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MINI REVIEW

Melatonin–estrogen interactions in breast cancer

Abstract: In this article, we review the experimental data supporting an oncostatic role of melatonin on hormone-dependent mammary tumors. Beginning with the evidence on the role of estrogens in breast cancer etiology and mammary tumor growth, we summarize the actual therapeutic strategies with estrogens as a target. Additionally, we demonstrate that melatonin fulfills all the requirements to be considered as an antiestrogenic drug which shares properties with drugs of the two main pharmacological groups of substances which interact with the estrogen-signaling pathways such as: (i) drugs that act through the estrogen receptor interfering with the effects of endogenous estrogens; and (ii) drugs that interfere with the synthesis of estrogens by inhibiting the enzymes controlling the interconversion from their androgenic precursors. Furthermore, melatonin decreases circulating levels of estradiol. These three antiestrogenic mechanisms suggest that melatonin may have an important role in the prevention and treatment of hormone-dependent mammary cancer.

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Introduction

For more than 25 yr many research groups, including our own, have been working on the hypothesis of the possible oncostatic role of melatonin on several kinds of tumors, but especially on hormone-dependent mammary cancer. There is general agreement that melatonin, in vivo, prevents promotion and growth of spontaneous or chemically induced mammary tumors in rodents, whereas in vitro melatonin inhibits breast cancer cell proliferation and invasiveness [1–3].

The oncostatic properties of melatonin could be explained in variety of ways based on the different known actions of the indoleamine. Thus, the antitumor actions of melatonin may be considered: (i) as indirect effects derived from its interaction with the neuroendocrine reproductive axis [4] leading to a down-regulation of some of the hormones influencing tumor growth, especially gonadal estrogens; (ii) as a consequence of its interaction with estrogen receptors (ER) on the epithelial mammary cells [5] in a similar way as classic antiestrogens act; (iii) dependent on its immuno-enhancing effects [6]; (iv) as a consequence of its antioxidant properties [7]; or (v) derived from its inhibitory effects on telomerase activity in tumor cells [8]. Of all these different mechanisms by which melatonin may influence mammary cancer, we have focused our interest on its interaction with estrogen-signaling pathways. In fact, either directly or indirectly, melatonin–estrogen interaction is involved in all the above proposed oncostatic mechanisms of melatonin, as telomerase activity and immune function are, in some way, related to the levels of estrogens, and some estrogen metabolites are among the agents responsible for DNA oxidative damage. In this review, we summarize the evidence that considers melatonin as an antiestrogen and aromatase inhibitor and, consequently, as a useful molecule in the prevention and treatment of hormone-dependent breast cancer.

Estrogens and mammary cancer

The role of the ovarian estrogens in mammary cancer has been known since 1896 when Beatson demonstrated that ovariectomy inhibited the growth of breast tumors [cited in 9]. It is important to recognize that, until now, most of the advances in the treatment of this malignancy came from experiments carried out on rodents and cell lines, most of them based, in some way, on this former idea.

Why are estrogens considered key molecules in mammary carcinogenesis? From the pioneer experiments of Beatson, considerable evidence from epidemiologic and experimental studies [10] left no doubt as to the role of estrogens on mammary carcinogenesis. However, it is still controversial as to whether these effects are dependent on the stimulatory actions of estrogens on epithelial cell proliferation (indirect carcinogenic effects), or whether estrogens or their metabolites act as mutagenic agents (direct carcinogenic effects). In the first case, estrogens, by stimulating cell proliferation mainly through the ERα could increase the probability of propagation of mutations induced by different carcinogenic agents [11]. Concerning the possible direct genotoxic effects of the estrogens, some estrogenic metabolites, especially the catechol-estrogens are considered carcinogens, as their oxidation generates free radicals which produce oxidative lesions in DNA [12, 13].

In relation to the role of estrogens in the genesis and evolution of mammary tumors, it is important to consider that two-thirds of breast cancer occurs in postmenopausal women, where ovaries have ceased to be functional and circulating levels of estrogens are low. Nevertheless, in these cases, the concentration of estradiol (E_2) in breast tumors is higher than in plasma and normal breast tissue [14]. This is presumably due to the in situ biosynthesis and accumulation of estrogens by breast tissue. Adrenal androgens are the substrate for this extra-gonadal biosynthesis of estrogens, and the 'aromatase pathway' is one of the principal enzymatic systems involved in the process [15, 16].

Therapeutic strategies for breast cancer treatment with estrogens as a target

After the above-mentioned surgical ovariectomy as the first 'antiestrogenic' treatment, different pharmacological strategies have been employed to selectively neutralize the effects of estrogens on mammary cells. These pharmacological approaches are summarized as follows: (i) development of drugs that act through the ER interfering with the effects of the endogenous estrogens; this group includes the so-called selective estrogen receptor modulators (SERMs) of which tamoxifen and its derivatives are the most representative examples. After the identification and characterization of two ERs (α and β), optimal SERMs would be those that selectively block the ERα but not the ERβ; (ii) development of drugs that interfere with the synthesis of steroid hormones by inhibiting the enzymes controlling the interconversion from androgenic precursors. These are selective estrogen enzyme modulators (SEEMs), which include steroidal (formestane, exemestane, etc.) as well as nonsteroidal (anastrozole, letrozole, etc.) compounds [17, 18].

Melatonin and estrogens

As is mentioned in the introduction of this review, the consideration of melatonin as a natural substance useful in the prevention or treatment of estrogen-dependent breast cancer is based on the different actions of this indoleamine; however, in all cases the interaction of melatonin with estrogens could be the key to understand its oncostatic properties. Estrogens are metabolized by cytochrome P450 enzymes to hydroxylated products such as 2-, 4-, and 16α-hydroxyestradiol. These two and four hydroxylated catechol-estrogens are oxidized to semiquinones which, in the presence of molecular oxygen, are oxidized to quinones with formation of superoxide anion radicals and hydroxyl radicals which are responsible for carcinogenicity of these steroids [19]. As melatonin has been shown to act as a potent free radical scavenger [7] and to attenuate the E_2-induced oxidative damage in tissues such as the kidney and liver [20], its oncostatic properties could possibly be explained, at least in part, as a consequence of its antioxidant actions [21]. However, immuno-enhancing properties of melatonin have been also considered as an explanation for its antitumor actions. It is well known that estrogens modulate immune function and that high concentrations of estrogens suppress cell-mediated immune responses [22]; thus, the antiestrogenic action of melatonin could be linked to its immuno-enhancing effects. Finally, the inhibitory effect of melatonin on telomerase activity in MCF-7 human breast cancer cells [8] has been considered as the basis for its antitumor action, but also in this case, the antiestrogenic effects of melatonin could be the link to its effects on telomerase, as recently it has been demonstrated that estrogens possess the ability to up-regulate telomerase activity [23, 24]. Without excluding the above-mentioned possibilities, here we focus on melatonin's oncostatic effects based on its interaction with either the synthesis of estrogens or with the estrogen-signaling pathways; these have been the most extensively studied hypotheses and research projects of our group.

Melatonin could influence estrogenic actions in three different ways (Fig. 1): (i) by down-regulating gonadal synthesis of steroids and, consequently, decreasing their circulating levels; (ii) by interacting with the ER, thus behaving as an SERM; and (iii) by down-regulating the activity of some enzymes, such as aromatase, involved in the synthesis of estrogens from androgens, i.e. behaving as a SEEM. The evidence supporting each of these three possibilities is now discussed.

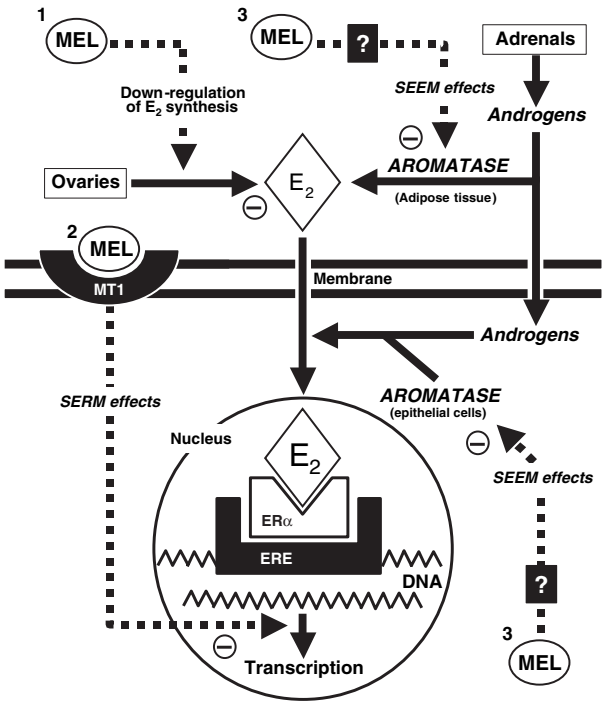

Fig. 1. Representation of the three mechanisms whereby melatonin reduces estrogen-mediated cancer growth: (1) by down-regulating gonadal synthesis of steroids by acting on receptor sites within the neuroendocrine reproductive axis, (2) by decreasing the expression of estrogen receptor (ERα) and inhibiting the binding of the E_2-ER complex to the estrogen response element (ERE) in the DNA, thus behaving as a selective estrogen receptor modulator (SERM). This effect depends on melatonin binding to specific membrane receptors (MT1), and (3) by acting as a selective estrogen enzyme modulator (SEEM), decreasing aromatase activity in the epithelial breast cancer cells and perhaps in the adipocytes, both responsible for the local biosynthesis of estrogens in mammary tissue. The mechanism of these SEEM effects is still unknown although it probably implies the melatonin binding to MT1 and changes in cAMP.

Melatonin modulation of plasma concentrations of estrogens

Melatonin was formerly considered as a hormone controlling seasonal reproduction in wild animals [4, 25]. In seasonally breeding mammalian species, melatonin controls reproductive function through the activation of receptor sites within the hypothalamic-pituitary axis thus driving the levels of gonadal activity [26–28]. Melatonin down-regulation of the ovarian estrogen secretion has been observed in a variety of mammals [4]. In one of the first published articles relating to the pineal gland and breast cancer [29], the authors proposed the hypothesis that 'impaired pineal secretion (hyposecretion of melatonin) results in unopposed estrogen secretion and an increased susceptibility to breast cancer', they concluded that 'melatonin, by suppression of estrogen secretion, or by direct inhibitory effects on breast tissue, might suppress induction of breast cancer'. Although in humans the role of melatonin on the reproductive system is not completely clear, an inverse relationship between melatonin and ovarian activity [30] and a certain role of melatonin in the modulation of neuroendocrine-reproductive axis has been also proposed [31, 32]. Furthermore, direct modulatory effects of melatonin on ovarian steroidogenesis have been demonstrated in human granulosa-luteal cells [33] as well as the presence of functional melatonin receptors in cells of antral follicles and corpora lutea of rat ovaries [34]. Together, these data suggest that melatonin modulates ovarian function by down-regulating the production of estrogens, thereby supporting the above-mentioned hypothesis of its role in breast cancer.

SERM properties of melatonin

In the previous section, we reviewed how melatonin could down-regulate circulating levels of estrogens, we now summarize the evidence supporting the theory that melatonin may counteract the effects of estrogens, thus behaving as a naturally occurring SERM.

Several years ago, our group demonstrated that the growth of chemically induced ER-positive mammary tumors in ovariectomized rats treated with exogenous E_2 was significantly reduced when these animals were subjected to some of the experimental manipulations known as enhancers of pineal-dependent effects (anosmia, underfeeding or exposure to cold, associated with light deprivation) [1, 2, 35]. These antitumor effects could not be explained by a pineal-dependent decline in circulating estrogens, as serum E_2 concentrations were kept stable because of the exogenous administration of steroids and the lack of changes in the rate of metabolism of steroids. The results of these experiments suggested that melatonin, the main pineal product, may counteract the effects of estrogens at the level of the tumor.

However, it was from in vitro studies, carried out basically with the estrogen-sensitive MCF-7 human breast cancer cells, that the direct antiestrogenic effects of melatonin were established. Melatonin, at concentrations similar to those found in serum of most mammals during the nocturnal period (1 nM), counteracts E_2-induced MCF-7 cell proliferation and invasiveness [1, 36, 37], augments the sensitivity of MCF-7 to antiestrogens such as tamoxifen [38], and down-regulates the expression of proteins, growth factors, and proto-oncogens regulated by estrogens [39, 40]. In humans, administration of melatonin together with tamoxifen induced objective tumor regression in metastasic breast cancer patients refractory to tamoxifen alone [41]. 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 in ERα-positive cells [42].

The mechanism involved in the antiestrogenic actions of melatonin is still being studied. 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 [5, 43, 44]. What melatonin seems to do is to decrease the expression of ERα and to inhibit the binding of the E_2-ER complex to the estrogen response element (ERE) on DNA [5, 44, 45]. These effects have been shown to be dependent on melatonin binding to specific melatonin (MT1) membrane receptors [46–48] and the overexpression of these receptors in MCF-7 cells enhances the response of these cells to the antiestrogenic effects of melatonin [42, 49]. The MT1 receptors have also been found in human breast tissue, both normal and tumoral [50]. Thus, melatonin behaves as an antiestrogen which does not bind to ER but to its own membrane receptors, and via this binding to its specific receptors it is able to interact with the ER-signaling pathway.

What are the links between the signaling pathways of melatonin and estrogens? A possible interplay between these two pathways could be the opposing modulation of cyclic adenosine monophosphate (cAMP) intracellular concentrations. The ERα may be activated by elevated intracellular concentrations of cAMP [51]. In MCF-7 cells, estrogens activate adenylate cyclase increasing intracellular cAMP by a nontranscriptional mechanism which involves steroid-induced modulation of cytoplasmic or cell membrane-bound regulatory proteins (nongenomic actions) [52]. The cAMP synergizes with the genomic actions of steroids as it enhances ER-mediated transcription [52]. Alternatively, melatonin, working through the membrane-bound Gi protein-coupled MT1 receptor, inhibits adenylate cyclase activity and decreases cAMP [53]. A melatonin-induced reduction in cAMP could be a mechanism by which the indoleamine decreases E_2-induced ERα 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 ERα gene transcription [54].

Another possible link for melatonin–E_2 interaction may be calmodulin (CaM). 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 [55, 56]. Interestingly, only ERα, but not ERβ, interacts with CaM [57] 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 [56, 57]. In this context, melatonin is known to exert modulatory effects on the Ca^{2+}/CaM-signaling pathway [58–60]. Melatonin binding to Ca^{2+}/CaM inactivates

the complex thus counteracting its positive effects on the estrogen-signaling pathway [44].

As indicated above, one of the desirable properties of an SERM is its ability to specifically block the ERα but not 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 [61]. The sensitivity of the MCF-7 human breast cancer cells to melatonin depends on the ERα/ERβ ratio and is abolished by ERβ overexpression [61]. Another important point supporting the mediation of CaM on the antiestrogenic effects of melatonin is that proliferation of cells expressing the ERα (K302, 303G), a mutant of the ERα that does not interact with CaM, is not inhibited by melatonin, behaving like cells expressing ERβ.

Melatonin properties as SEEM

What is the evidence which supports the hypothesis of the possible anti-aromatase actions of melatonin in breast cancer cells? In adipose tissue of tumor-bearing breasts as well as in MCF-7 human cancer cells, expression of the CYP19 gene, which encodes aromatase P450, the enzyme responsible for estrogen biosynthesis, is regulated by two proximal promoters, namely, I.3 and II [62, 63]. These promoters respond to cAMP [15, 64], which plays an important role in the positive regulation of the expression of aromatase in breast cancer cells. Consequently, any agent that modulates intracellular levels of cAMP could also influence aromatase expression on breast cancer cells. This is the case with prostaglandin E_2 which increases intracellular cAMP levels and stimulates aromatase and estrogen biosynthesis [65]. Estrogens also increase cAMP in breast cancer cells by a mechanism involving its binding to some membrane sites [52]. Thus, in breast cancer cells, but not in normal epithelial cells with different CYP19 promoters, estrogens may induce, through a paracrine loop, the local biosynthesis of estrogens via the increase of cAMP and expression of aromatase. At this point it is necessary to remember, as mentioned in the previous paragraph, that melatonin, after its binding to MT1 membrane receptor linked to Gi proteins, decreases the activity of adenylate cyclase and subsequently reduces cAMP synthesis [53] and that the incubation of homogenates of mice mammary tissue with melatonin decreases cAMP accumulation and increases cGMP, in a dose- and time-dependent manner [66].

Until now, the studies on the possible effects of melatonin on aromatase activity have been rare and limited mainly to the field of the andrology. Thus, the low sperm quality of human seminal plasma has sometimes been attributed to a low aromatase activity dependent on melatonin [67, 68]. We recently demonstrated, by using MCF-7 cells, which express aromatase [69] and MT1 melatonin receptors [42, 70], 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. Furthermore, we demonstrated by reverse transcriptase-polymerase chain reaction, that melatonin in MCF-7 cells down-regulates aromatase expression at the transcriptional level [71].

Conclusions

Melatonin is a neurohormone with different actions which include the down-regulation of the circulating levels of gonadal estrogens. Simultaneously, melatonin works as an antiestrogen with mechanisms of action different (and probably complementary) to those of the commercially available antiestrogens, and inhibits aromatase expression in human breast cancer cells (Fig. 1). These properties, collectively, make melatonin an interesting anticancer drug in the prevention and treatment of estrogen-dependent tumors, as it has the advantage of acting at different levels of the estrogen-signaling pathways. The reason clinical studies looking for the possible applicability of melatonin in breast cancer are still so rare is inexplicable in light of the results from experimental studies reviewed in this article. However, melatonin as an inhibitor of hormone-dependent cancers in humans is worthy of test.

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