

## Melatonin:

Cos, S., A. Gonzalez, et al. (2005). "Melatonin inhibits the growth of DMBA-induced mammary tumors by decreasing the local biosynthesis of estrogens through the modulation of aromatase activity." *Int J Cancer*.

**Melatonin inhibits the growth of breast cancer cells by interacting with estrogen-responsive pathways, thus behaving as an antiestrogenic hormone.** Recently, we described that **melatonin reduces aromatase expression** and activity in MCF-7 human breast cancer cells, thus modulating the local estrogen biosynthesis. To investigate the in vivo aromatase-inhibitory properties of melatonin in our current study, this indoleamine was administered to rats bearing DMBA-induced mammary tumors, ovariectomized (ovx) and treated with testosterone. In these castrated animals, the growth of the estrogen-sensitive mammary tumors depends on the local aromatization of testosterone to estrogens. Ovariectomy significantly reduced the size of the tumors while the administration of testosterone to ovx animals stimulated tumor growth, an effect that was suppressed by administration of melatonin or the aromatase inhibitor aminoglutethimide. Uterine weight of ovx rats, which depends on the local synthesis of estrogens, was increased by testosterone, except in those animals that were also treated with melatonin or aminoglutethimide. The growth-stimulatory effects of testosterone on the uterus and tumors depend exclusively on locally formed estrogens, since no changes in serum estradiol were appreciated in testosterone-treated rats. Tumors from animals treated with melatonin had lower microsomal aromatase activity than tumors of animals from other groups, and incubation with melatonin decreased the aromatase activity of microsomal fractions of tumors. Animals treated with melatonin had the same survival probability as the castrated animals and significantly higher survival probability than the uncastrated. We conclude that melatonin could exert its antitumoral effects on hormone-dependent mammary tumors by inhibiting the aromatase activity of the tumoral tissue. (c) 2005 Wiley-Liss, Inc.

Cos, S., C. Martinez-Campa, et al. (2005). **"Melatonin modulates aromatase activity in MCF-7 human breast cancer cells."** *J Pineal Res* **38**(2): 136-42.

Most of the current knowledge about the mechanisms by which melatonin inhibits the growth of breast cancer cells point to an interaction of melatonin with estrogen-responsive pathways, thus behaving as an antiestrogenic hormone. However, a possible effect of melatonin on the local synthesis of estrogens had not been examined. The objective of this work was to study whether melatonin may modify the aromatase activity in MCF-7 breast cancer cells thus modulating the local estrogen biosynthesis. In MCF-7 cells cultured with testosterone in estradiol-free

media, **melatonin (1 nM) counteracts the testosterone-induced cell proliferation dependent on the local biosynthesis of estrogens from testosterone by the aromatase activity of the cells.** We found that melatonin reduces the aromatase activity (measured by the tritiated water release assay) of MCF-7 cells both at basal conditions and when aromatase activity was stimulated by cAMP or cortisol. The greatest inhibition of the aromatase activity was obtained with 1 nM melatonin, the same concentration that gives the highest antiproliferative and anti-invasive effects of MCF-7 cells. Finally, by RT-PCR, we found that melatonin downregulates aromatase expression at the transcriptional level in the MCF-7 cells. **We conclude that melatonin, at physiological concentrations, decreases aromatase activity and expression in MCF-7 cells. This aromatase inhibitory effect of melatonin, together with its already known antiestrogenic properties interacting with the estrogen-receptor, makes this indoleamine an interesting tool to be considered in the prevention and treatment of hormone-dependent mammary neoplasias.**

Sanchez-Barcelo, E. J., S. Cos, et al. (2003). "Melatonin and mammary cancer: a short review." *Endocr Relat Cancer* **10**(2): 153-9.

Melatonin is an indolic hormone produced mainly by the pineal gland. The former hypothesis of its possible role in mammary cancer development was based on the evidence that melatonin down-regulates some of the pituitary and gonadal hormones that control mammary gland development and which are also responsible for the growth of hormone-dependent mammary tumors. Furthermore, melatonin could act directly on tumoral cells, as a naturally occurring antiestrogen, thereby influencing their proliferative rate. **The first reports revealed a low plasmatic melatonin concentration in women with estrogen receptor (ER)-positive breast tumors.** However, later studies on the possible role of melatonin on human breast cancer have been scarce and mostly of an epidemiological type. These studies described a low incidence of breast tumors in blind women as well as an inverse relationship between breast cancer incidence and the degree of visual impairment. Since light inhibits melatonin secretion, the relative increase in the melatonin circulating levels in women with a decreased light input could be interpreted as proof of the protective role of melatonin on mammary carcinogenesis. From in vivo studies on animal models of chemically induced mammary tumorigenesis, the general conclusion is that experimental manipulations activating the pineal gland or the administration of melatonin lengthens the latency and reduces the incidence and growth rate of mammary tumors, while pinealectomy usually has the opposite effects. Melatonin also reduces the incidence of spontaneous mammary tumors in different kinds of transgenic mice (c-neu and N-ras) and mice from strains with a high

tumoral incidence. In vitro experiments, carried out with the ER-positive MCF-7 human breast cancer cells, demonstrated that **melatonin, at a physiological concentration (1 nM) and in the presence of serum or estradiol: (a) inhibits, in a reversible way, cell proliferation, (b) increases the expression of p53 and p21WAF1 proteins and modulates the length of the cell cycle, and (c) reduces the metastatic capacity of these cells and counteracts the stimulatory effect of estradiol on cell invasiveness; this effect is mediated, at least in part, by a melatonin-induced increase in the expression of the cell surface adhesion proteins E-cadherin and beta(1)-integrin.** The direct oncostatic effects of melatonin depends on its interaction with the tumor cell estrogen-responsive pathway. In this sense it has been demonstrated that melatonin down-regulates the expression of ERalpha and inhibits the binding of the estradiol-ER complex to the estrogen response element (ERE) in the DNA. **The characteristics of melatonin's oncostatic actions, comprising different aspects of tumor biology as well as the physiological doses at which the effect is accomplished, give special value to these findings and encourage clinical studies on the possible therapeutic value of melatonin on breast cancer.**

Cos, S., M. D. Mediavilla, et al. (2002). "Does melatonin induce apoptosis in MCF-7 human breast cancer cells in vitro?" *J Pineal Res* **32**(2): 90-6.

Melatonin inhibits proliferation of the estrogen-responsive MCF-7 human breast cancer cells. **The objective of this work was to assess whether melatonin not only regulates MCF-7 cell proliferation but also induces apoptosis.** In this experiment we used 1,25-dihydroxycholecalciferol (D3) as a positive control because it inhibits MCF-7 cell proliferation and induces apoptosis. MCF-7 cells were cultured with either 1 nM melatonin, 100 nM D3 or its diluent to determine their effects on cell proliferation, cell viability, cell-cycle phase distribution, population of apoptotic cells, and expression of p53, p21WAF1, bcl-2, bcl-X(L) and bax proteins. After 24 or 48 hr of incubation, both melatonin and D3-treatment significantly decreased the number of viable cells in relation to the controls, although no differences in cell viability were observed between the treatments. The incidence of apoptosis, measured as the population of cells falling in the sub-G1 region of the DNA histogram, or by the TUNEL reaction, was similar in melatonin-treated and control cells whereas, as expected, apoptosis was higher among cells treated with D3 than in controls. **The expression of p53 and p21WAF1 proteins significantly increased after 24 or 48 hr of incubation with either melatonin or D3.** No significant changes in bcl-2, bcl-XL and bax mRNAs were detected after treatment with melatonin whereas in D3-treated cells, a significant drop in bcl-XL was observed. These data support the

hypothesis that melatonin reduces MCF-7 cell proliferation by modulating cell-cycle length through the control of the p53-p21 pathway, but without clearly inducing apoptosis.

Mediavilla, M. D., S. Cos, et al. (1999). "Melatonin increases p53 and p21WAF1 expression in MCF-7 human breast cancer cells in vitro." *Life Sci* **65**(4): 415-20.

The aim of the present work was to study whether melatonin, at physiological concentrations, exerts its antiproliferative effects on MCF-7 human breast cancer cells by inducing the expression of some of the proteins involved in the control of the cell cycle. MCF-7 cells were cultured for 48 h in DMEM media containing either melatonin (1 nM) or the diluent (0.001% ethanol). **At this concentration, after 48 hours of incubation, melatonin reduced the number of viable cells in relation to controls.**

The decreased cell proliferation was coincident with a significant increase in the expression of p53 as well as p21WAF1 proteins. These results demonstrate that melatonin inhibits MCF-7 cell proliferation by inducing an arrest of cell cycle dependent on an increased expression of p21WAF1 protein, which is mediated by the p53 pathway.

Lissoni, P., M. Chilelli, et al. (2003). **"Five years survival in metastatic non-small cell lung cancer patients treated with chemotherapy alone or chemotherapy and melatonin: a randomized trial."** *J Pineal Res* **35**(1): 12-5.

Numerous experimental data have documented the oncostatic properties of melatonin. In addition to its potential direct antitumor activity, **melatonin has proved to modulate the effects of cancer chemotherapy, by enhancing its therapeutic efficacy and reducing its toxicity. The increase in chemotherapeutic efficacy by melatonin may depend on two main mechanisms, namely prevention of chemotherapy-induced lymphocyte damage and its antioxidant effect, which has been proved to amplify cytotoxic actions of the chemotherapeutic agents against cancer cells.** However, the clinical results available at present with melatonin and chemotherapy in the treatment of human neoplasms are generally limited to the evaluation of 1-year survival in patients with very advanced disease. Thus, **the present study was performed to assess the 5-year survival results in metastatic non-small cell lung cancer patients obtained with a chemotherapeutic regimen consisting of cisplatin and etoposide, with or without the concomitant administration of melatonin (20 mg/day orally in the evening).** The study included 100 consecutive patients who were randomized to receive chemotherapy alone or chemotherapy and melatonin. **Both the overall tumor regression rate and the 5-year survival results were significantly higher in patients concomitantly treated with melatonin.** In particular, no patient treated with chemotherapy alone was alive after 2 years, whereas a 5-year survival

was achieved in three of 49 (6%) patients treated with chemotherapy and melatonin. **Moreover, chemotherapy was better tolerated in patients treated with melatonin.** This study confirms, in a considerable number of patients and for a long follow-up period, the possibility to improve the efficacy of chemotherapy in terms of both survival and quality of life by a concomitant administration of melatonin. This suggests a new biochemotherapeutic strategy in the treatment of human neoplasms.

Cerea, G., M. Vaghi, et al. (2003). "Biomodulation of cancer chemotherapy for metastatic colorectal cancer: a randomized study of weekly low-dose irinotecan alone versus irinotecan plus the oncostatic pineal hormone melatonin in metastatic colorectal cancer patients progressing on 5-fluorouracil-containing combinations." *Anticancer Res* **23**(2C): 1951-4.

**Recent advances in immunobiological knowledge have suggested the possibility of enhancing the therapeutic activity of various chemotherapeutic agents by a concomitant administration of anti-oxidant drugs and/or immunomodulating neurohormones.** In particular, the pineal neurohormone melatonin (MLT), which is able to exert both **antioxidant and immunomodulating effects, has been proven to enhance the efficacy of various chemotherapeutic drugs, namely cisplatin, anthracyclines and 5-fluorouracil,** whereas at present there are no data about its possible influence on cytotoxic drugs effective in the treatment of colon cancer other than 5-fluorouracil, such as irinotecan (CPT-11). The present study was performed to evaluate the influence of a concomitant administration of MLT on CPT-11 therapeutic activity in metastatic colorectal cancer. The study included 30 metastatic colorectal cancer patients progressing after at least one previous chemotherapeutic line containing 5-fluorouracil, who were randomized to be treated with CPT-11 alone or CPT-11 plus MLT. According to a weekly low-dose schedule, CPT-11 was given i.v. at 125 mg/m<sup>2</sup>/week for 9 consecutive weeks. MLT was administered orally at 20 mg/day during the dark period of the day. No complete response was observed. A partial response (PR) was achieved in 2 out of 16 patients treated with CPT-11 alone and in 5 out of 14 patients concomitantly treated with MLT. Moreover, a stable disease (SD) was obtained in 5 out of 16 patients treated with CPT-11 alone and in 7 out of 14 patients treated with CPT-11 plus MLT. **Therefore, the percent of disease-control achieved in patients concomitantly treated with MLT was significantly higher than that observed in those treated with chemotherapy alone** (12 out of 14 vs 7 out of 16,  $p < 0.05$ ). The only important toxicity was diarrhoea grade 3-4, which occurred in 6 out of 16 patients treated with CPT-11 alone and in 4 out of 14 patients treated with CPT-11 plus MLT, which required a 50% dose reduction. However, taken together, patients treated with CPT-11 at 50% of the planned dose showed a percent of disease

control comparable to that achieved in patients who had no dose reduction (6 out of 10 vs 13 out of 20). **This preliminary study shows that the efficacy of weekly low-dose CPT-11 in pretreated metastatic colorectal cancer patients may be enhanced by a concomitant daily administration of the pineal hormone MLT, according to the results previously reported for other chemotherapeutic agents.** Moreover, since the dose reduction of CPT-11 does not influence its efficacy, the dose of CPT-11 for successive studies might be not greater than 70 mg/m<sup>2</sup>.

Lissoni, P., F. Paolorossi, et al. (1997). "A randomized study of chemotherapy with cisplatin plus etoposide versus chemoendocrine therapy with cisplatin, etoposide and the pineal hormone melatonin as a first-line treatment of advanced non-small cell lung cancer patients in a poor clinical state." *J Pineal Res* **23**(1): 15-9.

**Recent studies suggest that the pineal hormone melatonin may reduce chemotherapy-induced immune and bone marrow damage.** In addition, melatonin may exert potential oncostatic effects either by stimulating host anticancer immune defenses or by inhibiting tumor growth factor production. **On this basis, we have performed a randomized study of chemotherapy alone vs. chemotherapy plus melatonin in advanced non-small cell lung cancer patients (NSCLC) with poor clinical status.** The study included 70 consecutive advanced NSCLC patients who were randomized to receive chemotherapy alone with cisplatin (20 mg/m<sup>2</sup>/day i.v. for 3 days) and etoposide (100 mg/m<sup>2</sup>/day i.v. for 3 days) **or chemotherapy plus melatonin (20 mg/day orally in the evening).** Cycles were repeated at 21-day intervals. Clinical response and toxicity were evaluated according to World Health Organization criteria. A complete response (CR) was achieved in 1/34 patients concomitantly treated with melatonin and in none of the patients receiving chemotherapy alone. Partial response (PR) occurred in 10/34 and in 6/36 patients treated with or without melatonin, respectively. **Thus, the tumor response rate was higher in patients receiving melatonin (11/34 vs. 6/35), without, however, statistically significant differences. The percent of 1-year survival was significantly higher in patients treated with melatonin plus chemotherapy than in those who received chemotherapy alone (15/34 vs. 7/36, P < 0.05).** Finally, chemotherapy was well tolerated in patients receiving melatonin, and in particular the frequency of myelosuppression, neuropathy, and cachexia was significantly lower in the melatonin group. **This study shows that the concomitant administration of melatonin may improve the efficacy of chemotherapy, mainly in terms of survival time, and reduce chemotherapeutic toxicity in advanced NSCLC, at least in patients in poor clinical condition.**

Lissoni, P., S. Barni, et al. (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**(4): 854-6.

Recent observations have shown that the pineal hormone melatonin (MLT) may modulate oestrogen receptor (ER) expression and inhibit breast cancer cell growth. On this basis, we have evaluated the biological and clinical effects of a concomitant MLT therapy in women with metastatic breast cancer who had progressed in response to tamoxifen (TMX) alone. **The study included 14 patients with metastasis who did not respond (n = 3) to therapy with TMX alone or progressed after initial stable disease (SD) (n = 11). MLT was given orally at 20 mg day-1 in the evening, every day starting 7 days before TMX,** which was given orally at 20 mg day-1 at noon. A partial response was achieved in 4/14 (28.5%) patients (median duration 8 months). The treatment was well tolerated in all cases, and no MLT-induced enhancement of TMX toxicity was seen; on the contrary, most patients experienced a relief of anxiety. **Mean serum levels of insulin-like growth factor 1 (IGF-1), which is a growth factor for breast cancer, significantly decreased on therapy, and this decline was significantly higher in responders than in patients with SD or progression. This pilot phase II study would suggest that the concomitant administration of the pineal hormone MLT may induce objective tumour regressions in metastatic breast cancer patients refractory to TMX alone.**

Brackowski, R., B. Zubelewicz, et al. (1994). "Preliminary study on modulation of the biological effects of tumor necrosis factor-alpha in advanced cancer patients by the pineal hormone melatonin." *J Biol Regul Homeost Agents* **8**(3): 77-80.

Previous experimental studies have suggested the possibility to modulate the biological activity and toxicity of cytokines by immunomodulating neurohormones. **In particular, the pineal hormone melatonin (MLT) has been proven to amplify the immune effects of IL-2 and to reduce its toxicity.** On this basis, we decided to investigate the effect of MLT on biological activity and toxicity of another important antitumor cytokine, TNF. The study was performed in 14 metastatic solid tumor patients, for whom no effective standard antitumor therapy was available. Informed consent was previously obtained from each patient. Patients were randomized to be treated with TNF or TNF plus MLT. Recombinant human TNF was given at a daily dose of 0.75 mg intravenously for 5 consecutive days. MLT was given orally at a daily dose of 40 mg, starting 7 days before TNF. Lymphocyte mean number observed at the end of TNF infusion was significantly higher in patients treated with TNF plus MLT than in those receiving TNF alone. On the contrary, no significant difference occurred in hemoglobin, platelet and neutrophil mean values.

**Asthenia and hypotension were significantly less frequent in patients treated with TNF plus MLT, whereas no difference occurred in the frequency of fever and chills. Even though limited to a small number of patients, this preliminary study would suggest the possibility to modulate TNF toxicity and biological activity by a concomitant treatment with the pineal hormone MLT.**