

# Anti-angiogenic activity of melatonin in advanced cancer patients

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## Abstract

**OBJECTIVES:** The anticancer activity of the indole melatonin has been explained to be due to its immunomodulatory, anti-proliferative and anti-oxidant effects, whereas at present no data are available about its possible influence on the angiogenesis, which has been shown to be one of the main biological mechanisms responsible for tumor dissemination. Vascular endothelial growth factor (VEGF) is the most active angiogenic factor, and the evidence of abnormally high blood levels of VEGF has been proven to be associated with poor prognosis in cancer patients. To investigate the influence of melatonin on angiogenesis, in this preliminary study we have evaluated the effects of melatonin therapy on VEGF blood levels in advanced cancer patients.

**MATERIAL & METHODS:** The study included 20 metastatic patients, who progressed on previous conventional antitumor therapies and for whom no other effective treatment was available. Melatonin was given orally at 20 mg/day in the evening for at least 2 months. Serum levels of VEGF were measured by an enzyme immunoassay on venous blood samples collected at 15-day intervals.

**RESULTS:** The clinical response consisted of minor response (MR) in 2, stable disease (SD) in 6 and progressive disease (PD) in the remaining 12 patients. VEGF mean levels decreased on therapy, without, however, statistical differences with respect to the pre-treatment values. In contrast, by evaluating changes in VEGF levels in relation to the clinical response, non-progressing patients (MR + SD) showed a significant decline in VEGF mean concentrations, whereas no effect was achieved in progressing patients.

**CONCLUSIONS:** This study, by showing that melatonin-induced control of the neoplastic growth is associated with a decline in VEGF secretion, would suggest that the pineal hormone may control tumor growth at least in part by acting as a natural anti-angiogenic molecule, with a following opposition or angiogenesis-dependent cancer proliferation.

## Introduction

Recent studies would suggest that, in addition to the well documented existence of a psycho-neuroendocrine regulation of the immune responses [1] and hemopoietic processes [2], the angiogenesis-related phenomena are also under a neuroendocrinoimmune control [3].

Since angiogenesis is promoted by several inflammatory cytokines [4], the psychoneuroendocrine system could influence the angiogenesis through its well known modulatory effects on cytokines secretion [3]. In addition, however, a direct neuroendocrine influence on the secretion of angiogenic factors cannot be excluded. At present, it is known that vascular endothelial growth factor (VEGF) represents the most active endogenous angiogenic molecule [5], namely its molecular form consisting of 165 aminoacids. *In vivo*, VEGF can induce angiogenesis by acting directly on the endothelium, as well as increase the micro vascular permeability [5].

As far as the neoplastic disease is concerned, it has been well established that the activation of angiogenesis represents one of the major mechanisms responsible for tumor growth [6], in addition to the well known role exerted by cancer-related immunosuppressive status. The pineal gland, which constitutes one of the main organs involved in the neuroendocrine control of the immunity [7], would represent at the same time one of the most evident antitumor organs [1, 2, 7], which may contribute to the natural resistance against tumor onset and dissemination. Several pineal hormones may play antitumor activity [8]. Melatonin, which is the most clinically investigated antitumor hormone released from the pineal gland [9], would exert anticancer activity through several mechanisms, including immunomodulatory action, oncostatic activity and anti-oxidant properties [3]. The recently demonstrated anti-inflammatory effect of melatonin [10] could also contribute to the antitumor effect of the pineal hormone, since several inflammation-related cytokines have been proven to suppress the anticancer immunity, namely IL-6 [11]. In contrast, at present there are no data about the possible pineal influence on the angiogenesis. From a clinical point of view, the angiogenetic processes may be analyzed *in vivo* by determining the blood concentrations of the most active angiogenic factor, VEGF. In fact, abnormally high blood levels of VEGF have appeared to correlate with poor prognosis and low survival time in advanced cancer patients [12] through the activation of cancer-related neovascularization. In addition, it has been shown that VEGF may induce immunosuppression of the anticancer immunity by inhibiting the maturation of dendritic cells (DC) [13], which have been proven to play an essential role in activating an effective antitumor immunity [14]. Therefore, VEGF could promote cancer growth by either inducing immunosuppression, or by stimulating angiogenesis-dependent cancer cell proliferation. Then, the inhibition of VEGF secretion could constitute one of the main mechanisms to biologically control cancer growth and dissemination. This statement is justified by the fact that recent studies would suggest that the angiogenesis is also under

a central neuroimmunoregulatory control [1, 2, 3]. On this basis, a study was planned in an attempt to analyze the effects of melatonin therapy on VEGF secretion in advanced cancer patients, and to evaluate the action of melatonin in relation to changes in VEGF blood concentrations.

## Materials and methods

The study included 20 consecutive metastatic cancer patients (M/F: 12/8; median age: 56 years, range 44–67), who did not respond to previous conventional antitumor therapies, and for whom no other effective standard therapy was available. The protocol was approved by the Ethical Committee. Moreover, it was explained to each patient, and written consent was obtained.

Tumor histotypes were, as follows: non-small cell lung cancer: 8; colorectal carcinoma: 7; hepatocarcinoma: 3; renal cell cancer: 2. All patients had metastatic disease, and dominant metastasis sites were, as follows: bone: 5; lung: 6; liver: 4; liver + lung: 3; serouses: 2. Melatonin was given orally at 20 mg/day during the dark period of the day, every day for at least two consecutive months. Patients were considered as evaluable when they were treated for at least two months. The clinical response, as assessed by radiological examinations, was established according to WHO criteria, as follows: complete response (CR) was defined as a complete regression of all neoplastic lesions for at least one month; partial response (PR) was a reduction of at least 50% in the sum of the products of the two longest perpendicular diameters for at least one month; minor response (MR) was defined as a reduction lower than 50%, but greater than 25% of the lesions for at least one month; stable disease (SD) was considered as no objective cancer regression or increase greater than 25%; progressive disease (PD) was an increase of at least 25% in measurable lesions or the appearance of new lesions.

To evaluate VEGF secretion, venous blood samples were collected in the morning before therapy, then at 15-day intervals for two consecutive months. Serum levels of VEGF-165 were measured in duplicate with an enzyme immunoassay and commercially available kits (Quantikine, R&D Systems, Minneapolis, MN, USA). Normal values of VEGF-165 (95% confidence limits) observed in our laboratory were ranged from 62 to 601 pg/ml. Intra-assay and inter-assay coefficients of variation were below 4% and 5% respectively. The results were statistically analyzed by the chi-square test, the Student's T test, and the analysis of variance, as appropriate.

## Results

No melatonin-related toxicity occurred. Moreover, no patient had weight-loss under melatonin treatment. The oncological clinical response, as assessed by WHO criteria, consisted of MR in two patients (lung cancer: 1; colon cancer: 1), SD in other six patients (lung cancer: 2; colon cancer: 2; hepatocarcinoma: 1; renal cancer: 1), whereas the remaining 12 patients had PD. Then, a con-

trol of the neoplastic progression was achieved in 8/20 (40%) patients in response to melatonin therapy.

Abnormally high serum pre-treatment concentrations of VEGF were found in 9/20 (45%) patients. Changes in mean serum levels of VEGF occurring with melatonin therapy are illustrated in Fig. 1. VEGF mean serum levels decreased with melatonin therapy, without, however, statistically significant differences with respect to the pre-treatment concentrations. In contrast, by considering VEGF profile in relation to the clinical response, no VEGF decline occurred in patients who had PD in response to melatonin administration, whereas in non-progressing patients (MR + SD) VEGF mean concentrations significantly diminished during melatonin therapy, and the minimum levels were achieved after 30–45 days of melatonin therapy. Finally, no significant difference in pre-treatment levels of VEGF was seen between progressing and non-progressing patients.

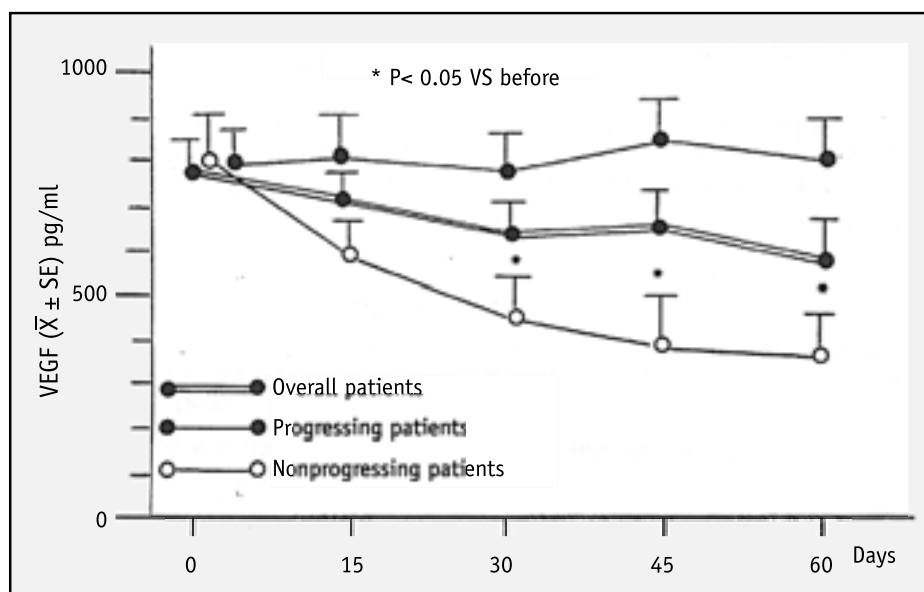
### Discussion

This preliminary study, by showing a possible decline in blood levels of the major angiogenic factor VEGF, would suggest that the pineal hormone melatonin may exert anti-angiogenic activity. Therefore, in addition to the previously demonstrated immunomodulating, anti-oxidant, cytodifferentiating and anti-proliferative effects, its potential anti-angiogenic action could also contribute to explain the antitumor role of melatonin. This statement is also justified by the evidence that VEGF decline on melatonin therapy tends to be associated with a control of the neoplastic growth. Moreover, the apparent inhibitory effect of melatonin on VEGF secretion, as shown by this study, could also contribute to explain the mechanisms responsible for the immuno-enhancing activity of the pineal hormone, since VEGF has been proven to suppress the immune responses by blocking DC maturation [13, 14]. Finally, from a physiopathological point of view, this study would

suggest that the pineal gland may be involved also in the regulation of the neoangiogenic processes, in addition to its well known modulatory role on immunity and hematopoiesis [2, 3, 7]. Therefore, this preliminary study would justify further efforts in the experimental and clinical investigations of the relationship among pineal gland, melatonin, angiogenesis and cancer.

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**Fig. 1.** Changes in vascular endothelial growth factor (VEGF) mean serum concentrations during melatonin therapy in metastatic cancer patients in relation to their clinical