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MELATONIN AND HUMAN CANCER

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Summary—A number of studies performed *in vitro* and on experimental animals supported the view that pineal gland inhibits neoplastic growth. Data in humans are scanty and controversial. In the present study we measured serum melatonin (MT), prolactin (PRL) and growth hormone (GH) concentrations, at 08.00 and 24.00, in 132 cancer patients and in 58 healthy control subjects. The patients were stratified according to histology and stage of disease as follows: 30 stage I–II and 45 stage III–IV breast cancer (BC); 39 stage III–IV lung cancer; 18 advanced gastrointestinal (GI) cancer. We also measured MT levels, at the same time-points, in 20 women with primary BC before and after radical mastectomy. Finally, we evaluated the circadian rhythm of serum MT in 18 patients with advanced cancer. On the whole, the patients with advanced tumors showed serum MT levels significantly higher than controls, without any correlation with PRL and GH values. When looking at stage III–IV vs stage I–II BC patients, significantly higher MT levels have been found in the former group. The surgical removal of the primary BC was not associated with any changes in MT values at both time points considered. A highly significant rhythm of serum MT was recorded in advanced cancer patients and the rhythmic parameters were substantially superimposable on those of the control subjects.

INTRODUCTION

The role of the pineal gland has been historically centered on its possible connection with mental processes and reproductive events [1], but the interest around this organ has always been scarce. Only recently, when sensitive assays for its major product melatonin (MT) have been developed, a new scenario for the pineal gland has been proposed. In brief it has been demonstrated that the pineal function is to transduce the changes of environmental lighting into neuroendocrine signals and serum MT has been found to oscillate with a circadian rhythm as a function of the pineal entrainment by the dark/light cycle.

The effects of the pineal gland upon neoplastic processes have been studied *in vitro* and more extensively in experimental animals. Of the several models investigated, sex steroid cancer, such as prostate or breast carcinoma, have received the greatest attention because of a possible influence of the pineal gland on the hypothalamic–pituitary–gonadal axis. According to Blask [2], the most consistent response of nearly all the experimental tumor types studied so far concerns increased growth and metastatic spread following pinealectomy. Moreover, pineal products, such as MT, appear to exert an inhibitory effect on tumor growth. The oncostatic effect of MT seems to be enhanced when the hormone is administered at a particular time of the day. It was suggested that the pineal gland regulates tumor growth through several mechanisms, including direct antimitotic action [3, 4], influence on neuroendocrine systems [5] and modulation of the immune system [6, 7]. MT was also found to inhibit cellular growth *in vitro* [3, 4] when incubated with normal and cancer-derived cell lines. Data coming from investigations on human neoplasia are still scarce and conflicting. Using chronobiological computation of data obtained in two populations at different racial/geographical risk of developing breast cancer (BC), Wetterberg *et al.* [8] found increased circadian MESOR (mean computed value) of urinary MT in Minnesotian women (high risk) in comparison with Japanese women (low risk); the subjects at higher risk also had a higher amplitude of the MT rhythm. This finding

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seems to reflect primarily a difference in nightly MT excretion [8]. When patients bearing malignant neoplasia were compared to control subjects, the majority of authors [9–14] found serum or urinary MT higher in the former group than in the latter. Bartsch *et al.* [15] reported on MT values in patients with prostate carcinoma; the highest values were found in very early disease (incidental carcinoma) in comparison to control subjects bearing benign tumor or locally advanced carcinomas. More recently, the same authors [16] have demonstrated that in women affected with primary BC the nocturnal peak of serum MT tends to decrease as a function of the tumor size, the significance being attained from T1 to T3 (TNM staging). These findings seem to support the hypothesis of Relkin [17], who suggested a changing pattern of the pineal secretion, namely a precocious activation in the attempt to control the malignant process and a subsequent exhaustion when the control over the malignant process is definitely lost.

On the other hand, in the same study [15] the patients bearing advanced tumors had higher MT levels (expressed as circadian MESOR) in comparison to the patients with earlier stages. Finally, Lissoni *et al.* [13] found that serum MT levels were lower in patients with advanced cancer than in those with early stages of the disease. The study procedures could account for discrepancies; in particular, Lissoni *et al.* [13] considered only one day-time blood sampling in the morning and this approach appears inadequate because it does not account for the large nocturnal increment of MT secretion.

In this paper we report on the results of three studies aimed to evaluate: (1) the clinical significance of MT determination in patients affected by neoplastic disease at different stages (early vs advanced); (2) the changes, if any, of MT secretion in patients with BC following the surgical removal of the primary tumor; (3) the circadian rhythm of serum MT in neoplastic and control subjects.

SERUM MT VARIATION IN HUMAN NEOPLASIA AS A FUNCTION OF CLINICAL PARAMETERS

In this study we have measured serum MT in patients stratified according to type and as well as stage and host conditions (age, performance status). In a large number of samples we also measured growth hormone (GH) and prolactin (PRL). Preliminary data have been published elsewhere [18]. The updating of our study concerns 132 patients (42 males, 90 females), aged 29–82 yr (median 62.5 yr), with histologically proven neoplastic disease, and 58 healthy controls (32 males, 26 females), aged 24–81 yr (median 35 yr). Histology was as follows: 30 stage I–II and 45 stage IV BC; 39 stage III–IV lung cancer—16 small cells lung cancer (SCLC), 23 non-small cells lung cancer (NSCLC); 18 advanced gastrointestinal (GI) cancer. The patients were studied during hospitalization, where they followed a typical ward schedule (lights off at 22.00, lights on at 06.00). The controls were studied under the same conditions. Blood samples were drawn at two predetermined time-points: 08.00 and 24.00. Serum MT was measured using a commercially available radioimmunoassay (RIA) procedure (Eurodiagnostic, Apendoorn, Holland), intra- and inter-assay coefficients of variation were 10 and 15%, respectively. Serum GH and PRL were measured by using standard RIA kits (CIS Diagnostici, Santhià, Italy); intra- and inter-assay coefficients of variation were $<10\%$. Each patient was scored for performance status (Karnofsky index), clinical course of the neoplasia (remission, stable or progressive disease) and, in the case of BC, menopausal status and estrogen receptor (ER) status. Statistical analyses were performed using non-parametric tests (Wilcoxon's signed rank sum test, Spearman test).

Cancer patients as a whole group showed mean MT levels significantly higher than controls

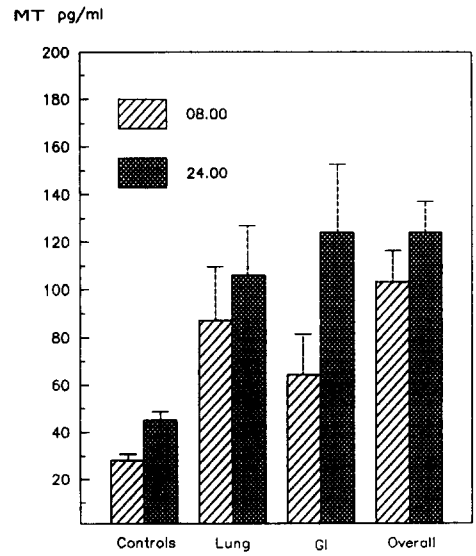


Fig. 1. Mean concentration of serum MT ± SE at two determined time-points (08.00 and 24.00) in 58 control subjects (controls) and in 102 patients bearing advanced cancer (overall), 39 of them with lung carcinoma (lung) and 19 with GI carcinoma.

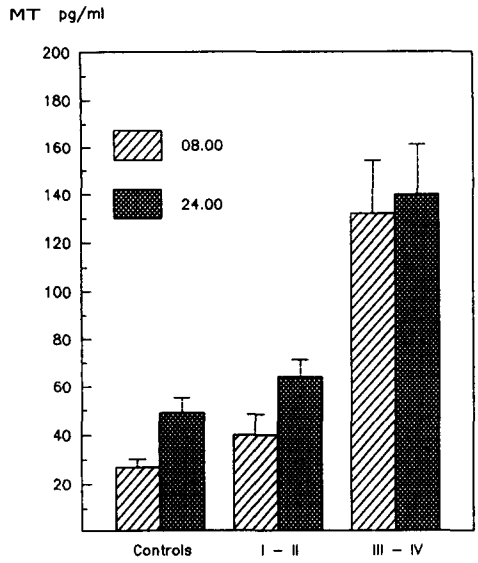


Fig. 2. Mean concentration of serum MT ± SE at two determined time-points (08.00 and 24.00) in 26 control women (controls) and in 30 stage I–II and 45 stage III–IV BC.

(Fig. 1), both at midnight and in the morning: 110.2 vs 45.4 pg/ml ($P < 0.0001$) at 24.00; 88.7 vs 27.6 pg/ml ($P < 0.0001$) at 08.00. Stage I–II BC patients showed mean MT levels higher than female controls both at 08.00 (39.7 vs 26.5) and 24.00 (63.5 vs 48.9), but significance was not attained. Stage IV BC patients had mean MT values significantly higher than controls (140.3 vs 48.9, $P < 0.0001$ at 24.00; 132.3 vs 26.5, $P < 0.0001$ at 08.00); mean MT levels of stage IV BC were significantly higher than stage I–II either at 24.00 (140.3 vs 63.5, $P < 0.002$) or at 08.00 (132.3 vs 39.7, $P < 0.0001$) (Fig. 2). These results are in contrast with those of Lissoni *et al.* [13], who found that the patients with metastatic tumors had serum MT levels significantly lower than those suffering from non-metastatized tumors. At variance with our preliminary results [18] and with those of Tamarkin *et al.* [19], who found lower MT in ER+ patients vs ER−, no difference was apparent between ER+ vs ER− patients at both time-points.

Advanced lung cancer patients as a whole group showed mean MT levels significantly higher than controls at both time-points: 106.2 vs 45.4 ($P < 0.001$) at 24.00; 87.4 vs 27.6 ($P < 0.0001$) at 08.00. The highest levels were recorded in patients having SCLC. The question could be raised whether this particular finding is compatible with a direct secretion (paraneoplastic) or is an indirect effect of the tumor cells. In this regard, the list of candidates for MT-stimulating substances should include ACTH-related or others propiomelanocortin-derived peptides that are potential products of SCLC cells. Finally, patients with advanced GI cancer had mean MT concentrations significantly higher than controls as well: 124.8 vs 45.4 ($P < 0.005$) at 24.00; 64.06 vs 27.6 ($P < 0.001$) at 08.00.

We did not find significant differences between patients and controls for PRL and GH concentrations. No correlation was apparent between MT and PRL and between MT and GH in any group examined.

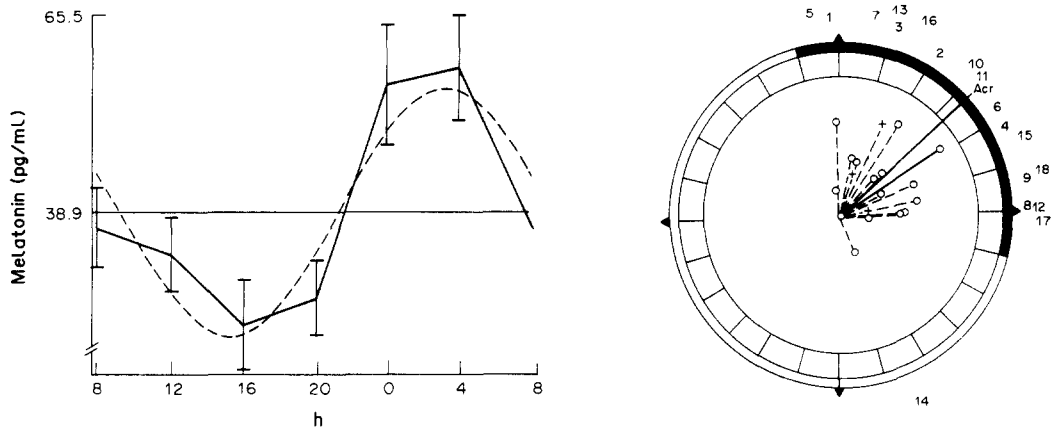
When the patients were considered in relation to their clinical course (progression vs no change or remission), the mean nocturnal levels of PRL were higher in the group with progressive disease as compared to the group with stable disease or remission ($P < 0.01$). This finding is in accord with previous data [20]. An inverse relationship between MT levels at 08.00 and performance status was also recorded ($r = -37$, $P < 0.01$). It is likely that the relationship is based on a common feature in the clinical setting of neoplastic subjects with poor performance status, namely the circulating levels of free tryptophan, an MT precursor that is reportedly higher in cancer patients suffering from anorexia [21].

SERUM MT IN PATIENTS WITH BC BEFORE AND AFTER RADICAL MASTECTOMY

The aim of this study was to check whether the surgical removal of primary BC was associated with MT changes. Recently, Lissoni *et al.* [22] have reported that MT levels are modified by surgery in about 50% of BC patients, both as an increase or a decrease. The study was carried out on 20 unselected patients aged 29–81 yr (median 60 yr): 12 had stage I, 6 stage II and 2 stage III BC. Blood samples were collected at two time-points (08.00 and 24.00), both 3 days before and 15 days after surgical removal of the tumor. We did not find any difference in MT concentrations measured pre- vs post-surgery at both time-points. We could conclude that the presence of the primary tumor does not influence MT concentrations.

CIRCADIAN RHYTHM OF SERUM MT IN PATIENTS WITH ADVANCED CANCER

To further clarify the relationship between the pineal gland and human cancer we thought it of interest to evaluate the circadian rhythm of serum MT in patients bearing different types of advanced cancer and to compare the chronobio-



Population-mean cosinor

Key	P	N OBS.	PR	MESOR ± SE	Amplitude	Acrophase
Cancer patients	<0.001	18	66.1	38.9 ± 4.9	16.8 ± 2.3	−48.4° (−26.6° − 72.6°)

Fig. 3. Circadian rhythm of serum MT, revealed by 4-h blood sampling, in 18 patients bearing advanced cancer. (a) The chronogram displays mean MT values ± SD of overall patients at each time-point considered; (b) the polar plot of population mean cosinor analysis shows the location of individual acrophases.

logical parameters with those obtained in control subjects; 18 patients entered the study: 3 had kidney cancer, 4 lung cancer, 4 breast cancer, 6 prostate cancer and 1 carcinoid tumor. Controls and patients were evaluated during hospitalization when they had the same ward schedule. A highly significant rhythm of serum MT was recorded in both groups. Blood was drawn every 4 h over a 24-h span. The circadian profile of MT concentrations was evaluated using the population mean cosinor analysis [23]. The computation for the cancer patients yielded a mean MESOR (rhythm determined average) of 38.2 ± 5.7 pg/ml, a mean amplitude (half the difference between the highest and the lowest values of the function) of 17.9 ± 2.4 pg/ml and an acrophase (timing of the crest point from a defined reference point expressed as a degree of cosinor diagram) located at 2.51 h (Fig. 3). The rhythmic parameters were substantially superimposable on those of the control subjects.

In conclusion, despite the fairly consistent results obtained in animal models, the available information obtained from studies performed on patients with cancer is conflicting, probably as a consequence of the large inter-individual variability of MT levels, methodological difficulties and the inhomogeneous selection of probands. Our data show that: (1) MT is higher in patients suffering from any kind of advanced cancer, particularly when they have a low performance status; (2) the presence or absence of primary breast tumor does not modify MT levels; (3) the rhythmic night/day pattern of MT secretion is maintained in patients with advanced cancer. Our results support the view that MT secretion in cancer patients is modified more as a consequence of metabolic changes due to the worsening of the host/tumor relationship, than as an upward resetting of the pineal function aimed to control the neoplastic growth.

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