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Chapter XV

Melatonin: An Endogenous Antiestrogen with Oncostatic Properties

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Abstract

Melatonin inhibits the growth of breast cancer cells by interacting with estrogen-responsive pathways, thus behaving as an antiestrogenic hormone. In this article we will review the evidence supporting three different kinds of melatonin antiestrogenic effects: a) the downregulation of the gonadal synthesis of estrogens, thus giving a reduction in the circulating levels of gonadal steroids, b) the interaction with the estrogenic receptors, in a similar way to the so called selective estrogen receptors modulators (SERMs), although by means of a different mechanism, and c) the inhibition of the enzymes involved in the biosynthesis of estrogens in peripheral tissues, mainly the aromatases, allowing melatonin to be considered as a selective estrogen enzyme modulator (SEEMs). We conclude that melatonin, because of the wide range of its antiestrogenic actions could be a valuable instrument in the prevention and treatment of hormone-dependent mammary tumors, either alone, or in combination with other SERMs and SEEMs drugs which share properties with melatonin but have different action mechanisms.

Introduction

Almost 50 years from the identification of melatonin as the main pineal hormone [47], the number of physiological functions attributed to this indoleamine is so great than the adjective "pleiotropic" is now frequently used to refer to the nature of its actions. However, in our opinion, perhaps the most solidly established role for melatonin in mammals is the control of the reproduction on seasonal breeders [63]. This control is exerted through a modulation, by melatonin, of the neuroendocrine reproductive axis, through different mechanisms [63]. In this way, melatonin reduces the gonadal function, including the synthesis of gonadal steroids. This fact, together with the evidence for the role of estrogens on mammary cancer [67], were the basis for considering a possible influence of melatonin on the development of some hormone dependent tumors and, particularly, on the estrogen-dependent mammary adenocarcinomas.

The first contribution on this line came from Cohen and collaborators [19]. These authors published in Lancet their hypothesis of the possible relationship between pineal function and mammary carcinogenesis. They proposed that, whatever its cause, any dysfunction of the pineal gland, resulting in a decrease of melatonin production, could participate in the etiology of breast cancer, because of the "relative hyperestrogenism" experienced by these subjects, due to the loss of the inhibitory actions of the pineal hormone on the endocrine-reproductive axis. This former hypothesis has since then been developed in numerous epidemiologic and experimental studies. Among the epidemiologic studies, one of the first was the demonstration of a decreased nocturnal plasma melatonin peak in patients with estrogen receptor (ER) positive breast cancer [78]. The relationship between pineal function and risk of breast cancer has also been proposed because of the low incidence of breast cancer among blind women [20,34,43], as well as the inverse association between breast cancer incidence and degree of visual impairment [80]. In both cases, the total or partial suppression of the light input could mediate an increase of melatonin circulating levels that could explain the low incidence of tumours. Recently [73], in a prospective case-control study in nurses, it has been demonstrated that higher melatonin levels, measured as 6-sulphatoxymelatonin concentration in the first morning urine, are associated with a lower risk of breast cancer. On the contrary, the high incidence of breast cancer among women exposed to light during night, such as shift workers [41], or exposed to low frequency electromagnetic fields [9,18] could be explained by the decreased melatonin synthesis under these environmental conditions [64] which, in turn, could represent a relative increase in the synthesis of estrogens by gonads, as well as a "circadian disruption" [19,77]. We have recently verified, by using the model of chemical induced mammary carcinogenesis in rats, the hypothesis of the possible effect of exposure to light-at-night as a factor which increases the risk of breast cancer, supported by the above mentioned epidemiological studies. Female rats bearing DMBA-induced mammary adenocarcinomas were exposed to different lighting environments, and animals exposed to light-at-night, especially those placed under a constant dim light during darkness, showed higher rates of tumor growth, lower survival and lower nocturnal excretion of 6-

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sulfatoxymelatonin than controls not exposed to "light pollution" during the period of darkness [26]. These results give experimental support to the previously mentioned epidemiologic data describing a possible influence of nocturnal light in mammary carcinogenesis, and encourage the study of melatonin-based treatments to reduce the risk of carcinogenesis in people exposed to light-at-night.

The experimental studies, carried out with different animal models of mammary carcinogenesis and tumoral cell lines [28,68-69] have confirmed that melatonin, *in vivo*, reduces the incidence and growth of chemically induced [29], or spontaneous [54] mammary tumors in rodents, whereas i*n vitro*, at concentrations corresponding to the physiological levels present in human blood during the night (1 nM), inhibits proliferation, increases expression of p53 and reduces the invasiveness of the estrogen-responsive MCF-7 human breast cancer cells [10,22-23,27,29,53,68].

In this article we will review the evidence supporting a possible oncostatic role of melatonin based on three different kinds of antiestrogenic actions of this indoleamine: a) the downregulation of the gonadal synthesis of estrogens, b) the interaction with the estrogenic receptors (ER), and c) the inhibition of the enzymes involved in the biosynthesis of estrogens in peripheral tissues, mainly the aromatases. Although other possible hypothesis, not related to the estrogen-signaling pathway, have been proposed to explain the oncostatic role of melatonin [1,45-46,65] we will focus our attention on the above mentioned antiestrogenic effects of melatonin.

Oncostatic Properties of Melatonin Based on its Ability to Downregulate the Neuroendocrine Reproductive Axis

In seasonally breeding mammalian species, melatonin controls reproductive function through the activation of receptor sites within the hypothalamic-pituitary areas driving the gonadal activity [33,50,79], and melatonin down-regulation of the ovarian estrogen secretion has been observed in a variety of mammals [63]. Furthermore, the presence of functional melatonin receptors in cells of antral follicles and corpora lutea of rat ovaries [75] also suggests direct regulatory action of melatonin on ovarian function. The presence of melatonin receptors in brain and gonads, together with the localization of sex-steroid receptors in the pineal gland, indicate a complex bidirectional interplay between melatonin and estrogen secretion [49].

In humans, the role of melatonin on ovarian function is still poorly understood, and most evidence of melatonin-gonadal hormone relationship came from the finding of abnormal melatonin secretion in disorders of the reproductive system or, conversely, from alterations of melatonin secretion associated with gonadal dysfunctions [49]. There is an inverse seasonal relationship between melatonin and estradiol (E_2) serum concentrations [40], and significantly increased levels of E_2 can be found in women exposed to light-at-night, which suppresses melatonin production [71-72]. Human granulose-luteal cells expresses the two forms of melatonin receptors [83] and, in these cells, melatonin modulates the expression of

LH and GnRH receptors [83]. In vitro, melatonin reduces the LH- and FSH-induced secretion of E_2 from cultured human granulose cells [11]. All these data are in favor of a possible downregulation of ovarian function by melatonin. However, some reports indicate no effect of melatonin on E_2 production by granulose cells [81] or even stimulatory effect [84]. Human follicular fluid has melatonin concentrations higher than serum [13,66], resulting not from a local synthesis but from an active uptake and local concentration. The melatonin concentration is higher in preovulatory follicles than in the small immature ones [58]. In short, the experimental data available up to date, with the reserves concerning those from humans, support a modulatory effect of melatonin on ovarian function, and this could be one of the explanations to the protective role of melatonin on the etiology of estrogen-dependent breast-cancer.

Oncostatic Properties of Melatonin Based on its Ability to Interact with the Estrogen Receptors

The growth of chemically-induced ER-positive mammary tumors in ovariectomized rats treated with exogenous E_2 is significantly reduced when these animals are subjected to experimental manipulations (anosmia, underfeeding or exposure to cold, associated with light deprivation) [28-29,70], that enhance the pineal-dependent effects. Since in these animals serum E_2 concentrations were kept stable because of its exogenous administration and the lack of changes in their metabolic rate, it was suggested that melatonin may counteract the effects of estrogens at the level of the tumor by interacting with the estrogen binding sites (ER). These kind of direct antiestrogenic effects of melatonin were established from *in vitro* studies, carried out basically with the estrogen-sensitive MCF-7 human breast cancer cells. Melatonin, at physiologic concentrations (1 nM), counteracts E_2-induced MCF-7 cell proliferation and invasiveness [23,28,68], augments the sensitivity of MCF-7 to antiestrogens such as tamoxifen [82], and down-regulates the expression of proteins, growth factors, and proto-oncogens regulated by estrogens [54,57]. In humans, administration of melatonin together with tamoxifen induced objective tumor regression in metastasic breast cancer patients refractory to tamoxifen alone [48]. A clear demonstration of the interaction of melatonin with the ER is that the transfection of MT1 melatonin receptors to MCF-7 cells (ERα positive) or MDA-MB-231 cells (ERα negative) enhances the growth suppressive effects of melatonin only in the ERα positive cells [85].

Unlike the "classic" antiestrogens such as tamoxifen and its derivates, melatonin neither binds to the ER nor interferes with the binding of estrogens to its receptor [55-56,62]. Melatonin, *in vitro*, decreases the expression of ERα and inhibits the binding of the E_2-ERα complex to the estrogen response element (ERE) on DNA [44,56,62], in a dose-dependent, saturable, and specific manner [62]. These effects of melatonin depend on its binding to specific melatonin (MT1) membrane receptors [4,38,60], which are present in normal and tumoral human breast tissue [32]. The overexpression of MT1 melatonin receptors in MCF-7 cells enhances the response of these cells to the antiestrogenic effects of melatonin [21,85]. The above mentioned data place melatonin among the substances known as selective estrogen receptors modulators (SERMs)

From the discovery of ERβ, it was possible to show two different kinds of biological action for estrogens, and pharmacologist look for SERMs with the ability to specifically block the ERα, overexpressed in more than 50% of breast cancer [59], but not the ERβ. Recently, it was demonstrated that whereas melatonin is a specific inhibitor of E_2-induced ERα-mediated transcriptional activation, it does not inhibit ERβ-mediated transactivation [31]. The sensitivity of the MCF-7 human breast cancer cells to melatonin depends on the ERα/ERβ ratio and is abolished by ERβ overexpression [31].

Calmodulin (CaM) has been proposed as the possible link for melatonin-E_2–ER interaction. This hypothesis is based on two facts: one, that the association of CaM with the E_2-ER complex facilitates its binding to an ERE, thus suggesting a role for CaM as a modulator of the transcriptional activity of the ER [12,18]; the second fact is that melatonin is able to bind to Ca^{++}/CaM and to inactivate the complex [7-8,30]. Only ERα, but not ERβ, interacts with CaM [35] stimulating the phosphorylation of the receptor, thus facilitating the binding of estrogen as well as that of the E_2-ER complex to the ERE [12,35]. In this context, melatonin binding to Ca^{++}/CaM inactivates the complex thus counteracting its positive effects on the estrogen signaling pathway [62]. Cells expressing the ERα (K302, 303G), a mutant of the ERα that does not interact with CaM, are not inhibited by melatonin, behaving like cells expressing ERβ. Upon binding of E_2, ER homodimerizes and interacts not only with specific ERE but also with other DNA bound proteins such as AP1 transcription factors; the ERα-mediated transcription in AP1 by E_2 depends on CaM and is also inhibited by melatonin [31].

Another possible interplay between melatonin and the ER could be the cAMP. The ERα may be activated by elevated intracellular concentrations of cAMP [2]. In MCF-7 cells, estrogens activate adenylate cyclase increasing intracellular cAMP by a non-transcriptional mechanism which involves steroid-induced modulation of cytoplasmic or cell membrane-bound regulatory proteins (non-genomic actions) [3]. The cAMP synergizes with the genomic actions of steroids since it enhances ER-mediated transcription [3]. Alternatively, melatonin, working through the membrane-bound Gi protein-coupled MT1 receptor, inhibits adenylate cyclase activity and decreases cAMP [36]. In this way, a melatonin-induced reduction in cAMP could be a mechanism by which the indoleamine decreases E_2-induced ER" transcriptional activity. In this sense, it has been demonstrated that melatonin inhibits forskolin-induced and E_2-induced elevation of cAMP in MCF-7 cells, and inhibits ER" gene transcription [42]. Furthermore, in murine mammary tissue, our group demonstrates that melatonin decreases cAMP and increases cGMP in a dose- and time-dependent way [17].

Over the last few years, numerous environmental pollutants with estrogenic activity have been identified; among them, some heavy metals such as cadmium (Cd) which have been so called “metalloestrogens”. Cd has been demonstrated to behave as an estrogen by binding to the ER and inducing the growth of estrogen-dependent organs such as uterus or mammary glands [39], this being one of the reasons for its consideration as carcinogenic agent. We have recently demonstrated that melatonin inhibits MCF-7 cell growth induced by Cd, by interacting with the metal in the ERα binding to both ERE and AP1sites [52]. These results constitute one more demonstration of the antiestrogenic nature of melatonin actions.

Oncostatic Properties of Melatonin Based on its Ability to Inhibit the Enzymes Involved in the Biosynthesis of Estrogens in Peripheral Tissues

The high incidence of hormone-dependent breast cancer in postmenopausal women suggests an important role of extragonadal steroids on mammary carcinogenesis [67]. The local estrogen synthesis in normal and neoplasic breast tissue depends on the aromatization of androgens by the activity of enzymes of the aromatase complex [14,37,74,86]. The aromatase activity in breast cancer tissue has been demonstrated to be higher than in non-malignant breast tissue or tissue distal to tumors, thus leading to the hypothesis that an increased production of estrogens within breast tumors may exert a biological effect and thereby stimulate tumor growth [14,74]. These are the reasons for the interest in developing drugs able to interfere with the synthesis of steroid hormones by inhibiting the enzymes controlling the interconversion from androgenic precursors, the so-called selective estrogen enzyme modulators (SEEMs) [5].

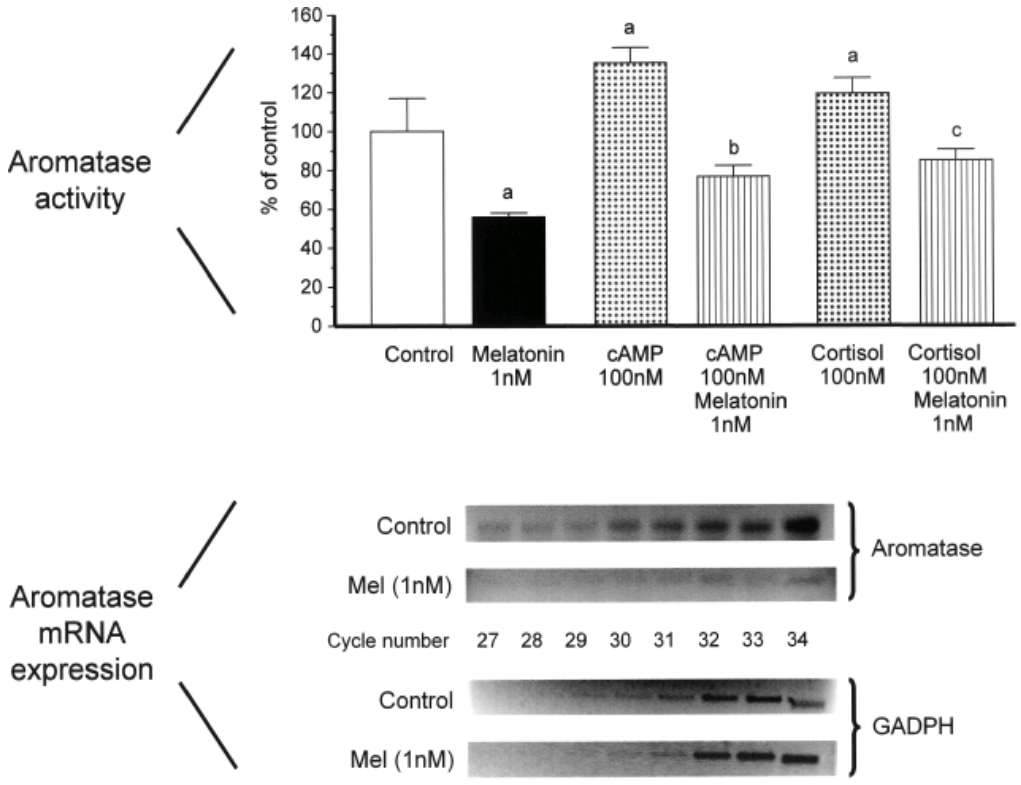

Figure 1. (Upper panel) Effects of melatonin on aromatase activity in MCF-7 human breast cancer cells. Melatonin inhibits aromatase activity both in basal conditions as well as under stimulation by either cAMP or cortisol. $^{a}p<0.001$ vs C, $^{b}p<0.001$ vs 100 nM cAMP, $^{c}p<0.01$ vs 100 nM cortisol. (Lower panel). Melatonin-induced inhibition of mRNA aromatase in MCF-7 cells. Modified from Cos et al., 2005 [25].

Recently, our group demonstrated, by using MCF-7 human breast cancer cells in culture, which express aromatase [76,89] and MT1 melatonin receptor [61,85], that melatonin, at physiological concentrations, reduces aromatase activity in these cells both under basal conditions and when aromatase activity is stimulated by cAMP or cortisol [25] (Figure 1, upper panel). Furthermore, the inhibitory effects of aminoglutethimide on the aromatase activity of MCF-7 cells is enhanced by pre-incubating the cells with melatonin [51]. This increased sensitivity of MCF-7 cells to the antiaromatase effect of aminoglutethimide after treatment with melatonin could be dependent of the melatonin induced decrease in the aromatase mRNA expression (Figure 1, lower panel), and encourages the study of the possible utility of the association of melatonin with other known antiaromatase drugs in the treatment of breast cancer.

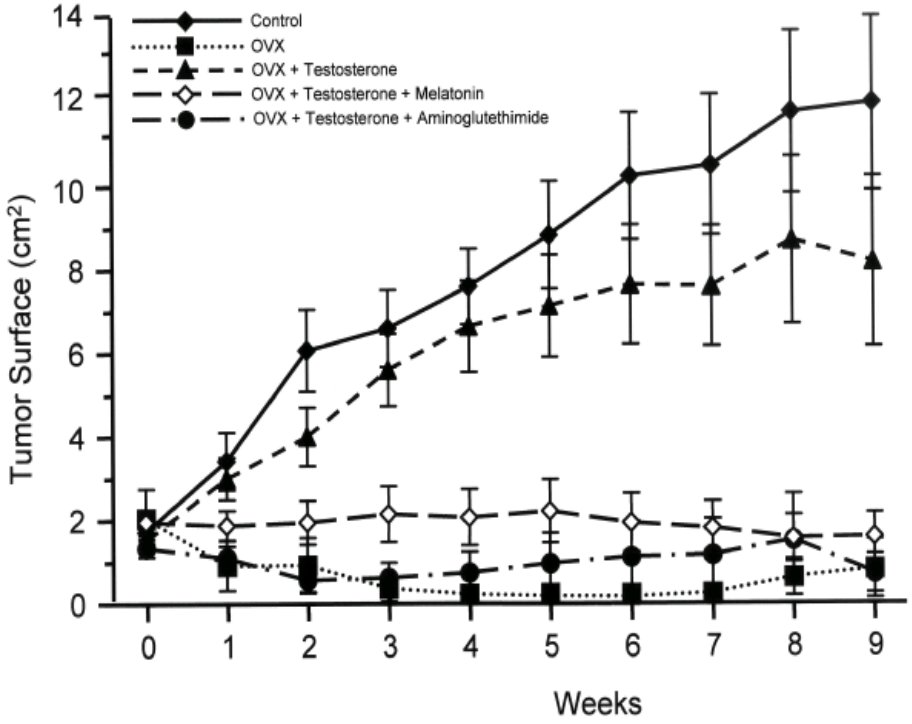

Figure 2. Evolution of estrogen-dependent DMBA-induced mammary adenocarcinomas in rats which, when bearing 1 cm diameter tumors, were ovariectomized (OVX) or sham-operated (control). OVX rats were then treated with testosterone, melatonin or aminoglutethymide. The tumors of control rats showed a progressive increased of surface, whereas those of OVX animals, because of the loss of gonadal steroids, did not grow. Treatment of OVX with testosterone let the tumor grow because the conversion of androgens to estrogens. Melatonin, as well as aminoglutethymide, (a well known aromatase inhibitor) counteract the stimulatory effects of testosterone on tumor growth. Modified from Cos et al., 2006 [24].

To investigate the in vivo aromatase-inhibitory properties of melatonin, we administered this indoleamine to rats bearing DMBA-induced mammary tumors, ovariectomized, and treated with testosterone. In castrated animals, the growth of the estrogen-sensitive mammary

tumors basically depends on the local aromatization of testosterone to estrogens. Melatonin significantly reduced the tumor size in rats treated with testosterone similar to the way the aromatase inhibitor aminoglutethimide does (Figure 2) [24]. Tumors from animals treated with melatonin had lower microsomal aromatase activity than tumors of animals from other groups (Figure 3), and the incubation of microsomal fractions of mammary tumors with melatonin decreased its aromatase activity [24]. These data support that melatonin could also exert its antitumoral effects on hormone-dependent mammary tumors by inhibiting the aromatase activity of the tumoral tissue.

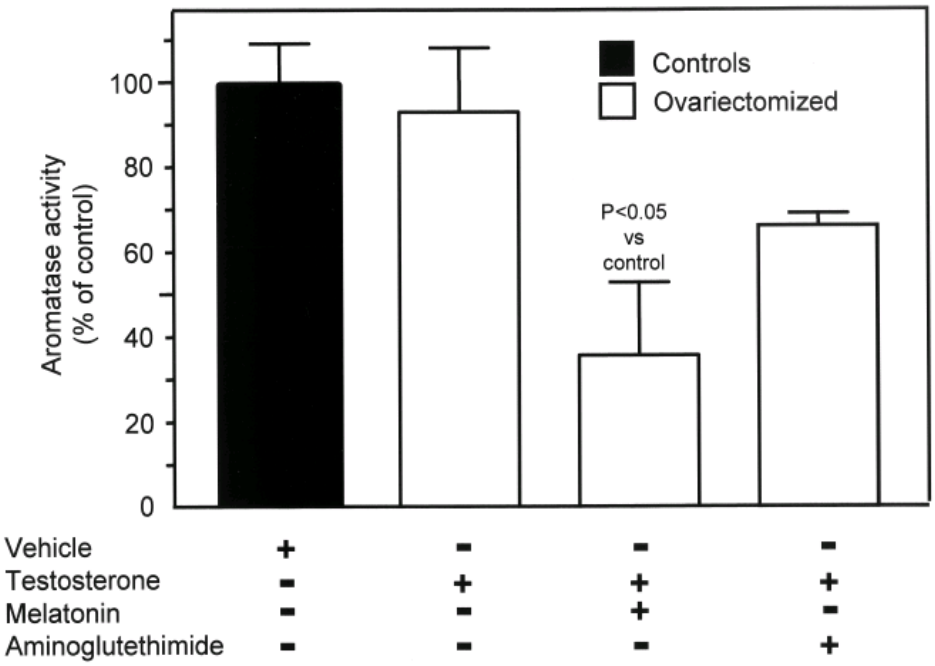

Figure 3. Microsomal aromatase activity on DMBA-induced mammary tumors in rats. The tumors were obtained from animals bearing DMBA-induced mammary adenocarcinomas, nine weeks after ovariectomy and treatment with testosterone, melatonin or aminoglutethimide. Modified from Cos et al., 2006 [24]

The mechanisms involved in the antiaromatase effects of melatonin are still unknown. However we have proposed some hypothesis for study. The aromatase gene (CYP19) is, in mammary cancer cells, under the control of promoters II and I.3, regulated by cAMP [15,87-88]. Melatonin, through a membrane-bound Gi protein-coupled receptor (MT1) downregulates cAMP in different cell types [6,36,42]. In MCF-7 cells, melatonin at nanomolar concentration reduces the forskolin-induced increase of cAMP [42] and our group described that melatonin, in murine mammary tissue, decreases cAMP and increases cGMP in a dose- and time-dependent way [17]. Taken together, these data suggest that melatonin could influence aromatase through its modulatory activity on cAMP.

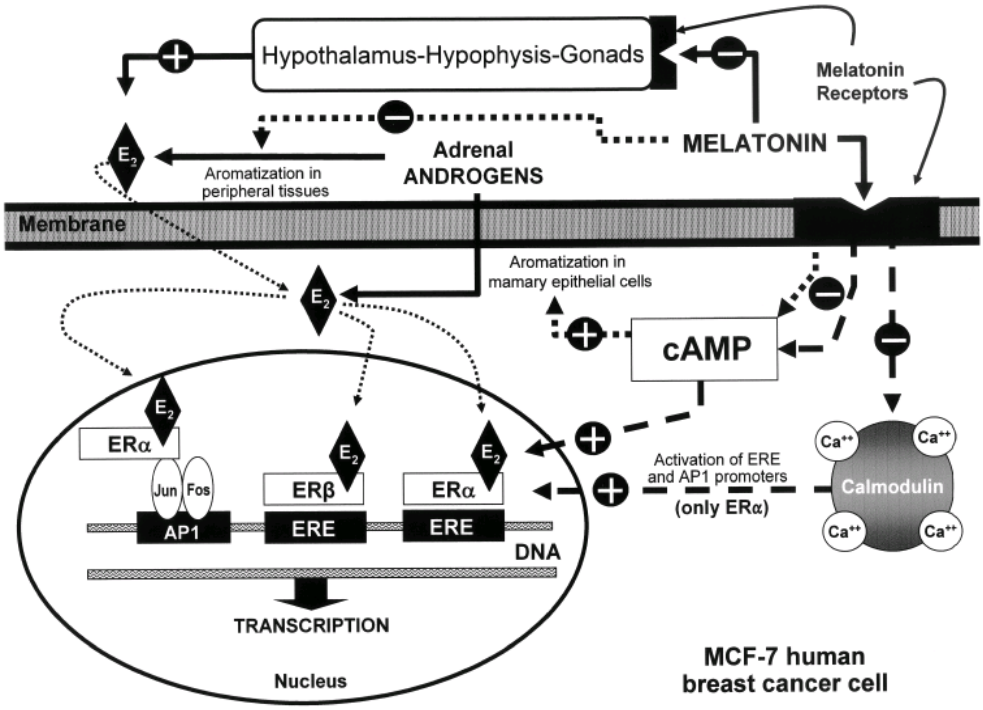

Figure 4. Diagram showing the three different mechanisms involved in the melatonin antiestrogenic actions. 1. (solid lines) Downregulation of gonadal synthesis of estrogens by acting on specific receptors at different levels of the neuroendocrine-gonadal axis; 2. (Broken lines) Interaction with the ERα (not with ERβ) at level of ERE and AP1 promoters; these actions are probably mediated by the inhibition of CaM or by the decreased formation of cAMP. 3. (Dotted lines) Inhibition of the local conversion of adrenal androgens to estrogens by modulating the activity and expression of aromatase; these effects are probably dependent on the decrease of cAMP induced by melatonin.

Conclusion

Among the therapeutic strategies for breast cancer treatment and prevention, those based on the selective neutralization of the effects of estrogens on mammary cells are the most widely used. Melatonin is a naturally occurring antiestrogen which share properties with most of the synthetic drugs used to either interact with the estrogens at the estrogenic-receptor level (SERMs), or to decrease the biotransformation of estrogens from adrenal androgens (SEEMs). We have here reported experimental data supporting that melatonin has properties of both SERMs and SEEMs and, because of its different mechanism of action (especially in the case of SERMs) could be complementary to these drugs in the treatment of mammary tumors. Furthermore, the specificity of the interactions of melatonin with E_2 in ERα but not the ERβ, gives to melatonin one of the features looked for an optimal SEEM. Figure 4 summarizes the three mechanisms involved in the antiestrogenic effects of melatonin.

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References

[1] Anisimov, VN. The role of pineal gland in breast cancer development. *Crit Rev Oncol Hematol*, 2003, 46:221-234.

[2] Aronica, SM; Katzenellenbogen, BS. Stimulation of estrogen receptor mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-I. *Mol Endocrinol,* 1993, 7:743-752.

[3] Aronica, SM; Kraus, WL; Katzenellenbogen, BS. Estrogen action via the cAMP signaling pathway: stimulation of adenylate cyclase and cAMP-regulated gene transcription. *Proc Nat Acad Sci USA*, 1994, 91:8517-8521.

[4] Baldwin, WS; Barrett, JC. Melatonin: receptor-mediated events that may affect breast and other steroid hormone-dependent cancers. *Mol Carcinogen*, 1988, 21:149-155.

[5] Barker, S. Anti-estrogens in the treatment of breast cancer: current status and future directions. *Curr Opin Investig Drugs,* 2003, 4:652-657.

[6] Barrett, P; Conway, S; Morgan, PJ. Digging deep-structure-function relationships in the melatonin receptor family. *J Pineal Res*, 2003, 35:221-230.

[7] Benitez-King, G; Huerto-Delgadillo, L; Anton-Tay F. Binding of 3H-melatonin to calmodulin. *Life Sci*, 1993, 53:201-207.

[8] Benitez-King, G; Rios, A; Martinez, A; Antón-Tay, F. In vitro inhibition of $Ca2^{+}$/calmodulin dependent kinase II activity by melatonin. *Biochim Biophys Acta*, 1996, 1290**:**191-196.

[9] Brainard, GC; Beacham, S; Sanford, BE; Hanifin, JP; Streletz, L; Sliney, D. The relationship between electromagnetic field and light exposures to melatonin and breast cancer risk: a review of the relevant literature*. J Pineal Res*, 1999, 26:65-100.

[10] Blask, DE; Hill, SM. Effects of melatonin on cancer: studies on MCF-7 human breast cancer cells in culture. *J Neural Transm*, 1986, 21:433-449.

[11] Bodis, J; Koppan, M; Kornya, L; Tinneberg, HR; Torok, A. Influence of melatonin on basal and gonadotropin-stimulated progesterone and estradiol secretion of cultured human granulosa cells and in the superfused granulosa cell system. *Gynecol Obstet Invest*, 2001, 52:198-202.

[12] Bouhoute, A; Leclercq, G. Modulation of estradiol and DNA binding to estrogen receptor upon association with calmodulin. *Biochem Biophys Res Commun,* 1995, 208:748-755.

[13] Brzezinski, A; Seibel, MM; Lynch, HJ; Deng, MH; Wurtman, RJ. Melatonin in human preovulatory follicular fluid. *J Clin Endocrinol Metab*, 1987, 64:865-867.

[14] Bulun, SE; Zeitoun, K; Sasano, H; Simpson, ER. Aromatase in aging women. *Semin Reprod Endocrinol*, 1999, 17,4:349-358.

[15] Bulun, SE; Sebastian, S; Takayama, K; Suzuki, T; Sasano, H; Shozu, M**.** The human CYP19 (aromatase P450) gene: update on physiologic roles and genomic organization of promoters. *J Steroid Biochem Mol Biol*, 2003, 86:219-224.

[16] Caplan, LS; Schoenfeld, ER; O´Leary, ES; Leske, MC. Breast cancer and electromagnetic fields-a review. *Ann Epidemiol,* 2000, 10:31-44.

[17] Cardinali, DP; Bonanni Rey, RA; Mediavilla, MD; Sánchez-Barceló, EJ. Diurnal changes in cyclic nucleotide response to pineal indoles in murine mammary glands. *J Pineal Res*, 1992, 13:111-116.

[18] Castoria, G; Migliaccio, N; Nola, E; Auricchio, F. *In vitro* interaction of estradiol receptor with C2+ calmodulin. *Mol Endocrinol*, 1988, 2:167-174.

[19] Cohen, M; Lippman, M; Chabner, B. Role of pineal gland in aetiology and treatment of breast cancer. *Lancet*, 1978, 2:814-816, 1978.

[20] Coleman, MP; Reiter, RJ. Breast cancer, blindness and melatonin. *Eur J Cancer,* 1992, 28:501-503.

[21] Collins, A; Yuan, L; Kiefer, TL; Cheng, O; Lai, L; Hill, SM. Overexpression of the MT1 melatonin receptor in MCF-7 human breast cancer cells inhibits mammary tumor formation in nude mice. *Cancer Lett*, 2003, 189:49-57.

[22] Cos, S; Blask, DE; Lemus-Wilson, A; Hill, SM. Effects of melatonin on the cell cycle kinetics and estrogen rescue of MCF-7 human breast cancer cells in culture. *J Pineal Res*, 1991, 10:36-42.

[23] Cos, S; Fernández, R; Güézmes, A; Sánchez-Barceló, EJ. Influence of melatonin on invasive and metastatic properties of MCF-7 human breast cancer cells. *Cancer Res*, 1998, 58:4383 ! 4390.

[24] Cos, S; González, A; Güezmes, A; Mediavilla, MD; Martínez-Campa, CM; Alonso-González, C; Sánchez-Barceló, EJ. 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, 2006, 118:274-278.

[25] Cos, S; Martínez-Campa, C; Mediavilla, MD; Sánchez-Barceló, EJ. Melatonin modulates aromatase activity in MCF-7 human breast cancer cells. *J Pineal Res*, 2005, 38:136-142.

[26] Cos, S; Mediavilla, MD; Martínez-Campa, CM; González, A; Alonso-González, C; Sánchez-Barceló, EJ. Exposure to light-at-night increases the growth of DMBA-induced mammary adenocarcinomas in rats. *Cancer Lett,* 2006, 235:266-271.

[27] Cos, S; Recio, J; Sánchez-Barceló, EJ. Modulation of the length of the cell cycle time of MCF-7 human breast cancer cells by melatonin. *Life Sci,* 1996, 58:811-816.

[28] Cos, S; Sánchez-Barceló, EJ. Melatonin and mammary pathological growth. *Front Neuroendocrinol*, 2000, 21:133-170.

[29] Cos S, Sánchez-Barceló EJ. Melatonin, experimental basis for a possible application in breast cancer prevention and treatment. *Histol Histopathol*, 2000, 15:637-647.

[30] Dai, J; Inscho, EW; Yuan, L; Hill, SM. Modulation of intracellular calcium and calmodulin by melatonin in MCF-7 human breast cancer cells. *J Pineal Res,* 32:112-119, 2002.

[31] Del Rio, B; Garcia-Pedrero, OJ; Martínez-Campa, C; Zuazua, P; Lazo, PS, Ramos S. Melatonin: An endogenous specific inhibitor of estrogen receptor α via calmodulin. *J Biol Chem*, 2004, 279:38294-38302, 2004.

[32] Dillon, DC; Easley, SE; Asch, BB; Cheney, RT; Brydon, L; Jockers, R; Winston, JS, Brooks JS, Hurd, T; Asch, HL. Differential expression of high-affinity melatonin receptors (MT1) in normal and malignant human breast tissue. *Am J Clin Pathol,* 2002, 118:451-458.

[33] Dubocovich, ML; Rivera-Bermudez, MA; Gerdin, MJ; Masana, MI. Molecular pharmacology, regulation and function of mammalian melatonin receptors. *Front Biosci*, 2003, 8:1093-1108.

[34] Feychting, M; Osterlund, B; Ahlbom, A. Magnetic fields and breast cancer in Swedish adults residing near high-voltage power lines, *Epidemiology*, 1998, 9:392-397.

[35] García-Pedrero, J; Martínez, MA; Del Rio, B; Martínez-Campa, CM; Muramatsu, M; Lazo, PS; Ramos, S. Calmodulin is a selective modulator of estrogen receptors. *Mol Endocrinol*, 2002,16:947-960.

[36] Godson, C; Reppert, SM. The Mel_{1A} melatonin receptor is coupled to parallel signal transduction pathways. *Endocrinology*, 1997, 138:397-404.

[37] Harada N. Aberrant expression of aromatase in breast cancer tissues. *J Steroid Biochem Mol Biol*, 1997,61:175-84.

[38] Jones, MP; Melan, MA; Witt-Enderby, PA. Melatonin decreases cell proliferation and transformation in a melatonin receptor-dependent manner. *Cancer Lett* 2000, 151:133-143.

[39] Johnson, MD; Kenney, N; Stoica, A; Hilakivi-Clarke, L; Singh, B; Chepko, G; Clarke, R; Sholler, PF; Lirio, AA; Foss, C; Reiter, R; Trock, B; Paik, S; Martin, MB. Cd mimics the in vivo effects of estrogen in the uterus and mammary gland. *Nat Med*, 2003, 9:1081-1084.

[40] Kauppila, A; Kivela, A; Pakarinen, A; Vakkuri, O. Inverse seasonal relationship between melatonin and ovarian activity in humans in a region with a strong seasonal contrast in luminosity. *J Clin Endocrinol Metab*, 1978, 65:823-8.

[41] Kheifets, LI; Matkin, CC. Industrialization, electromagnetic fields, and breast cancer risk. *Environ Health Perspect*, 1999, 107:145-54.

[42] Kiefer, T; Ram, PT; Yuan, L; Hill, SM. Melatonin inhibits estrogen receptor transactivation and cAMP levels in breast cancer cells. *Breast Cancer Res Treat*, 2002; 71:37-45.

[43] Kliukiene, J; Tynes, T; Andersen, A. Risk of breast cancer among Norwegian women with visual impairment, *Br. J. Cancer*, 2001, 84:397-399.

[44] Lawson NO, Wee BE, Blask DE; Castles, CG; Spriggs, LL; Hill, SM. Melatonin decreases estrogen receptor expression in the medial preoptic area of inbred (LSH/SsLak) golden hamsters. *Biol Reprod* 1992; 47:1082-1090.

[45] Leon-Blanco, MM; Guerrero, JM; Reiter, RJ; Calvo, JR; Pozo, D. Melatonin inhibits telomerase activity in the MCF-7 tumor cell line both in vivo and in vitro. *J Pineal Res*, 2003, 35:204-11

[46] León-Blanco, MM; Guerrero, JM; Reiter, RJ; Pozo. RNA expression of human telomerase subunits TR and TERT is differentially affected by melatonin receptor agonists in the MCF-7 tumor cell line. *Cancer Lett*, 2004, 216:73-80.

[47] Lerner, AB; Case, JD, Takahashi, Y; Lee, TH; Mori, W. Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J Am Chem Soc*, 1958, 80:2587.

[48] Lissoni, P; Barni, S; Meregalli, S; Fossati, V; Cazzaniga, M; Eposti, D; Tancini, G. 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*, 1995, 71:854-856.

[49] Luboshitzky, R; Lavie, P. Melatonin and sex hormones interrelationships, a review. *J Pediatr Endocrinol Metab*, 1999, 12:355-362.

[50] Malpaux, B; Migaud, M; Tricoire, H; Chemineau, P. Biology of mammalian photoperiodism and the critical role of the pineal gland and melatonin. *J Biol Rhythms*, 2001, 16:336-347.

[51] Martínez-Campa CM, González A, Mediavilla MD, Alonso-González C, Sánchez-Barceló EJ, Cos S. Melatonin enhances the inhibitory effect of aminoglutethimide on aromatase activity in MCF-7 human breast cancer cells. *Breast Cancer Res Treat*, 2005, 94:249-254.

[52] Martínez-Campa, CM; Alonso-González, C; Mediavilla, MD; Cos, S; González, A; Ramos, S; Sánchez-Barceló EJ. Melatonin inhibits both ERα activation and breast cancer cell proliferation induced by a metalloestrogen, Cadmium. J Pineal Res, 2006, 40:291-296.

[53] Mediavilla, MD; Cos, S; Sánchez-Barceló, EJ. Melatonin increases p53 and p21WAF1 expression in MCF-7 human breast cancer cells in vitro. *Life Sci* 1999, 65:415-420.

[54] Mediavilla, MD; Güezmez, A; Ramos, S; Kothari, L; Garijo, F; Sánchez-Barceló, EJ. Effects of melatonin on mammary gland lesions in transgenic mice overexpressing N-ras proto-oncogene. *J Pineal Res*, 1997; 22:86-94.

[55] Molis, TM; Walters, MR; Hill SM. Melatonin modulation of estrogen receptor expression in MCF-7 human breast cancer cells. *Int J Oncol*, 1993, 3:687-694.

[56] Molis, TM; Spriggs, LL; Hill, SM. Modulation of estrogen receptor mRNA expression by melatonin in MCF-7 human breast cancer cells. *Mol Endocrinol*, 1994, 8:1681-1690.

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

[58] Nakamura, Y; Tamura, H; Takayama, H; Kato, H. Increased endogenous level of melatonin in preovulatory human follicles does not directly influence progesterone production. *Fertil Steril*, 2003, 80:1012-1016.

[59] Pujol, P; Rey, JM; Nirde, P; Roger, P; Gastaldi, M; Laffargue, F; Rochefort, H; Maudelonde, T. Differential expression of estrogen receptor-alpha and -beta messenger RNAs as a potential marker of ovarian carcinogenesis. *Cancer Res,* 1998 ;58:5367-73.

[60] Ram, PT; Day, J; Yuan, L; Dong, C; Kiefer, TL, Lai, L; Hill, SM. Involvement of the mt1 melatonin receptor in human breast cancer. *Cancer Lett*, 2002, 179:141-150.

[61] Ram, PT; Kiefer, TL; Silverman, M; Song, Y; Brown, GM; Hill, SM. Estrogen receptor transactivation in MCF-7 breast cancer cells by melatonin and growth factors. *Mol Cell Endocrinol*, 1998;141:53-64.

[62] Rato, AG; García-Pedrero, JM; Martínez, MA; Del Rio, B; Lazo, PS; Ramos, S. Melatonin blocks the activation of estrogen receptor for DNA binding. *FASEB*, 1999, 13:857-868.

[63] Reiter, RJ.The pineal gland and its hormones in the control of reproduction in mammals. *Endocr Rev*, 1980, 1:109-131.

[64] Reiter, R. The mammalian pineal gland as an end organ of the visual system, In: Wetterberg, L, editor. *Light and biological rhythms in man*. Oxford, England: Pergamon Press, 1993; 145-160.

[65] Reiter, RJ. Mechanisms of cancer inhibition by melatonin. J. Pineal Res, 2004, 37:213-214.

[66] Rönnberg, I; Kauppila, A; Leppäluoto, J; Martikainen, H; Vakkuri, O. Circadian and seasonal variations in human preovulatory follicular fluid melatonin concentration. *J Clin Endocrinol Metab*, 1990, 71:493-496.

[67] Russo, IH; Russo, J. Role of hormones in mammary cancer initiation and progression. *J Mammary Gland Biol Neoplasia*, 1998, 3:49-61.

[68] Sánchez-Barceló, EJ; Cos, S; Fernández, R; Mediavilla, MD. Melatonin and mammary cancer: a short review. *Endocr Relat Cancer*, 2003,10:153-159.

[69] Sánchez-Barceló, EJ; Cos, S; Mediavilla, MD; Martínez-Campa, CM; González, A; Alonso-González, C. Melatonin-estrogen interactions in breast cancer. *J Pineal Res,* 2005, 38:217-222.

[70] Sánchez-Barceló, EJ; Cos, S; Mediavilla, MD. Influence of pineal gland function on the initiation and growth of hormone-dependent breast tumors. Possible mechanisms. In: Gupta, D; Attanasio, A; Reiter, RJ, editors. *The Pineal Gland and Cancer*. Tübingen, Germany: Brain Research Promotion; 1988; 221-232.

[71] Schernhammer, ES; Rosner, B; Willett, WC; Laden, F; Colditz, GA; Hankinson, SE. Epidemiology of urinary melatonin in women and its relation to other hormones and night work. *Cancer Epidemiol Biomarkers Prev*, 2004, 13:936-43.

[72] Schernhammer, ES; Schulmeister, K. Melatonin and cancer risk: does light at night compromise physiologic cancer protection by lowering serum melatonin levels? *Br J Cancer*, 2004, 90:941-3.

[73] Schernhammer, ES; Hankinson, SE. Urinary melatonin levels and breast cancer risk. *J Natl Cancer Inst*, 2005, 97:1084-1087.

[74] Simpson, E; Rubin, G; Clyne, C; Robertson, K; O'Donnell, L; Davis, S; Jones, M. Local estrogen biosynthesis in males and females. *Endocr Relat Cancer*, 1999, 6:131-137.

[75] Soares, JM; Masana, MI; Ersahin, C; Duvocovich, ML. Functional melatonin receptors in rat ovaries at various stages of the estrous cycle. *J Pharmacol Exp Ther*, 2003, 306:694-702.

[76] Sonne-Hansen, K; Lykkesfeldt, AE. Endogenous aromatization of testosterone results in growth stimulation of the human MCF-7 breast cancer cell line. *J Steroid Biochem Mol Biol*, 2005, 93:25-34.

[77] Stevens, RG. Circadian disruption and breast cancer. In: Bartsch, C; Bartsch, H; Blask, DE; Cardinali, DP; Hrushesky, WJM; Mecke, D, editors. *The Pineal Gland and Cancer. Neuroimmunoendocrine Mechanisms in Malignancy.* Berlin:Springer; 2001; 511-517.

[78] Tamarkin, L; Danforth, D; Lichter, A; DeMoss, E; Cohen, M; Chabner, B; Lippman M. Decreased nocturnal plasma melatonin peak in patients with estrogen receptor positive breast cancer. *Science*, 1982, 216:1003-1005.

[79] Vanecek, J. Melatonin inhibits increase of intracellular calcium and cyclic AMP in neonatal rat pituitary via independent pathways. *Mol Cell Endocrinol*, 1995, 107:149-153.

[80] Verkasalo, PK; Pukkala, E; Stevens, RG; Ojamo, M; Rudanko, SL. Inverse association between breast cancer incidence and degree of visual impairment in Finland. *Br J Cancer*, 1999, 80:1459-1460.

[81] Webley, GE; Luck, MR. Melatonin directly stimulates the secretion of progesterone by human and bovine granulosa cells in vitro. *J Reprod Fertil,* 1986, 78:711-717

[82] Wilson, ST; Blask, DE; Lemus-Wilson, AM. Melatonin augments the sensitivity of MCF-7 human breast cancer cells to tamoxifen in vitro. *J Clin Endocrinol Metab*, 1992, 75:669-670.

[83] Woo, MM; Tai, CJ; Kang, SK; Nathwani, PS; Pang, SF; Leung, PCK. Direct actions of melatonin in human granulose-luteal cells. *J Clin Endocrinol Metab,* 2001, 86:4789-4797.

[84] Yie, SM; Niles, LP; Younglai, EV. Melatonin receptors on human granulose cell membranes. *J Clin Endocrinol Metab*, 1995, 80:1747-1749.

[85] Yuan, L; Collins, AR; Dai, J; Dubocovich, ML; Hill, SM. MT1 melatonin receptor overexpression enhances the growth suppressive effects of melatonin in human breast cancer cells. *Mol Cell Endocrinol*, 2002, 192:147-156.

[86] Yue, W; Wang, JP; Hamilton, CJ; Demers, LM; Santen, RJ. In situ aromatization enhances breast tumor estradiol levels and cellular proliferation. *Cancer Res*, 1998, 58:927-932

[87] Zhao, Y; Agarwal, VR; Mendelson, CR; Simpson, ER. Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE_2 via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene. *Endocrinology*, 1996, 137:5739-5742.

[88] Zhou, D; Clarke, P; Wang, J; Chen, S. Identification of a promoter that controls aromatase expression in human breast cancer and adipose stromal cells. *J Biol Chem*, 1996, 271:15194-15202.

[89] Zhou, D; Wang, J; Chen, E; Murai, J; Siiteri, PK; Chen, S. Aromatase gene is amplified in MCF-7 human breast cancer cells. *J Steroid Biochem Mol Biol*, 1993, 46:147-153.