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Biotherapy with the Pineal Immunomodulating Hormone Melatonin versus Melatonin plus *Aloe vera* in Untreatable Advanced Solid Neoplasms

Key Words
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Biotherapy
Melatonin
Natural cancer therapy
Pineal gland

Abstract
The possibility of natural cancer therapy has been recently suggested by advances in the knowledge of tumor immunobiology. Either cytokines such as IL-2, or neurohormones, such as the pineal indole melatonin (MLT), may activate anticancer immunity. In addition, immunomodulating substances have also been isolated from plants, particularly from *Aloe vera*. Preliminary clinical studies had already shown that MLT may induce some benefits in untreatable metastatic solid tumor patients, whereas, for the time being, no clinical trial has been performed with aloe products. We have carried out a clinical study to evaluate whether the concomitant administration of aloe may enhance the therapeutic results of MLT in patients with advanced solid tumors for whom no effective standard anticancer therapies are available. The study included 50 patients suffering from lung cancer, gastrointestinal tract tumors, breast cancer or brain glioblastoma, who were treated with MLT alone (20 mg/day orally in the dark period) or MLT plus *A. vera* tincture (1 ml twice/day). A partial response (PR) was achieved in 2/24 patients treated with MLT plus aloe and in none of the patients treated with MLT alone. Stable disease (SD) was achieved in 12/24 and in 7/26 patients treated with MLT plus aloe or MLT alone, respectively. Therefore, the percentage of nonprogressing patients (PR + SD) was significantly higher in the group treated with MLT plus aloe than in the MLT gorup (14/24 vs. 7/26, $p < 0.05$). The percent 1-year survival was significantly higher in patients treated with MLT plus aloe (9/24 vs. 4/26, $p < 0.05$). Both treatments were well tolerated. This preliminary study would suggest that natural cancer therapy with MLT plus *A. vera* extracts may produce some therapeutic benefits, at least in terms of stabilization of disease and survival, in patients with advanced solid tumors, for whom no other standard effective therapy is available.

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Introduction

The recent advances in the knowledge of the mechanisms involved in antitumor immunity have demonstrated the existence of several natural substances which may be effective in the activation of host anticancer immune defenses [1, 2]. Immunomodulating molecules may be obtained from either animals or plants. Endogenous immunomodulating substances mainly consist of cytokines and neurohormones [1–6]. Within the cytokine group, IL-2, which is released from T-helper-1 lymphocytes (Th-1), would represent one of the most effective antitumor cytokines in the treatment of human neoplasms [1, 2, 6]. Its antitumor activity may be amplified by IL-12, at least in experimental observations [7]. In contrast, activation of the inflammatory response may suppress IL-2-induced anticancer immune reactions, and this event would be mainly mediated by IL-6, which has been proven to generate the inflammatory status and to inhibit IL-2-induced lymphocyte cytotoxicity [8]. In fact, the evidence of abnormally high blood concentrations of IL-6 has been shown to correlate with resistance to IL-2 in advanced cancer patients [9]. This finding would suggest that the neutralization of the inflammatory status could enhance the anticancer efficacy of IL-2 [1, 2]. In addition, the psychoneuroendocrine system, particularly the pineal gland, so appears to produce several immunomodulating substances [3]. In fact, the pineal hormone melatonin (MLT), the most investigated neurohormone released by the pineal, has been shown to contribute to the generation of an effective anticancer immune response through several mechanisms, including the stimulation of IL-2 production, the inhibition of macrophage-mediated suppressive events and the modulation of an inflammatory status [4, 5].

At the other side, within the exogenous substances, several immunomodulating or anti-inflammatory molecules have been isolated from plants, particularly *Aloe vera* [10], which contains various agents that possess antitumor immune activity. The main immunomodulatory substances of *A. vera* are: glycomannan, acemannan, aloenin, aloesin, barboloin, gibberelin and indole-3-acetic acid [10, 11]. The mechanisms responsible for the immunoactivating activity of *A. vera* need to be better defined. However, they would be mainly mediated by the activation of T-cytotoxic lymphocytes or the suppression of the inflammatory response, with a following enhanced antitumor activity of the endogenous IL-2. In addition, the administration of *A. vera* in various forms has been shown to inhibit cancer growth in experimental conditions, either in vitro or in vivo [12, 13]. The antitumor activity of *A. vera* would be mainly mediated by the immune system [10–13].

Our previous clinical studies with MLT alone in patients with untreatable metastatic solid tumors had already shown the efficacy of MLT, at least as an effective palliative therapy in prolonging survival and improving the performance status (PS), with a stabilization of the neoplastic growth in about 25% of cancer patients progressing on standard anticancer therapies and percent 1-year survival in about 15% of patients with a life expectancy <6 months [14]. In contrast, despite the promising experimental results, no clinical trial of *A. vera* has been performed in human neoplasms. Since aloe-related molecules may suppress the activation of the inflammatory status in advanced neoplastic disease [2, 8, 9], they might be successfully associated with MLT in the treatment of metastatic cancer patients who failed to respond to standard therapies. This proposal is further justified by the fact that no toxicity has been described for both MLT and *A. vera* compounds [10–14],

even though they were administered at high pharmacological doses. The rationale of the MLT-*A. vera* association mainly rests on the possibility to further enhance the antitumor activity of IL-2 released in response to MLT [3–5] by counteracting the inflammatory status with the aloe compounds [10–13]. Therefore, on this basis, we have performed a clinical trial with MLT plus *A. vera* in patients with untreatable advanced solid tumors, either to establish the efficacy of the treatment, or to investigate the possible mechanisms of action in a preliminary way.

Materials and Methods

The study included 50 consecutive patients with locally advanced or metastatic solid tumors, for whom no other effective standard therapy was available, because of lack of response to previous chemotherapies or a poor clinical status precluding chemotherapy. The experimental protocol was explained to each patient, and informed consent was obtained. Eligibility criteria were as follows: histologically proven advanced lung cancer, gastrointestinal tract tumor, breast cancer or brain glioblastoma, measurable neoplastic lesions, progression on classical chemotherapy, radiotherapy or endocrine therapy or poor clinical conditions precluding chemotherapy, no double tumor, no other concomitant immunomodulating therapy, and life expectancy generally <6 months. Patients were eligible for the protocol after at least 1 month from the last chemotherapy course.

After stratification according to tumor histotype, patients were treated with MLT alone or MLT plus *A. vera*. In agreement with its physiological nocturnal secretion and with the experimental studies showing its maximum oncostatic activity when it is given during the dark period of the day [3–5], MLT was administered orally at a dose of 20 mg/day, every day until disease progression. *A. vera* was administered as a tincture (*A. vera* leaves: 10%; 40° alcohol: 90%) at a dose of 1 ml twice/day (morning and evening), every day until disease progression. The dosage of both MLT and *A. vera* was established according to the previous experimental studies [10–14].

The clinical response was evaluated according to WHO criteria, by repeating the radiological examinations at 2-month intervals. The PS was evaluated according to Karnofsky's score. The immunoinflammatory status of patients was preliminarly evaluated by determining the erythrocyte sedimentation rate (ESR) and the serum levels of the macrophage marker neopterin, soluble IL-2 receptor (SIL-2R) and IL-6, either before or after 2 months of treatment.

The results were statistically analyzed by the χ^2 test, Student's t test and an analysis of variance, as appropriate. Moreover, the survival curves were plotted according to the Kaplan-Meier method, and the differences between curves were evaluated by the log-rank test.

Results

As reported in table 1, the two groups of patients treated with MLT or MLT plus *A. vera* were well balanced for the main prognostic variables, including histotype, sites of disease, PS and previous chemotherapies.

No objective tumor regression was achieved in patients treated with MLT alone. In contrast, 2/24 (8%) patients treated with MLT plus *A. vera* had a partial response (PR). The first patient had liver metastases due to biliary tract adenocarcinoma, while the second patient had liver metastases due to breast cancer (duration of response: 19 and 16 months, respectively). Stable disease (SD) was obtained in 12/24 (50%) patients treated with MLT plus *A. vera* and in only 7/26 (27%) patients treated with MLT alone. The remaining 7/24 (42%) and 19/26 (73%) patients treated with MLT plus *A. vera* or MLT alone, respectively, had progressive disease (PD). The percentage of nonprogressing patients (PR + SD) obtained with MLT plus *A. vera* was significantly higher than that found with MLT alone (14/24 vs 7/26, $p < 0.05$). In addition, the percent 1-year survival achieved in patients treated with MLT plus *A. vera* was also significantly higher than that seen in the MLT group (9/24 vs. 4/26, $p < 0.05$). The clinical results are reported in table 2. Moreover,

Table 1. Clinical characteristics of untreatable solid tumor patients receiving melatonin alone or melatonin plus *A. vera* extracts

Characteristic	Melatonin	Melatonin + *A. vera*
n	26	24
M/F	16/10	15/9
Median age, years	61	63
Range, years	46–79	48–80
Median PS (Karnofsky)	50	50
Range, years	30–80	20–80
Tumor histotype		
Non-small cell lung cancer	11	12
Gastrointestinal tract tumors	9	7
Colorectal cancer	4	4
Gastric cancer	2	1
Pancreatic adenocarcinoma	0	1
Hepatocarcinoma	1	0
Biliary tract adenocarcinoma	2	1
Breast cancer	4	3
Brain glioblastoma	2	2
Dominant metastasis sites		
Lung	5	4
Liver	5	6
Lung + liver	3	3
Seruses	2	2
Brain	1	1
Bone	6	4
Soft tissues	2	2
No distant organ metastases	2	2
Previous chemotherapies	21/26	20/24

Table 2. Clinical results achieved in untreatable solid tumor patients receiving melatonin alone or melatonin plus *A. vera* extracts

D	Melatonin	Melatonin + *A. vera*
Clinical response (WHO criteria)		
Partial response	0	2/24 (8)[1]
Stable disease	7/26 (27)[2]	12/24 (50)[3]
Partial response + stable disease	7/26 (27)	14/24 (58)*
Progressive disease	19/26 (73)	7/24 (42)
Percent 1-year survival	4/26 (15)	9/24 (37)*

Figures in parentheses are percentages. * $p < 0.05$ versus melatonin alone.

[1] Biliary tract cancer: 1; breast cancer: 1.

[2] Lung: 4; gastrointestinal tumors: 2; breast cancer: 1.

[3] Lung: 6; gastrointestinal tumors: 4; breast cancer: 1; glioblastoma: 1.

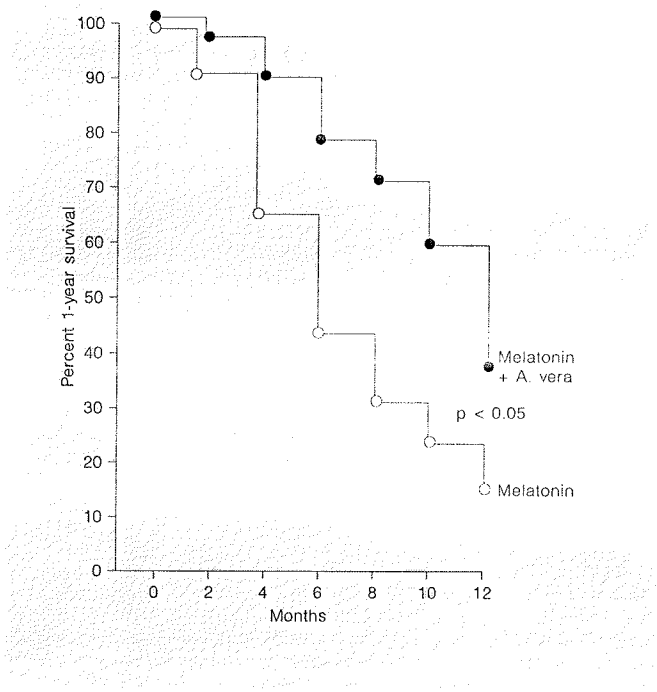

Fig. 1. Survival curves observed in patients with untreatable advanced solid tumors treated with melatonin alone or melatonin plus *A. vera* extracts.

as illustrated in figure 1, the survival curve found in patients treated with MLT plus *A. vera* was significantly longer than in the MLT group ($p < 0.05$). Finally, the percent improvement in PS achieved in patients treated with MLT plus *A. vera* was higher than that obtained in those treated with MLT alone, even though the difference was not statistically significant (11/24 vs. 8/26). In addition, complete resolution of severe disseminated intravascular coagulation (DIC) was obtained in 1 patient treated with MLT plus *A. vera*, whereas no benefit was seen in 2 patients with DIC treated with MLT alone, but only a stabilization of platelet decline.

As far as the immunoinflammatory response are concerned, as shown in table 3, both MLT alone and MLT plus *A. vera* induced a significant decline in mean serum levels of neopterin, SIL-2R and IL-6, even though the decline seen in response to MLT plus *A. vera* was more pronounced than that obtained with MLT alone. Finally, ESR mean values diminished on both MLT or MLT plus *A. vera*, but only the decline obtained with MLT plus *A. vera* was statistically significant with respect to the pretreatment levels.

No MLT-related toxicity occurred. Grade-1 diarrhea occurred in 4/24 (17%) patients treated also with *A. vera*, but it was limited to the first days of therapy.

Table 3. Mean (± SE) values of immunoinflammatory parameters observed before and after 2 months of treatment in patients with advanced solid tumors treated with melatonin or melatonin plus *A. vera*

Patients	ESR, mm/h		Neopterin, ng/ml		SIL-2R, U/ml		IL-6, pg/ml	
	before	after	before	after	before	after	before	after
Melatonin (n = 26)	83±11	61±7	4.3±0.3	3.1±0.2*	2,138±166	1,426±104*	85±7	62±4*
Melatonin + Aloe (n = 24)	78±9	42±6*	4.6±0.4	2.7±0.2**	2,296±159	1,342±118**	81±6	43±5**

* $p < 0.05$ vs. before; ** $p < 0.01$ vs. before.

Normal values (95% confidence limits): ERS, below 20 mm/h; neopterin: below 2.5 ng/ml; SIL-25: below 900 U/ml; IL-6: below 31 pg/ml.

Discussion

Even though limited to a small number of patients affected with different tumor histotypes, this preliminary clinical study would suggest that the immunomodulating and oncostatic activities of the pineal hormone MLT, already shown by both experimental [3–5] and clinical studies [14], may be increased by the concomitant administration of *A. vera*, which may augment the percent of patients with PR or SD. The percentage of patients with SD cannot be classified as clinical response. However, according to the recent criteria of the American Society of Clinical Oncology [15], a prolonged survival time and an improvement in PS may also be considered as an effective result. The mechanisms responsible for the apparent additive action of *A. vera* and MLT need to be better explained. However, according to the preliminary immunobiological results shown by this study, *A. vera* could act at least in part by further enhancing the anti-inflammatory action of MLT [5], with a subsequent potentially increased antitumor efficacy of IL-2 released. Further studies, however, by evaluating changes in IL-2 concentrations on treatment, will be required to better define the impact of MLT and *A. vera* on IL-2-activated anticancer immune response. In any case, even though the immunomodulating mechanisms need to be understood, the biotherapeutic combination of MLT plus *A. vera* might constitute a clear example of natural cancer therapy, which may be recommended also in patients with very advanced untreatable neoplastic disease and poor clinical conditions, because of the absolute lack of toxicity, the low cost and an apparent efficacy at least in terms of survival time and quality of life.

In conclusion, this preliminary study represents the first evidence of a modulatory effect of *A. vera* on the immunoinflammatory response in humans, with an apparent increase in the activity of the pineal hormone MLT.

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