 1 year, then suitable for the only supportive care by the simple administration of the immunostimulating pineal hormones would suggest that the separation between palliative and curative has to be abrogated by the existence in the nature of several non-toxic anticancer agents, namely within the same human body, which could be administered to untreatable cancer patients with respect to the only palliative therapy. Moreover, further studies by evaluating other immune parameters, such as tumor infiltrating lymphocytes, will be required to better define the immunomodulating effects of pineal therapy.

References

1. Riley V (1981) Psychoneuroendocrine Influences On Immunocompetence And Neoplasia. Science 212: 1100-1109.
2. Rubinow DR (1990) Brain, Behaviour and Immunity: An Interactive System. J Natl Cancer Inst Monoghr 10: 79-82.
3. Antony MH (2003) Psychoneuroimmunology of Cancer. Brain Beav Immun 17: 84-91.
4. Manfredi B, Sacerdote P, Pianchi M (1993) Evidence For An Opioid Inhibitory Tone On T Cell Proliferation. J Neuroimmunol 44: 43-46.
5. Zou W (2006) Regulatory T Cells, Tumor Immunity And Immunotherapy. Nat Rev Immunol 6: 295-307.
6. Antony PA, Restito NP (2005) CD4+CD25+ T Regulatory Cells, Immunotherapy Of Cancer And Interleukin-2. J Immunother 28: 120-128.
7. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA (1982) Lymphokine-Activated Killer Cell Phenomenon. J Exp Med 155: 1823-1841.
8. Banks RE, Patel PM, Selby PJ (1995) Interleukin-12: A New Clinical Player in Cytokin Therapy. Br J Cancer 71: 655-659.
9. Sze SF, Ng TB, Liu WK (1993) Antiproliferative Effect of Pineal Indoles on Cultured Tumor Cell Lines. J Pineal Res 14: 27-33.
10. Song Y, Wang J, Teng SF, Kesuma D, Deng Y, et al. (2002) Beta-Carbolines As Specific Inhbitors of Cyclin-Dependent Kinases. Biorg Med Chem Lett 12: 1129-1132.
11. Lissoni P, Resentini M, Mauri R, Esposti D, Esposti G, et al. (1986) Effects Of Tetrahydrocannabinol On Melatonin Secretion In Man. Horm Metabol Res 18: 77-78.
12. Grotenhermen F (2004) Pharmacology of Cannabinoids. Neuroendocrinol Lett 25: 14-23.
13. Regelson W, Pierpaoli WM (1987) A Rediscovered Antitumor Hormone? Cancer Invest 5: 379-385.
14. Guerrero JM, Reiter RJ (2002) Melatonin-Immune System Relationships. Curr Topics Med Chem 2: 167-180.
15. Conti A, Maestroni GJM (1995) The Clinical Neuroimmunotherapeutic Role Of Melatonin In Oncology. J Pineal Res 19: 103-110.
16. Mantovani A, Allavena P, Sica A, Balkwill F (2008) Cancer-Relòated Inflammatioopn. Nature 454: 436-444.
17. Grivennikov SI, Greten FR, Karin M (2010) Immunity, Inflammation, And Cancer. Cell 295: 883-899.
18. Lissoni P (2000) Is There A Role For Melatonin In Supportive Care? Supp Care Cancer 10: 110-116.

19. Bartsch C, Bartsch H (1999) Melatonin in Cancer Patients And In Tumor-Bearing Aninals. Adv Exp Med Biol 467: 247-264.
20. Brzezinski A (1997) Melatonin In Humans. N Engl J Med 336: 186-195.
21. Lissoni P (1999) The Pineal Gland as A Central Regulator Of Cytokine Network. Neuroendocrinol Lett 20: 343-349.
22. Hadjiu SI, Porro RS, Lieberman PH (1972) Degeneration Of The Pineal Gland Of Patients With Cancer. Cancer 29: 706-709.
23. Reiter RJ (2004) Mechanisms of Cancer Inhibition By Melatonin. J Pineal Res 37: 213-214.
24. Millis E, Wu P, Seely D, Guyatt G (2005) Melatonin In the Treatment of Cancer: A Systematic Review Of Randomized Controlled Trials And Meta-Analysis. J Pineal Res 39: 360-366.
25. Lissoni P, Messina G, Lissoni A, Rovelli F (2017) The Psychoneuroendocrine-Immunotherapy Of Cancer: Historical Evolution And Clinical Results. J Res Med Sci 22: 45-52.
26. Wakaskar RRJ (2018) Brief Overview Of Nanoparticulate Therapy In Cancer. J Drug Target 26: 123-126.
27. Wakaskar RRJ (2017) Passive And Active Targeting In Tumor Microenvironment. Int J Drug Dev & Res 9: 2.
28. Wakaskar RRJ (2018) Promising Effects of Nanomedicine in Cancer Drug Delivery. J Drug Target 26: 319-324.
29. Nishijma TF, Muss HB, Shachar SS, Tamura K, Takamatsu Y (2015) Prognostic Value Of Lymphocyte-To-Monocyte Ratio In Patients With Solid Tumors: A Systematic Review And Meta-Analysis. Cancer Treat Rev 41: 971-978.
30. Shibakita M, Tachibana M, Dhar DK, Kotoh T, Kinugasa S, et al. (1999) Prognostic Signifiifance Of FAS And FAS Ligand Expression In Human Esophageal Cancer. Clin Cancer Res 5: 2464-2469.
31. Messina G, Rovelli F, Brivio F, Lissoni P, Fumagalli L, et al. (2017) A Study On The Influence Of Spirituality On The Efficacy Of Antitumor Therapies With Natural Anticancer Agents In Untreatable Metastatic Camncer Patients. Cancer Stud Ther J 2: 1-5.
32. Lissoni P, Messina G, Rovelli F, Vigorè L, Lissoni A, et al. (2018) Low Lymphocyte-To-Monocyte Ratio Is Associated with an Enhanced Regulatory T lymphocyte Function In Metastatic Cancer Patients. Int J Rec Adv Mult Res 5: 3353-3356.
33. Paolo Lissoni (2016) Therapy Implications of the Role of Interleukin-2 in Cancer. Expert Rev Clin Immunol 13: 491-498.

Global Drugs and Therapeutics

Mini Review ISSN: 2399-9098

The modulation of the endocannabinoid system in the treatment of cancer and other systemic human diseases

Paolo Lissoni*, Giusy Messina, Giorgio Porro, Roberto Trampetti, Arianna Lissoni, Franco Rovelli, Vezika Cenay, Enrica Porta and Giuseppe Di Fede

Institute of Biological Medicine, Milan, Italy

Abstract

Despite cancer is at present considered as a systemic disease, in the clinical management of patients the neoplastic disease is often up to now generally considered and treated as a loco-regional pathology. The systemic nature of cancer is documented by the evidence of the fundamental role of the immune system in the control of tumor onset and dissemination. However, it must be taken into consideration that the in-vivo immune responses are under a physiological neuroendocrine control, namely played by brain opioid and cannabinoid systems. The endogenous cannabinoid system has been proven to exert a fundamental anti-inflammatory action. Then, since the chronic inflammatory status has appeared to promote cancer growth and dissemination, it could be clinically important to evaluate the functional status of the endogenous cannabinoid system in cancer patients. The endogenous cannabinoids, the most important of them are anandamide and 2-arachidonyl glycerol, are destroyed by the fatty acid amide hydrolase (FAAH) enzyme, whose levels are inversely correlated to those of the endogenous cannabinoids. Moreover, it has been observed that the evidence of abnormally high blood concentrations of FAAH, which reflect low levels of cannabinoids, has appeared to predict a poor prognosis in cancer patients. Therefore, the determination of FAAH blood levels would have to be included within the laboratory analyses of cancer patients in an attempt to synthetically evaluate their neuroimmunomodulatory status. Finally, the inhibition of FAAH synthesis and activity could represent a new possible approach in the bio-immunotherapy of human tumors.

Introduction

It is known that the endocannabinoid system exerts a fundamental role in the regulation of most biological functions by inducing metabolic, immunomodulatory and psychochemical effects, and in particular it has been shown that brain cannabinoid system plays an essential role in both pleasure perception and pain control [1]. Moreover, the recent discoveries in the area of the Psycho-neuro-endocrino-immunology (PNEI) have demonstrated that immune system-mediated systemic human diseases, including cancer and autoimmunity, would be due to an altered neuroendocrine regulation of the immune responses rather than to a primary alteration of immune cell functions themselves [2]. Despite its great complexity, the psycho-neuroendocrine regulation of the immunity has appeared to mainly depend on two major brain interneuronal systems, consisting of the cannabinoid [1] and the opioid system [3,4]. There are three essential opioid receptors, mu-, delta- and kappa receptors, and two main cannabinoid receptors, CB1 and CB2. The psychedelic psychotropic effect of cannabinoids is mediated by the activation of the CB1 receptor, whereas CB2 receptor, which is namely expressed by the immune cells, is involved in the modulatory effects of cannabinoids on the immuno-inflammatory biological response [1]. The two main endocannabinoids are represented by the arachidonyl-ethanol-amide (AEA), the so-called anandamide, and the 2-arachidonyl-glycerol (2-AG) [1].The main enzyme involved in cannabinoid degradation is the fatty acid amide hydrolase (FAAH) [1]. The importance of the neuroimmunomodulatory processes exerted by brain opioid and cannabinoid systems is confirmed by the evidence of a possible enhanced or diminished activity of both brain opioid and cannabinoid systems in the pathogenesis of cancer, as well as other systemic immune-mediated diseases, including autoimmunopathologies and cardiovascular diseases. In particular, it has been shown that stress-induced promoting effect on tumor development has appeared to be mediated by an enhanced opioid system activity, since the administration of mu-receptor opioidantagonists may abrogate the influence of stress on tumor progression [4]. Moreover, it is known that the progressive lack of pleasure perception, the so-called anaedonia, represents one of the main and most frequent cancer progression-related symptoms. Then, because of the fundamental role of endocannabinoids in the perception of pleasure [1], the evidence of cancer-related anaedonia would suggest that tumor diffusion may be characterized by a progressive failure of brain cannabinoid function, with consequent alterations in immune system function. On the contrary, other diseases, such as the acute schizofrenia, has been proven to be associated with an enhanced brain cannabinoid system activation [5]. Obviously, before analyzing the modulatory effects of the endocannabinoid system and most in general of the neuroendocrine system, it has to be synthetically considered the functionless of the immune system and the cytokine network, since the influence of the neuroendocrine system on the immunity is mainly mediated by its influence on the cytokine network.

Correspondence to: Paolo Lissoni, Pharm.D, Institute of Biological Medicine, Milan, Italy, E-mail: paolo.lissoni@gmx.com

Key words: *anticancer immunity, brain cannabinoid system, cannabinoids, Fatty acid amide hydrolase (FAAH), neuroimmunomodulation, pinealgland*

Received: October 22, 2018; **Accepted:** October 29, 2018; **Published:** October 31, 2018

The functional structure of the immune system and the anticancer immunity

Despite its great complexity, the immune system is mainly constituted by two major cell systems, the old or innate immunity, mainly mediated by the granulo-monocyte system and NK cells, and the new or aquired immunity, mainly mediated by the lymphocyte system. The immune status is the end results of two major dynamics, represented by the immunostimulation and the immunosuppression, which are respectively mainly exerted by the lymphocyte and the macrophage systems. Within the lymphocyte system, the only regulatory T lymphocytes (T reg) (CD4+CD25+) exert an immunosuppressive anti-inflammatory action, and their generation has appeared to be under a macrophage stimulatory regulation. The connection between innate and acquired immunity is namely represented by the dendritic cells (DC), mainly through the release of IL-12 [6], which would represent the main link between old and new immunity, then between macrophage and lymphocyte systems, whose interactions are responsible for the overall types of immune response. The immune response is mainly activated by T helper-1 (TH1) (CD4+) lymphocytes through the release of IL-2 and gamma-IFN. TH1-induced immune activation allows two different types of cytotoxic response, antigen-dependent and antigen-independent cytotoxicity, respectively mediated by cytotoxic T lymphocytes (CD8+) after IL-12 stimulation and NK cells after their IL-2-induced evolution into LAK cells [7]. Cancer would be characterized by a decline in TH1 and DC count and activity in association with an enhanced macrophage and T reg cell function. On the other side, the autoimmune diseases are characterized by a decline in T reg cell system activity, with following low levels of IL-10 and TGF-beta, in association with an enhanced activation of TH17 lymphocytes (CD4+CD17+) and a consequent enhanced secretion of IL-17, which would constitute the main inflammatory cytokine involved in the pathogenesis of the autoimmune disorders. From a PNEI point of view, the cytokine alterations occurring in cancer, autoimmunity, cardiovascular diseases, and neurodegenerative pathologies could be due at least at the beginning of the pathology to an altered neuroendocrine control of cytokine network itself [2]. Then, the cytokine network could be influenced by acting on its psychoneuroendocrine regulation, which is mainly exerted by the opioid and cannabinoid systems, rather than to directly act on the various cytokine secretions. IL-12, in addition to its importance in the relations between innate and acquired immunity, would also play an essential role in the interactions between cannabinoid system and immunity [8], since IL-12 has been proven to inhibit FAAH activity, with a consequent increase in cannabinoid endogenous content, whereas IL-10 may stimulate FAAH, with a consequent diminished cannabinoid concentration. Then, the immune system may modulate the function of brain cannabinoid system by simply influencing FAAH synthesis and activity, by enhancing the activity of the cannabinoid system through the release of IL-12, and by decreasing its function through that of IL-10, respectively responsible for the immunoactivation or the immunosuppression. The secretion of IL-10, which exerts an anti-inflammatory immunosuppressive activity [2], is stimulated by the opioid system [3], which is active in stress, pain and depressive conditions, whereas the cannabinoid system is involved in pleasure and spiritual sensitivity conditions [1]. Then, these evidences would explain the immunosuppressive effects of stress and the immunostimulatory one of the pleasure, and the spiritual expansion of mind.

The neuroendocrine regulation of the immune system

The central nervous and the neuroendocrine systems influence the immune system by acting on the cytokine network and modulating cytokine secretions [1-5]. The neuroimmunomodulation is namely exerted by the two major brain interneuronal systems, represented by the opioid and cannabinoid systems. The mu-opioid agonists, such as morphine and beta-endorphin, play an immunosuppressive activity [3,4] by stimulating the secretion of IL-10 and TGF-beta, which inhibit the antitumor immunity [9], as well that of IL-17 [10], and by inhibiting that of the two main anticancer cytokines in humans, IL-2 and IL-12. More controversial are the immunomodulating effects of delta-and kappa-opioid agonists. On the same way, contradictory results have been reported about the immune effects of cannabinoids, since both stimulatory and inhibitory effects on IL-2 , IL-12, TGF-beta and IL-10 secretions have been observed, whereas most studies have confirmed the inhibitory action of cannabinoids on TNF-alpha and IL-17 secretions, which would explain their anti-cachectic and anti-inflammatory activities, respectively. The controversial results concerning the immune effects of cannabinoids would depend by the fact that they are mediated by the interactions between brain cannabinoid system and pineal gland [11], whose essential role in the modulation of the immune system has been well proven.

Clinical applications of the knowledge of the cannabinoid system

Clinical investigation of the endocannabinoid system

The endocannabinoid system may be clinically investigated by measuring the blood and liquoral concentrations of the two major endocannabinoid agents, consisting of AEA and 2-AG, or probably in a more simple and synthetic manner by determining the blood concentrations of the enzyme responsible for cannabinoid degradation and metabolism, the FAAH, whose enhanced production may allow a decline in the endogenous cannabinoid content [1]. On the contrary, a diminished synthesis of FAAH would allow an increased cannabinod system activation. The acute phase of schizophrenia would be associated with an enhanced cannabinoid activity, as confirmed by the evidence of abnormally high levels of AEA in association with low concentrations of FAAH [5]. Because of the well demonstrated anticancer properties of cannabinoid agonists [1], due to several mechanisms, including direct anti-proliferative cytotoxic effect, anti-angiogenic activity, and inhibitory action on macrophage-mediated immunosuppressive inflammatory events, the evidence of a schizophrenia-related cannabinoid system hyperactivation could explain the low frequency of cancer in schizophrenic patients [5]. On the contrary, an enhanced production of FAAH, with a consequent decline in cannabinoid system activity, could constitute a risk factor for cancer onset and development, because of the fundamental role of the endocannabinoid system in the natural biological resistance against cancer in the optimal psycho-neuroendocrino-immune status of health [1,11]. In fact, it has been demonstrated that tumor expression of FAAH is associated with a more biological malignancy and a poor prognosis in some tumor histotypes, including prostate cancer [12]. Then, the inhibition of FAAH synthesis, with a consequent increase in cannabinoid concentrations, could constitute a new possible biological approach in the treatment of tumors. Moreover, by considering that FAAH activation may induce an enhanced inflammatory response by suppressing the anti-inflammatory action of the endogenous cannabinoids and a consequent enhanced production of inflammatory cytokines, such as IL-1beta, IL-6, TNF-alpha and IL-17, the inhibition of FAAH synthesis and activity could exert therapeutic benefits also in the cure of cardiovascular diseases [13], and neurodegenerative disorders [14], which are also determined at least in part by an enhanced inflammatory response. Psychiatric diseases themselves are

also characterized by the evidence of an enhanced inflammatory status, as suggested by the possible evidence of high levels of inflammatory cytokines, namely IL-6 [15]. By summarizing, FAAH inhibitors or stimulators could be successfully employed in the treatment of human diseases, respectively characterized by an abnormally low or abnormally high function of the endocannabinoid system. As far as the cardiovascular system is concerned, the endocannabinoid system may influence heart and endothelium functions by the simple regulation of FAAH synthesis, whose increase would allow an enhanced production of inflammatory cytokines, which negatively influence both cardiac and nervous activities. Then, from a therapeutic point of view, the inhibition of FAAH activity to enhance brain cannabinoid functionis more important than its eventual stimulation to reduce brain cannabinoid content. Another important enzyme in the control of the cardiovascular system, which is connected to FAAH through several neuroendocrine interactions, is neprilysin (NEP), also called enkephalinase, a zinc-dependent membrane peptidase involved in the metabolism and degradation of several vasoactive peptides, including atrial natriuretic peptide (ANP), endothelin-1 (ET-1), and enkephalins [15]. NEP synthesis and activity may influence the cardiovascular functions by affecting the inflammatory response, whose end-result would depend on its major degradation of molecules, suchas ANP [16] or ET-1 [17], which are provided by anti-inflammatory immunostimulatory or inflammatory and immunosuppressive effects, respectively. The inhibition of NEP activity has appeared to enhance the vasodilator effect of the angiotensin II-type 1 receptor antagonists, such as valsartan, as well as their inhibitory action on ET-1 secretion [17].

Therapeutic implications

At present, the most simple manner to modulate the functionless of the endogenous cannabinoid system, whose alterations have been proven to be involved in cancer, autoimmunity, neuropsychiatric and cardiovascular diseases, is represented by the control of FAAH synthesis and activity. Several FAAH inhibitors have been elaborated [18], and the pineal hormone MLT has appeared to cooperate with FAAH inhibitors to counteract FAAH activity with a following further increase in brain cannabinoid content [19]. On the contrary, leptin, a chemokine produced by the adipocytes provided by a stimulatory effect on inflammatory cytokine production, may stimulate FAAH activity, with a following decline in brain endocannabinoid content, which allows a decline in appetite and food intake [20]. Most of FAAH inhibitors are profen or phenyl-alkyl-sulfonyl-fluoride derivatives, and preliminary clinical studies seem to suggest the potential therapeutic efficacy of FAAH inhibitors in several human diseases, including cancer, depression, and cardiovascular pathologies, all characterized by a chronic enhanced inflammatory status, due at least in part to an endocannabinoid system deficiency. By summarizing, the measurement of FAAH blood concentrations is already sufficient to investigate the inflammatory status of patients, since the evidence of abnormally high levels of FAAH reflects and allows an enhanced inflammatory response because of the decline in the endogenous cannabinoid content and function induced by the high levels of FAAH. Then, it is possible to modulate the inflammatory status of patients by simply acting on FAAH synthesis and activity. Moreover, since the hyperactivation of the inflammatory response may be considered as the common pathological mechanism involved in the main human systemic diseases, including cancer and autoimmune diseases, the control of the inflammatory status by cannabinoids agents provided by anti-inflammatory effects would represent a fundamental point in the treatment of the already now untreatable human systemic pathologies.

Conclusions

It is already known that the cannabinoid agents may play a fundamental role in the treatment of most advanced cancer-related symptoms, including cachexia, anorexia, anaedonia, vomiting and pain. In addition to these therapeutic properties, at present it has to be also taken into consideration the important role of the cannabinoid system in the systemic control of the inflammatory response, and the functional status of the cannabinoid system may be clinically established by the simple determination of FAAH levels, whose concentrations are inversely correlated to those of the endogenous cannabinoids. Then, the determination of FAAH levels would have to be included within the routinary laboratory analyses of cancer patients, since the evidence of high FAH levels has been proven to be associated with an enhanced inflammatory response and with a suppression of the anticancer immunity [2], then with a worse prognosis in terms of both response to therapy and survival time.

References

1. Grotenhermen F (2004) Pharmacology of cannabinoids. *Neuro Endocrinol Lett* 25: 14-23. [Crossref]
2. Lissoni P, Messina G, Tantarelli R, Lissoni A, Tantarelli O, et al. (2017) The psychoneuroimmunotherapy of human immune-mediatedsystemicdiseases, including cancer and autoimmune diseases. *J Mol Oncol Res* 1: 7-13.
3. Manfredi B, Sacerdote P, Bianchi M, Locatelli L, Veljic-Radulovic J, et al. (1993) Evidence for an opioidinhibitorytone on T cellproliferation. *J Neuroimmunol* 44: 43-48. [Crossref]
4. Lewis JW, Shavit Y, Terman GV, Nelson LR, Gale RP, et al. (1983) Apparentinvolvement of opioidpeptides in stress-induced enhancement of tumor growth. *Peptides* 4: 635-638. [Crossref]
5. De Marchi N, De Petrocellis L, Orlando P, Daniele F, Fezza F, et al. (2003) Endocannabinoidsignalling in the blood of patients with schizofrenia. *Lipid Health Dis* 2: 5-8. [Crossref]
6. Banks RE, Patel PM, Selby PJ (1995) Interleukin-12: a new player in cytokine therapy. *Br J Cancer* 71: 655-659. [Crossref]
7. Grimm EA, MazumderA, Zhang HZ, Rosenberg SA (1982) Lymphokine-activated killer cellphenomenon. *J ExpMed* 155: 1823-1841. [Crossref]
8. Maccarone M, Valensise H, Bari M, Lazzarin N, Romanini C, et al. (2001) Progesterone up-regulatesanandamidehydroxilase in human lymphocytes: role of cytokines and implications in fertility. *J Imunol* 166: 7183-7189. [Crossref]
9. Reiss M (1999) TGF-beta and cancer. *Microbes Infect* 1: 1327-1347. [Crossref]
10. Lissoni P, Messina G, Cenay V, Rovelli F, Porro G, et al. (2018) The role of IL-17 secretion in mediartingtyheinfluebnceiof stress n cabncer and otgherhuansystemicduiseases. *MOJ Lymphol Phlebol* 2: 31-34.
11. Lissoni P (1999) The pinealglandas a central regulator of cytokine network. *Neuro Endocrinol Lett* 20: 343-349. [Crossref]
12. Endsley M, Thill R, Choudhry I, Williams CL, Kajdacsy-Balla A, et al. (2008) Expression and function of fatty acid amide hydrolase in prostate cancer. *Int J Cancer* 123: 1318-1326. [Crossref]
13. Godlewski G, Alapafuja SO, Batkai S, Nikas SP, Cinar R, et al. (2010) Inhibitor of fatty acid amide hydrolasenormalizescardiovascularfunction in hypertensionwithoutadverse metabolic effects. *Chem Biol* 17: 1256-1266. [Crossref]
14. Ahn K, Johnson DS, Cravatt BJ (2009) Fatty acid amide hydrolaseas a potential therapeutic target for the treatment of pain and CNS disorders. *Expert Opin Drug Discov* 4: 763-784. [Crossref]
15. Schiering N, D'Arcy A, Villard F, Ramage P, Logel C, et al. (2016) Structure of neprilysin in complex with activemetabolite of sacubitril. *Sci Rep* 6: 27902-27912. [Crossref]
16. De Vito P (2014) Atrialnatriuretic peptide: an holdhormone, or a new cytokine? *Peptides* 58: 108-116. [Crossref]
17. Grant K, Loizidou M, Taylor I (2003) Endothelin-1: a multifunctionalmolecule in cancer. *Br J Cancer* 88: 163-166. [Crossref]
18. Ogawa S, Kunugi H (2015) Inhibitors of fatty acid amide hydrolase and mono-acyl-glycerol-lipase: new targets for future antidepressant therapy. *Curr Neuropharmacol* 13: 760-775. [Crossref]

19. Spadoni G, Bedini A, Furiassi L, Mari M, Mor M, et al. (2018) Identification of bivalentligands with melatoninrecveptoragionist and fattuy acid amide hydrolase (FAAH) inhiitory activity that exhibit ocularhyptensive effect in the rabbit. *J Med Chem* 61: 7902-7916. [Crossref]

20. Balsevich G, Sticht M, Bowles NP, Singh A, Lee TTY, et al. (2018) Role of fatty acid amide hydrolase in the leptin-mediated effects on feeding and energy balance. *Proc Natl Acad Sci USA* 115: 7605-7610. [Crossref]

A phase-2 study of high-dose pineal antitumor hormone melatonin as an adjuvant therapy in triple negative breast cancer.

Paolo Lissoni*, Franco Rovelli, Giusy Messina, Vezika Cenaj, Giorgio Porro, Giuseppe Di Fede

Institute of Biological Medicine, Milan, Italy

Abstract

It is known that triple negative breast cancer (TNBC) is the most prognostically negative mammary tumor, because of its lack of sensitivity to the main growth factors for breast cancer, including estrogens and EGF. However, at least three other hormones would have to be considered, consisting of prolactin (PRL), oxytocin (OT), and the pineal hormone melatonin (MLT). PRL would stimulate TNBC growth, whereas MLT and OT would play an inhibitory action in several tumor histotypes, including TNBC, even though at present only clinical studies with MLT have been performed, by demonstrating that it's in human anticancer activity is a dose-dependent phenomenon. On these bases, a study was planned to evaluate the effects of high-dose MLT chronic administration as an adjuvant therapy on the percent of 3-year progression-free period (PSF) in TNBC after adjuvant chemotherapy. The study included 14 consecutive TNBC patients, who were treated with MLT at 40 mg/day orally in the evening every day without interruption, by comparing the results to those observed in a control group of 16 TNBC patients with comparable clinical characteristics. The 3-year PFS percentage achieved in MLT group was significantly higher than that found in the control group, either in patients with or without node involvement. No MLT-related biological toxicity occurred. On the contrary, most patients referred a mood improvement. These preliminary results justify further randomized study with or without high-dose MLT in TNBC patients, in an attempt to prolong their survival.

Keywords: Breast cancer, Melatonin, Pineal gland, Prolactin, Triple negative breast cancer.

Accepted on December 17, 2018

Introduction

Until few years ago, the endocrine oncological researches have been mainly performed in an attempt to identify possible hormones and growth factors involved in the stimulation of tumor growth, including estrogens for breast cancer and endometrial adenocarcinoma, androgens for prostate cancer, prolactin (PRL) PRL for breast and prostate tumors, and EGF and GH for several tumor histotypes. However, in the last years it has been identified also the existence of endogenous hormones provided by antitumor activity, namely oxytocin (OT) [1] and the pineal hormone melatonin (MLT) [2]. Moreover, it is known that the classical endocrine therapy of breast cancer has no efficacy in triple negative breast cancer (TNBC) because of its lack of sensitivity to hormonal stimulation. On the same way, anti-HER-2 monoclonal antibodies have no therapeutic activity in TNBC [3]. However, it has to be remarked that at least three other hormones would have to be taken into consideration because of their involvement in the control of breast growth, including PRL, OT and the pineal hormone MLT, which have been proven to exert opposite effects, consisting of a stimulatory effect of PRL [4] and an inhibitory action of OT and MLT on breast cancer cell proliferation, including that of TNBC [5]. In more detail, the effects of PRL on TNBC growth are yet controversial, since some authors have also reported an inhibitory action of PRL on TNBC growth [6]. On the same way, the biological and prognostic significance of PRL receptor (PRL-R) expression in TNBC is still unclear, even though most studies have shown that PRL-R expression may be associated with a more biological malignancy [4,6]. Cannabinoid agents have also appeared to inhibit the growth of TNBC expressing cannabinoid receptors [7]. The anticancer effect of OT is still only experimental evidence. On the contrary, all experimental and clinical studies performed up to now have constantly demonstrated the inhibitory activity of the pineal hormone MLT on several tumor histotypes, including breast tumors, including the TNBC. Moreover, it has been shown that tumor expression of MLT receptor (MT-R) may predict a less malignancy and a more favourable prognosis in terms of both response to therapy and survival times [5], even though the antitumor action of MLT is at least in part independent from MT-R expression [8]. The antitumor mechanisms of MLT are multiple and complex [9,10], and however, they include a direct cytotoxic antiproliferative action, a cell differentiating effect, an anti-angiogenic activity, an immuno stimulatory action on the anticancer immunity, namely consisting of stimulation of TH1 lymphocytes (TH1) and dendritic cells, with a consequent enhanced production of the two main antitumor cytokines in humans, consisting of IL-2 and IL-12, respectively [11,12]. Then, MLT would constitute at present the only natural molecule potentially able to counteract the overall phases responsible for cancer progression. Moreover, MLT is the only molecule, which has shown no lethal dose, because of the down-regulation of MT-R exerted by the normal cells, whereas tumor cells are unable to modulate MT-R expression,

then there are exposed to the cytotoxic action of MLT in a dose-dependent manner [13]. On these biological bases, as well as by considering the complete lack of toxicity by MLT, an experimental clinical study was performed in an attempt to evaluate the influence of an adjuvant endocrine therapy with high-dose MLT on 3-year progression-free survival (PFS) in a group of non-metastatic TNBC women after the classical adjuvant chemotherapy.

Patients and Methods

The phase-2 study included 14 consecutive non-metastatic TNBC women (median age 53 years, range 28-68). Eligibility criteria were, as follows: histologically proven TNBC other than the apocrine tumor, measurable lesions, no metastatic location, no double tumor, and previous adjuvant chemotherapy. The experimental protocol after approvation of the Ethical Committee was explained to each patient, and written consent was obtained. Depending on the different oncological Institutions, the adjuvant chemotherapy was consisted of carboplatin plus gemcitabine in 8, carboplatin plus taxolin 4, and 5-fluorouracil, epirubicin and cyclophosphamide in the remaining 2 patients. MLT was given orally at a dose of 40 mg/day during the dark period of the day according to its physiological light/dark circadian rhythm [6]. If we consider that the physiological daily endogenous production of MLT is less than 2 mg, a dosage of 40 mg/day may be retained as a mild pharmacological schedule. MLT was administered every day without interruption until disease recurrence. Patients were monitored for a minimum follow of 3 years. The results were compared with those observed in a control group of 16 non-metastatic TNBC women, who had also received the adjuvant chemotherapy. Data were statistically analyzed by the chi-square test. Moreover, the PFS curves were calculated according to Kaplan Meir method, and analyzed by the log-rank method.

Result

Table 1 shows the clinical characteristics of TNBC women and the 3-year PFS percentage in MLT group and in controls. The two groups of patients were well comparable for the main biological characteristics, including age, menopause status, node involvement and type of adjuvant chemotherapy. The 3-year percentage of PFS achieved in MLT group was significantly higher than that found in the control group, who did not received MLT (10/14 (71%) *vs.* 6/16 (37%), $P<0.05$). The percentage of relapse found in MLT group was significantly lower than that occurring in the control group (4/14 (29%) *vs.* 10/16 (63%), $P<0.05$). The percentage of recurrence was lower in MLT group than in controls also in relation to node involvement (node involvement: 1/6 (17%) *vs.* 3/7 (43%); node involvement: 3/8 (38%) *vs.* 7/9 (78%), $P<0.05$). On the contrary, no significant difference occurred between visceral and non-visceral sites of relapse (visceral recurrence: 3/4 (75%) *vs.* 7/10 (70%). However, the percentage of brain recurrence observed in MLT group was lower than that found in controls (1/14 (7%) *vs.* 3/16 (19%), even though the difference was not statistically significant. Finally the 3-year PFS achieved in MLT group was significantly longer that that found in the control groups ($P<0.05$). No MLT-related toxicity occurred. On the contrary, most patients referred a mood improvement and a more regular sleep quality.

Table 1. *Clinical characteristics of TNBC patients and 3-year progression-free survival (PFS) in MLT group and in controls.*

Characteristics	MLT Group(n=14)	Control Group (n=16)
Median age (years)	53 (28-68)	55 (34-70)
Node involvement	8/14 (57%)	9/16 (56%)
Adjuvant chemotherapy Carboplatin-Gemcitabine Carboplatintaxol FEC	8 4 2	8 5 3
Recurrence ratio Sites of relapse Node Bone Lung Liver Brain	4/14 (29%) ---------- 1 0 1 1 1	10/16 (63%) *P<0.05 ---------- 2 1 1 3 3

Discussion

The results of this preliminary study would seem to *in vivo* confirm the antitumor properties of the pineal hormone MLT also against the TNBC, as suggested by the lower percentage of recurrence in patients chronically treated by MLT as a potential endocrine adjuvant therapy of TNBC. Obviously, further studies in a greater number of patients and with a longer follow up period will be required to confirm the potential efficacy of MLT as an adjuvant endocrine therapy of TNBC. In any case, by also considering the complete lack of MLT toxicity, the results of this study would be already sufficiently promising to justify a randomized study with or without MLT, either alone or in association to the classical adjuvant chemotherapy in the treatment of TNBC women. The typical cancer endocrine therapies on the basis of their action mechanisms are in the reality anti-endocrine treatments, since their action consists of blocking the activity of potential protumoral hormones, such as estrogens for breast cancer and androgens for prostate cancer. On the contrary, the endocrine therapy of MLT, as well that with somatostatin for somatostatin receptor expressing neuroendocrine tumors [14], would represent a direct antiproliferative endocrine therapy of cancer. More predictive clinical information concerning the possible efficacy of MLT as a possible adjuvant endocrine therapy for TNBC may be drawn from the immunochemistry detection of MT-R expression on TNBC cells, since MT-R tumor expression would predict a greater efficacy of MLT itself. Finally, because of the dose-dependency of the antitumor activity of MLT [13], more promising results in reducing the percentage of recurrence in TNBC women could be achieved by a greater dosage of MLT, which has been proven to have no lethal dose [9-12].

References

1. Cassoni P, Sapino A, Papotti M, et al. Oxytocin and oxytocin-analogue F314 inhibit cell proliferation and tumor growth of rat and mouse mammary carcinomas. Int J Cancer. 1996; 66(6): 817-20.
2. Brzezinski A. Melatonin in humans. N Engl J Med. 1997. 336(3): 186-95.
3. Chavez KJ, Garimella SV, Lipkowitz S. Triple negative breast cancer cell lines: one tool in the search for better treatment of triple negative breast cancer. Breast Dis. 2010; 32(1-2): 35-48.
4. Clevenger CV, Furth PA, Hankinson SE, et al. The role of prolactin in mammary carcinoma. Endocr Rev. 2003; 24(1): 1-27.
5. Jablonska K, Pula B, Zemla A, et al. Expression of melatonin receptor MT1 in cells of human invasive ductal breast carcinoma. J Pineal Res. 2013; 54(3): 334-45.
6. Lopez-Ozuna VM, Hachim IY, Hachim MY, et al. Prolactin pro-differentiation pathway in triple negative breast cancer: Impact on prognosis and potential therapy. Sci Rep. 2016; 6: 30934.
7. Morales P, Blasco-Benito S, Andradas C, et al. Selective, nontoxic CB(2) cannabinoid o-quinone with in vivo activity against triple-negative breast cancer. J Med Chem. 2015; 58(5): 2256-64.
8. Reiter RJ. Mechanisms of cancer inhibition by melatonin. J Pineal Res. 2004; 37(3): 213-4.
9. Buswell RS. The pineal and neoplasia. Lancet. 1975; 1(7897): 34-5.
10. Regelson W, Pierpaoli W. Melatonin: a rediscovered antitumor hormone? Its relation to surface receptors; sex steroid metabolism, immunologic response, and chronobiologic factors in tumor growth and therapy. Cancer Invest. 1987; 5(4): 379-85.
11. Maestroni GJ. The immunoneuroendocrine role of melatonin. J Pineal Res. (1993); 14(1): 1-10.
12. Lissoni P. The pineal gland as a central regulator of cytokine network. Neuro Endocrinol Lett. 1999; 20(6): 343-9.
13. Lissoni P, Messina G, Rovelli F, et al. Dose-dependency of antitumor effects of the pineal hormone melatonin in untreatable metastatic solid tumor patients. Int J Immunol Immunobiol. 2018; 1(1): 104-6.
14. He Y. The antiproliferative effects of somatostatin receptor subtype 2 in breast cancer cells. Acta Pharmacol Sin. 2009; 30(7): 1053-9.

*Correspondence to:

Paolo Lissoni

Institute of Biological Medicine,

Milan, Italy.

E-mail: paolo.lissoni@gmx.com

Open Access
Volume 1 Issue 1
Review Article

Journal of Medical and Clinical Studies

PUBTEXTO

The Antitumor Endocrine Molecules of Human Body

Lissoni P*, Cusmai R, Messina G, Porro G, Brivio F, Pelizzoni F, Monzon A, Roselli MG and Di Fede G
Department Institute of Biological Medicine, Milan, Italy

Article Info

Article History:
Received: 21 November, 2018
Accepted: 22 December, 2018
Published: 28 December, 2018

Corresponding author: Paolo Lissoni, Institute of Biological Medicine, Milan, Italy;
E-mail: paolo.lissoni@gmx.com

Abstract

Several potential antitumor plants have been proposed and employed in the complementary medicine of tumors, but paradoxically only few attemption has been spent to investigate possible anticancer endocrine-like molecules within human body itself. In fact, at least ten antitumor hormones, provided by antiproliferative, antiangiogenic and immune-modulating effects, have been already identified up to now within the human body, including the pineal hormones melatonin [MLT], 5-methoxytryptamine and pinealine, the neurohypophyseal hormone oxytocin, the endocannabinoids anandamide and 2-arachidonyl-glycerol, the thymic hormones thymosin-alpha 1 and thymulin, somatostatin and the cardiac hormone atrial natriuretic peptide. At present, the only sufficiently investigated hormone from a clinical point of view is the pineal indole MLT, which has appeared to be effective in the treatment of several cancer-related symptoms, namely piastrinopenia, cachexia, mood disorder and asthenia's and to prolong the survival time in patients with disseminated cancer and life expectancy less than 1 year. Therefore, at least MLT would have to be included within the commonly used drugs in the medical Oncology.

Keywords: Anticancer agents; Anticancer resistance; Atrial natriuretic peptide; Beta-Carbolines; Cannabinoids; Metatonin; Oxytocin; Pineal indoles

Introduction

It is known that most antitumor molecules commonly used in the treatment of human neoplasms are drawn from plants and mushrooms, or chemically synthetized, whereas the human body has been namely investigated up to now to identify possible endogenous tumor growth factors and hormones provided by potential pro-tumoral activity rather than the antitumor ones, such as estrogens for breast cancer, androgens for prostate cancer, beta-endorphin for brain tumors [1], FSH for ovarian and endometrial tumors [2,3], PRL for breast and prostate carcinomas [4], and GH and IGF-1 [5] for several tumor histotypes. On the contrary, only few attemption has been spent to identify possible antitumor endogenous endocrine hormones, which could be involved in maintaining the natural anticancer resistance of the human body, and to clinically evaluate their potential efficacy in the therapy in human tumors. However, even though their clinical application is still at the beginning and very few clinical studies are available, at present at least ten endogenous anticancer endocrine molecules, whose action mechanisms have been already well documented in a great number of experimental studies, have been identified, including the pineal indoles melatonin [MLT] [6], corresponding to the N-acetyl-5-methoxytryptamine, andt he 5-methoxytryptamine [5-MTT] [7], the pineal beta-carboline pinealine [PNL] [8], the neurohypophyseal hormone oxytocin [OT] [9, 10], the endogenous cannabinoids arachidonyl-ethanol-amide [AEA], the so-called anandamide, and 2-arachidonyl-glycerol [2-AG] [11], the cardiac hormone atrial natriuretic peptide [ANP] [12], the thymic hormones thymosin-alpha 1 and thymulin [13], as well as somatostatin, at least for somatostatin receptor expressing neuroendocrine tumors [14]. Within the group of the endogenous molecules potentially provided by antitumor activity, in addition to the endocrine-like or neuro active molecules, cytokines have also to be considered, and at present the most important anticancer cytokines in humans are represented by IL-2 [15] and IL-12 [16], because of their capacity of activating the antigen-independent and the antigen-dependent anticancer cytotoxicity, respectively. Then, the human body may potentially control tumor development and growth through either the immune response, namely by the secretion of IL-2 and IL-12, or the production of endocrine molecules capable of exerting cytotoxic or antiproliferative activity, namely through the pineal gland [17-20], which represents the main source of anticancer hormones of the human body, and which constitutes with brain endocannabinoid system a fundamental functional axis involved in the perception of pleasure and in the spiritual expansion of mind [11, 21]. Moreover, it has to be remarked that cytokine network and neuroendocrine system are linked by complex and reciprocal interactions, which influence the *in vivo* immune functional status. Therefore, the natural anticancer resistance of the human body substantially would depend on the functionless of the psycho-neuro-endocrino-immune [PNEI] system [22,23], that may be considered as the synthesis among immune function, neuroendocrine activity and psycho-spiritual life.

The Neuroendocrine Control of the Antitumor Immunity

The influence of the psychological life, consisting of all possible emotions and sexual fancies, on the immune system would be mainly mediated by brain opioid system, namely through the mu-opioid receptor. In fact, brain opioid system has been proven to be involved in most cancer-promoting conditions, including stress and depression [24]. On the contrary, brain endo cannabinoid system with its connections with the pineal gland plays a fundamental role in mediating the perception of pleasure and the spiritual sensitivity [11]. Brain opioid hyper activation may predispose to cancer by both a direct stimulation of cancer cell proliferation, and a suppression of the anticancer immunity through an inhibition of IL-2 and IL-12 secretion and a stimulation of immunosuppressive cytokines, namely IL-10 and TGF-beta [25]. On the contrary, the pineal-cannabinoid system axis may play an anticancer role by stimulating IL-2 and IL-12 production, with a following activation of the antitumor immunity, and inhibiting cancer cell proliferation [11,21], since either the pineal hormone MLT, or the cannabinoid agents have been proven to inhibit cancer growth through a direct cytotoxic effect by inducing the apoptotic process by acting on a specific cell receptor, consisting of MLT receptor [MT-R] for MLT and cannabinoid receptor [CB-R] for the cannabinoid agonists. In contrast to the anticancer role of the pineal gland, which represents the main anticancer organ in the human body [17-20], it is known since many years that the hypophysis gland plays a major tumor promoting effect, since PRL may be a tumor growth factor at least for mammary and prostate carcinomas [4], FSH and LH may indirectly promoting breast and prostate tumors by stimulating the production of sexual hormones, and at least FSH could be a direct tumor growth factor for gynecologic neoplasms [2,3], beta-endorphin may be a growth factor for glioblastoma [1], and finally GH and IGF-1 may be potentially involved in the stimulation of several tumor histotypes through complex interactions with the various endogenous tumor growth factors, namely EGF itself, even though the relation between GH and EGF and other tumor growth factors has still to be better investigated and defined [5]. Finally, from the point of view of its effects on tumor growth, the neurohypophysis with respect to the anticancer activity of the pineal gland and the tumor promoting activity of the adeno hypophysis, the neuro hypophysis may exert both activities, since OT has been proven to exert antiproliferative effects against several tumor histotypes [9,10], whereas the other neurohypophyseal hormone, the vasopressin [ADH], would exert a preferential protumoral activity, namely by situating the angiogenic processes [26]. Moreover, in addition to the opposite preferential influence of pineal-cannabinoid axis and hypophysis-opioid system unity on tumor growth, heart itself may influence tumor growth in an opposite way by modulating both anticancer immunity and cancer cell proliferation through its endocrine activity, by stimulating the anticancer immunity [27] and inhibit cancer cell proliferation by the secretion of ANP [12], or in an opposite way by that of endothelin-1 [ET-1], which may inhibit the anticancer immunity by counteracting lymphocyte activation, and stimulate tumor growth by acting as a direct tumor growth factor, enhancing the potency of other tumor growth factors, and playing an angiogenic activity, due to a direct stimulation of VEGF secretion, which in turns stimulates ET-1 production, then VEGF and ET-1 are reciprocally linked by a positive feedback circuit [28]. Therefore it is possible to identify into the human body, two opposite ways with respect to tumor onset and growth, a protumoral way, which is linked to the psych emotional life, and which is chemically mediated by brain opioid system, adenohypophysis, ADH and ET-1, and on the other side an antitumor way, which is the expression of both pleasure, including the sexual one, and spirituality, and which is biochemically mediated by brain cannabinoid system, pineal, OT and ANP. ADH and ET-1 are linked by a reciprocal stimulatory action [29], as well the secretion of OT and ANP [30]. Finally, MLT and ANP secretions are connected by a positive feedback mechanism, because of their reciprocal stimulation [31,32]. The evidence of the antitumor activity of the pineal gland, which is connected with both spiritual life and pleasure perception through its relation with brain end cannabinoid system, as well as the evidence, even though in an opposite manner, of the preferential photomural role of the hypophysis, which is involved in the regulation of the biological life in normal and stress conditions, would have to be already sufficient for both Physicians and Tologists to recognize that human body, and most in general the Biology, is structured for both pleasure and spiritual expansion of self-consciousness, whose repression negatively influences the functionless of the physiological natural anticancer resistance, and predispose to tumor development or recurrence. As far as the importance of each single potential anticancer molecule of the human body in the treatment of human tumors is concerned, the promising studies on the anticancer and immune-modulating properties of the thyme hormones have been interrupted since may years ago, then at present no define conclusion may be proposed about the possible use of thyme hormones in the treatment of human tumors. The anticancer properties of somatostatin and its analogues are well known in the clinical oncology. Therefore, the major attempt concerning the natural anticancer agents of the human body has to namely concern the pineal hormones, OT, the endogenous cannabinoid agents, and the cardiac hormone ANP.

The Main Anticancer Agents of Human Body

Most clinical studies are limited to the only pineal indole MLT [33]. MLT is namely produced during the dark period of the day. However, MLT is not the only anticancer hormone produced by the pineal gland, since at least another indole hormone has to be considered for its documented anticancer properties, the 5-MTT, which is mainly produced during the light period of the day. All pineal indoles are originated from tryptophan and serotonin. Moreover, the pineal indoles may be transformed into beta-carbolines, which originate from the condensation of indole-ethyl-amines and aldehydes. Beta-carbolines are provided by both anticancer and psychedelic effects, by playing a fundamental role in self-consciousness processes and in the chemical mediation of the spiritual life. At present, more than 20 beta-carbolines have been identified, the most known of them is the 6-methoxy-1,2,3,4-tetrahydro-beta-carboline, the so-called pinealine [PNL] [8], and the main endogenous source of beta-carbolines is represented by pineal itself. At present, the main mechanisms involved in the control of tumor growth are consisting of possible direct cytotoxic action, induction of cancer cell apoptosis, inhibition of tumor growth factor production or growth factor receptor activation, inhibition of the action of protumoral hormones, inhibition of tumor neo-angiogenesis, induction of an effective anticancer immune reaction, and resolution of cancer-related chronic inflammatory status [34], which is mainly mediated by the macrophage system. In fact, cancer related-chronic inflammatory status has been proven to constitute the main mechanism responsible for suppression of the anticancer immunity, due to the production of immunosuppressive inflammatory cytokines, such as IL-1-beta, IL-6, and TNF-alpha, or to the secretion of anti-inflammatory cytokines, but also provided by immunosuppressive

activity, namely IL-10 and TGF-beta, because of their stimulation of T regulatory [T reg] lymphocyte generation [35], which would represent the main immune cells responsible for cancer-related immunosuppression. By considering these possible anticancer mechanisms of action, it has been demonstrated that the anticancer molecules of the human body, as well as the anticancer principles of the most investigated antitumor plants, including Aloe, Myrrh, Boswellia, Magnolia, Graviola Curcuma and Cannabis, may exert their anticancer activity through several biological mechanisms, which included both antiproliferative cytotoxic and immune stimulatory effects.

The Pineal Hormone Melatonin

MLT is the main natural anticancer molecule clinically investigated in the curative or palliative treatment of human neoplasms [33]. From a palliative point of view, MLT has appeared to be effective in the treatment of cancer-related thrombocytopenia, neoplastic cachexia by inhibiting TNF-alpha secretion, mood depression, anxiety, including the anticipatory vomiting on chemotherapy, asthenia, sleep disorders, and to partially prevent cardio-toxicity and neuro-toxicity of the various chemotherapeutic agents, while no relevant effect has been observed in the treatment of neutropenia, anemia, and alopecia. On the other hand from a curative point of view, MLT would represent up to now the only existing molecule potentially able to counteract the overall biological mechanisms responsible for tumor onset, growth and dissemination, including both spontaneous and chemically-induced malignant transformation, intercellular joint alteration with the following change in intracellular matrix characteristics, which allows the neo-angiogenic process, and abrogation of the mechanisms responsible for cancer-related immunosuppression, including cancer cell Fas-L expression, which is responsible for T cytotoxic lymphocyte apoptosis in the case of its cell contact with the neoplastic cell. The great variety of biological effects played by MLT may be explained on the basis of its capacity of controlling DNA gene expression. Then, the anticancer activity of MLT is due to the overall three main mechanisms involved in the control of tumor growth, including cytotoxic-antiproliferative, anti-antigenic and imuno-stimulatory effects. MLT may exert several immune-modulatory effects [36], but from an oncologic point of view, the most important immune-modulating properties of MLT are represented by the stimulation of IL-2 and IL-12 secretion from T helper-1 [TH1] lymphocytes and dendritic cells, respectively, as well as by counteracting macrophage-mediated immunosuppression. MLT may act in an antitumor way by either acting on specific MT-R expressed by tumor cells, or in independent receptor manner as a free-radical scavenger [37]. Then, with respect to the overall common palliative therapies of cancer, which are limited to the treatment of each single cancer-related symptom, MLT medical therapy may not only contribute to the relief of cancer-related symptoms, but also prolong the survival time of untreatable disseminated cancer patients, in association with an acceptable quality of life, by abrogating the paradoxical separation between cure and palliative therapy of cancer. *In vivo*, MLT has been proven to exert an evident anticancer action only at pharmacological doses and with an administration once day during the dark period of the day, corresponding the daily phase, during which its production is physiologically maximal [38]. In humans, the anticancer efficacy of MLT has appeared to be a dose-dependent phenomenon [39], and at present no dose-limiting toxicity has been observed until at a dosage greater than 500 mg/day. MLT may be administered orally, intramuscularly or intravenously. At present, within the great variety of cancer complementary medicines, the treatment with high-dose MLT, either alone or in association with other pineal in doles and antitumor plants, is the only non-standard medical oncological therapy, which has been proven to prolong the 5-year survival in patients with metastatic cancer, for whom no conventional antitumor therapy was available and with life expectancy less than 1 year [40]. Most human tumors may potentially respond to MLT, since promising results have been observed in melanoma, non-small cell lung cancer, gastric cancer, prostate cancer, triple negative breast cancer, pancreatic adenocarcinoma, sarcoma, brain tumors and brain metastases due to solid tumors. The antitumor properties of MLT are not surprising, since the pineal failure represent the main cancer-related endocrine deficiency [41], and it may involve the whole pinel gland, because of the evidence of pineal histological damage in patients died from cancer [42], even though at present the evidence of abnormally low blood levels has been documented for the only MLT [41]. The possible importance of the deficiency of pineal antitumor hormones other than MLT is also suggested by the evidence that the association of 5-MTT or PNL to MLT may improve the clinical efficacy of MLT itself [43]. By synthetizing, the significance of MLT cancer therapy is justified by either its anticancer properties at pharmacological doses, or as a substitute treatment to correct cancer-related pineal deficiency.

The Pineal Indole 5-methoxytryptamine and the Beta-Carboline Pinealine

Both 5-MTT and PNL may play a direct anticancer anti proliferative activity. *In vitro*, 5-MTT has been proven to exert an anticancer action superior to that of MLT itself [7], while its immune-modulating effects need to be further investigated and defined. The same anti-inflammatory activity of both 5-MTT and PNL needs to be better analyzed. Moreover, at present is still unknown whether 5-MTT and PNL may act on specific receptors, or by modulating MT-R or neurotransmitter and benzodiazepine receptors [BNZ-R], as well as demonstrated for the beta-carboline molecules. Finally, PNL has appeared to exert antidepressant effects by inhibiting MAO activity, but paradoxically in association with anxiety, due to block of BNZ-R [44].

The Endocannabinoid Anandamide and 2-Arachidonyl-Glycerol

The endogenous cannabinoids AEA and 2-AG are synthetized at cell surface levels starting from the arachydonic acid and by the anandamide synthase, and they were catabolized by the fatty acid amide hydrolase [FAAH], whose increased levels are associated with a reduced cannabinoid content [11].The cannabinoid agonists may exert a direct cytotoxic effect, which is mediated by the CB1 receptor, on most cancer cell histo types by inducing the apoptosis or inhibiting growth factor receptor activation. On the contrary, the immune-modulating effects of cannabinoids are namely mediated by the CB2 receptor. The psychdelic effect is only a CB1 receptor-dependent event. In contrast to MLT, whose main target immune cell would be represented by the TH1 lymphocyte, cannabinod immune-inflammatory activity is namely due to the inhibition of macrophage production of inflammatory immunosuppressive cytokines, such as IL-1-beta, IL-6 and TNF-alpha, as well that of IL-17 from TH17 lymphocytes [11]. At present, the only documented efficacy of cannabinoid agents in the care of human tumors is that in brain glioblastoma [45]. In any case, cannabinoids agents hav been proven to be effective at least in the treatment of the following cancer-related symptoms:

anaedonia, neoplastic cachexia, anorexia, vomiting, and pain, including the neuropathic one [11]. No clinical study has been performed up to now with AEA or 2-AG in the treatment of cancer. However, it is probable that their effects may be similar to that of the exogenous cannabinoids.

The Neurohypophyseal Hormone Oxytocin

Until few years ago, OT was considered only for its importance in partum and lactation. OT has also appeared to play an important role in behavioral functions, including affective life, maternal profile and social relationships. Finally, OT may exert antalgic and memory inhibition activities. In addition to these endocrine and psychological effects, OT has appeared to act as a growth regulator and to exert an proliferative effect on several tumor histotypes [9,10,46], including breast cancer, prostate cancer, gynecologic tumors, and brain neoplasms in experimental conditions by acting on a specific OT receptor, whereas it could potentially stimulate the growth of trophoblast-derived tumors. OT may also exert anti-angiogenic [10] and anti-inflammatory effects, while its influence on the immune system has still to be investigated. Finally, in experimental studies OT has appeared to be effective in preventing chemotherapy-induced neurotoxicity at a dose of about 100 micrograms/kg b.w. [47]. At present, however, no clinical study of OT has been performed in cancer treatment.

The Cardiac Hormone Atrial Natriuretic Peptide

ANP is produced by cardiac myocytes. In addition to its vasodilator, natriuretic and cardiac protective effects, ANP would also play an important anticancer activity, due to several mechanisms[48], including the inhibition of cancer cell proliferation of ANP-receptor expressing tumors and tumor growth factor-induced tumor cell proliferation, the inhibition of angiogenesis by blocking VEGF secretion, and the stimulation of the anticancer immunity by counteracting cancer-related chronic inflammation and activating T lymphocyte system. Unfortunately, no clinical study of ANP in cancer cure has been performed. In any case, because of its short half-life, long acting ANP analogues will be required in the clinical practices.

The Anti-Mullerian Hormone

Within the endogenous endocrine-like molecules potentially provided by anticancer activity, the anti-mullerian hormone [AMH] would have also to be included, a glycoprotein of TGF-beta family. AMH is produced by fetal Sertoli cells, and it is responsible for the regression of Mullerian ducts in males, with the following inhibition of the development of uterus and Fallopian tubes. AMH may be also produced by ovarian granulosa cells, and it would play an important role in ovarian folliculogenesis. From a clinical point of view [49], the evidence of abnormally low blood levels of AMH is the more precocious and adequate marker of menopausal status with respect to FSH itself. AMH decrease also during pregnancy. On the other side, high levels of AMH have been reported in polycystic ovarian syndrome, and namely in granulosa cell ovarian tumors. AMH has also appeared to inhibit aromatase activity, then, because of its importance in influencing testosteron metabolism, AMH could influence the sexual differentiation and identity also during the adult life. Preliminary studies would also suggest an antiproliferative activity of AMH, namely in endometrial cancer and other gynecologic tumors, by inhibiting epidermal growth factor-induced cancer cell proliferation [50].

Conclusion

The well documented existence of several natural anticancer nontoxic molecules, either in the human body, or from plants, would have to interrupt the separation between palliative and curative therapies of human neoplasms, since most natural anticancer agents may exert both palliative and curative effects by counteracting tumor growth, with a consequent improvement not only in cancer-related symptomatology and quality of life, but also in the survival time. In particular, on the basis of the great number of experimental and clinical studies confirming its anticancer properties, the refusal of MLT use in the clinical therapy of cancer patients would have to be considered as an incredible medical failure of the medical sciences.

References

1. Westphal M, Li CH. Beta-endoprphin: characterization of binding sites specific for the human hormone in human glioblastoma SF 126 cells. Proc Natl Acad Sci. 1984; 81: 2931-33.
2. Zheng W, Lu JJ, Luo F, Zheng Y, Feng YJ, Felix LC, et al. Ovarian epithelial tumor growth promotion by follicle-stimulating hormone ad inhibition of the effect by luteinizing hormone. Gynecol Oncol. 2000; 76: 80-8.
3. Huang Y, Hua K, Zhou X, Jin H, Chen X, Lu X, et al. Activation of the PI3K/AKT pathway mediates FSH-stimulated VEGF expression in ovarian serous cystoadenocarcinoma. Cell Res. 2008; 18: 780-91.
4. Welsch CW, Nagasawa H. Prolactin and murine tumorigenesis: a review. Cancer Res. 1997; 17: 951-63.
5. Ben-Shlomo A, Melmed S. Growth hormone excess and cancer. J Antiaging Med. 2001;4: 301-9.
6. Brzezinski A. Melatonin in humans. N Engl J Med. 1997; 336: 186-95.
7. Sze SF, Ng TB, Liu WK, Antiproliferative effects of pineal indoles on cultured tumor cell lines. J Pineal Res. 1993; 14: 27-3.
8. Airaksinen MM, Kari I. Beta-carbolines, psychoactive compounds in the mammalian body. Med Biol. 1981; 59: 190-11.
9. Cassoni P, Marrocco T, Deaglio S, Sapino A, Bussolati G. Biological relevance of oxytocin and oxytocin receptors incancer cells and primary tumors. Ann Oncol. 2001; 12: S37-S39.
10. Ji H, Liu N, Yin Y, Wang X, Chen X, Li J, et al. Oxytocin inhibits ovarian cancer metastases by repressing the expression of MMP-2 and VEGF. J Cancer. 2018; 9: 1379-84.
11. Grotenhermen F. Pharmacology of cannabinoids. Neuroendocrinol Lett. 2004; 25: 14-23.
12. De Vito P. Atrial natriuretic peptide: A hold hormone, or a new cytokine? Peptids. 2014; 58: 108-16.
13. McClure JE, Lameris N, Wara DW, Goldstein AL. Immunochemical studies on thymosin radioimmunoassay of thymosin alpha-1. J Immunol. 1982; 128: 368-71.
14. Maton PN, Gardner JD, Densen RT. Use of long-acting somatostatin analog SMS 201-995 in patients with pancreatic islet cell tumors. Dig Dis Sci. 1989; 34: 28-39.
15. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA. Lymphokine-activated killer cell phenomenon. J Exp Med. 1982; 155: 1823-41.

16. Banks R, Patel RM, Selby PJ. Interleukin-12: a new clinicalplayer in cytokine therapy. Br J Cancer. 1995; 71: 655-59.
17. Buswell RS. The pineal and neoplasia. Lancet. 1975; 1: 34-5.
18. Regelson W, Pierpaoli W. Melatonin: a rediscovered antitumor hormone? Cancer Invest. 1987; 5: 379-5.
19. Russel RJ. Mechanisms of cancer inhibition by melatonin. J Pineal Res. 2004; 37: 213-14.
20. Danielczyk K, Dzjgiel P. MT 1 melatonin receptors and their role in the oncostatic action of melatonin. Postepy Hig Med Dosw. 2009; 63: 425-34.
21. Lissoni P, Resentini M, Muri R, Esposti D, Esposti G, Rossi D, et al. Effects of tetrahydrocannabinol on melatonin secretion in man. Horm Metab Res. 1986; 18: 77-8.
22. Riley V. Psychoneuroendocrine influences on immunocompetemnce and neoplasia. Science. 1981; 212: 1100-1109.
23. Antony MH. Psychoneuroimmmunology of cancer. Brain Behav Immunol. 2003; 17: 84-91.
24. Lewis JW, Shavit Y, Terman GW, Nelson IR, Gale RP, Liebeskind JC. Apparent involvement of opioid peptides in stress-iduced enhancemenrt of tumor growth. Peptides. 1983; 4: 635-38.
25. Reiss M. TGF-beta and cancer. Microbes Infect. 1999; 1: 1327-47.
26. Alonso G. Vasopressin and angiogenesis. J Soc Biol. 2009; 203: 39-7.
27. Lissoni P, Pittalis S, Vigoré L, Rovelli F, Vezzo R, Bramati S, et al. The heart as an immunomodulator organ: in vitro immune effects of the cardiac hormone atrial natriuretic peptide-alpha and their possible relevance in cardiac failure and aging. Cardiol Eld. 1993; 1: 227-31.
28. Grant K, loizidou M, Taylor I. Endothelin-1: a multifunctional molecule in cancer. Br J Cancer. 2003; 88: 163-6.
29. Yamamoto T, Kimura T, Ota K, Shoji M, Inoue M, Sato K, et al. Central effects of endothelin-1 on vaospresin release, blood pressure, and renal solute excretion. Am J Physiol. 1992; 262: 856-62.
30. Evrard A, Hober C, Racadat A, Levefre J, Wantyghem MC. Atrial natriuretic hormone and endocrine functions. Ann Biol Clin. 1999; 5: 149-55.
31. Lissoni P, Pelizzoni F, Grugni G, Guzzaloni G, Mauri R, Archili O, et al. Melatonin response to atrial natriuretic peptide adminIstration in healthy volunteers. J Cardiol Pharmacol. 1990; 16: 850-52.
32. Sallinen P, Manttari S, Leskinen H, Vakkuri O, Ruskoaho H, Saarela S. long-term pmelatonin administration alters the expression of DHPR, SERCA 2, and MT2, and elevates ANP levels in the rat left ventricle. J Pineal Res. 2008; 45: 61-9.
33. Millis E, Wu P, Seely D, Guyatt G. Melatonin in the treatment of cancer: a systematic review of randomized controlled trials and meta-analysis. J Pineal Res. 2005; 39: 360-66.
34. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008; 454: 436-44.
35. Lissoni P, Messina G, Rovelli F, Vigoré L, Lissoni A, et al. Low lymphocyte-to-monocyte ratio is associated with an enhanced regulatory T lymphocyte function in metastatic cancer patients. Int J Rec Adv Multi Res. 2018; 5: 3353-56.
36. Maestroni JGM. The immunoneuroendocrine role of melatonin. J Pineal Res. 1993; 14: 1-10.
37. Reiter RJ, Tan DX, Sainz RM, Mayo JC, Lopez-Burillo S. Melatonin: reducing the toxicity and increasing the efficacy of drugs. J Pharm Pharmacol. 2002; 54: 1299-21.
38. Bartsch H, Bartsch C. Effect of melatonin on experimental tumors under different photoperiods and times of administration. J Neural Transm. 1981; 52: 269-79.
39. Lissoni P, Messina G, Rovelli F, Brivio F, Di Fede G. Dose-dependency of antitumor effects of the pineal hormone melatonin in untreatable metastatic solid tumor patients. Int J Immunol Immunobiol. 2018; 1: 1-3.
40. Lissoni P, Rovelli F, Brivio F, Messina G, Lissoni A, Pensato S, et al. Five-year survival with high-dose melatonin and other antitumor pineal hormones in advanced cancver patients eligible for the only palliative therapy. Res J Oncol. 2018; 2: 1-7.
41. Bartsch C, Bartsch H, Lippert TH. The pineal gland and cancer: facts, hypotheses and perspectives. Cancer J. 1992; 5: 194-99.
42. Hadiju SI, Porro RS, Lieberman PH. Degeneration of the pineal gland of patients with cancer. Cancer. 1972; 29: 706-9.
43. Di Fede G, Messina G, Monzon A, Meli O, Gavazzeni C, Rovelli F, et al. Clinical effects of the pineal antitumor and psychedelic beta-carboline pinealine in the palliative therapy of untreatable metastatic cancer patients. Integr Canc Biol Res. 2017; 1: 1-3.
44. Cain M, Weber RW, Guzman F, Cook JM, Barker SA, Rice KC, et al. Beta-carbolines: synthesis and neurochemical and pharmacological actions on brain benzodiazepine receptors. J Med Chem. 1982; 25: 1081-91.
45. Lissoni P, Messina G, Porro G, Porta E, Nosetto L, Mancuso M, et al. A psycho-neuro-endocrino-immune (PNEI) approach to enhance the efficacy of radiochemotherapy in glioblastoma. JJ Rad Oncol. 2016; 3: 29-32.
46. Imanieh MH, Bagheri F, Alizadeh AM, Ashkani-Esfahani S. Oxytocin has therapeutic effects on cancer, a hypothesis. Eur J Pharmacol. 2015; 741: 112-23.
47. Akman T, Akman L, Erbas O, Terek MC, Taskiran D, Ozsaran A. The preventive effect of oxytocin to cisplatin-induced neurotoxicity: an experimental rat mode. Biomed Res Int. 2015; 167-235.
48. Kong X, Wang X, Xu W, Behera S, Hellermann G, Kumar A, et al. Natriuretic peptide receptor A as a novel anticancer target. Cancer Res 68: 249-256, 2008.
49. Ozzola G. Anti-mullerian hormone: a brief review of the literature. Clin Ther. 2017; 168: 14-22.
50. Renaud EJ, MacLaughlin DT, Oliva E, Rueda BR, Donahoe PK. Endometrial cancer is a receptor-mediated target for mullerian inhibiting substance. Proc Natl Acad Sci USA. 2005; 102: 111-116.

Per la tua salute ascolta la natura.

Dal 1987 NATUR
sostiene il benessere dell'uomo
con una gamma completa
di esclusivi integratori
di origine naturale,
all'avanguardia nella concezione,
nella qualità delle sostanze impiegate
e nell'innovativa
tecnologia di produzione.

NATUR®
Scienza e ricerca per coltivare la salute.

Via M. Macchi, 10 - 20124 Milano
Tel. 02 6693950 - Fax 02 6700708
e-mail: info@natur.it
www.natur.it