Histology and Histopathology
Cellular and Molecular Biology

Invited Review

Melatonin, experimental basis for a possible application in breast cancer prevention and treatment

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Summary. The role of the pineal as an oncostatic gland has been studied in animal models of tumorigenesis, especially on those concerning the mammary gland. The general conclusion is that experimental manipulations activating pineal gland, or the administration of melatonin, reduce the incidence and growth rate of chemically-induced murine mammary tumors, while pinealectomy or situations which implicate a reduction of melatonin production usually stimulate mammary carcinogenesis. The direct actions of melatonin on mammary tumors have been suggested because of its ability to inhibit, at physiological doses (1nM), the in vitro proliferation of MCF-7 human breast cancer cells. In this article we review the outstanding findings related to melatonin actions on mammary which, taken together, support a possible usefulness of this indoleamine in the prevention and treatment of mammary gland malignancy.

Key words: Melatonin, Pineal gland, Breast cancer, Mammary cancer, MCF-7 cells

Introduction

The role of the pineal gland, mediated by its hormonal secretion melatonin, in the growth and spread of different kinds of tumors has been the subject of numerous studies from the first quarter of this century (Reiter, 1988; Bartsch et al., 1992; Blask, 1993). Among the neoplastic processes influenced by melatonin, those affecting the mammary gland have been the most extensively studied. More than twenty years ago, Cohen et al. (1978) were the first to suggest that a decrease in pineal activity (and consequently a low melatonin production) would contribute to the ethiology of breast cancer by leading to a state of relative hyperestrogenism. From this time, most studies on melatonin antitumoral effects have been based on the inhibitory actions of melatonin on the neuroendocrine-reproductive axis, decreasing the circulating levels of gonadal estrogens, which are considered to play a major role in promoting the proliferation of neoplastic mammary epithelium (Nandi et al., 1995; Russo and Russo, 1998). The aim of this article is to review some of the experimental evidence which gives support to the hypothesis that melatonin could serve as an oncostatic drug of possible utilization in the prevention and treatment of breast cancer, the most frequent oncological malignancy diagnosed in women in the Western world.

Melatonin as a modifier of the susceptibility of the mammary gland to neoplasia

The ability of the pineal gland to influence mammary gland susceptibility to the action of chemical carcinogens, through the secretion of melatonin, has been studied with different experimental approaches, mostly based on the induction of changes in the pineal gland function and evaluation of its effects on the carcinogenic process. In our laboratory, we compared the mammary carcinogenesis induced by a chemical carcinogen (7,12 dimethylbenz[a]anthracene; DMBA) in rats in which pineal actions (induced by light deprivation) had been previously either enhanced or suppressed. The treatments to increase the sensitivity to the pineal activation consisted in rendering the animals anosmic (Reiter and Klein, 1969; Sorrentino et al., 1971b; Blask, 1993). Among the neoplastic processes influenced by melatonin, those affecting the mammary gland have been the most extensively studied. More than twenty years ago, Cohen et al. (1978) were the first to suggest that a decrease in pineal activity (and consequently a low melatonin production) would contribute to the ethiology of breast cancer by leading to a state of relative hyperestrogenism. From this time, most studies on melatonin antitumoral effects have been based on the inhibitory actions of melatonin on the neuroendocrine-reproductive axis, decreasing the circulating levels of gonadal estrogens, which are considered to play a major role in promoting the proliferation of neoplastic mammary epithelium (Nandi et al., 1995; Russo and Russo, 1998). The aim of this article is to review some of the experimental evidence which gives support to the hypothesis that melatonin could serve as an oncostatic drug of possible utilization in the prevention and treatment of breast cancer, the most frequent oncological malignancy diagnosed in women in the Western world.

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developed were fibroadenomas and when adenocarcinomas were diagnosed, they presented signs of regression. On the contrary, pinealectomized rats developed mammary adenocarcinomas and in animals without palpable tumors, the presence of ductule hyperplasia (considered as a premalignant lesion) was observed more frequently than in controls or animals with potentiation of pineal actions (Cos et al., 1989).

These experiments suggested that pineal gland activity could prevent the development of chemically-induced mammary tumors, presumably by means of its modulatory effects on the hormones of the reproductive neuroendocrine axis involved in the control of mammary gland growth.

The development of breast cancer in women depends on a complex combination of genetic and environmental factors. It is accepted that early menarche and late menopause, nulliparity or late pregnancy, exposure to ionizing radiations at young age, and familiar history of breast or ovary cancer are factors which increase the risk of breast cancer (Russo and Russo, 1996). The period of time elapsed between the menarche and the first full term pregnancy seems to be critical for the initiation of breast carcinogenesis. The reason for the high susceptibility of breast to carcinogenic agents during this period could be the presence in mammary glands during this time interval, of an elevated number of structures known as terminal ductal lobular units (TDLU), or type 1 lobules. These TDLUs, which represent the most undifferentiated structures present in the breast of nulliparous women (Russo et al., 1990), are the sites where breast tumors originate. The TDLUs are equivalent in their carcinogenic potential to the terminal end buds (TEB) of rodents mammary glands (Russo et al., 1990; Russo and Russo, 1996). It has been demonstrated that the incidence of mammary cancer in rats correlates directly with the density of relatively undifferentiated TEB in the mammary gland at the time of carcinogen administration (Russo and Russo, 1978). The possible role of melatonin in preventing mammary gland carcinogenesis could be found in its ability to modulate mammary gland development and, specifically, to decrease the number of TEB. In mice the TEB in mammary gland reach their maximum number at about the 5th week of age. Interestingly, melatonin, at pharmacological doses, suppresses mammary morphogenesis in rats and mice (Mhatre et al., 1984; Sánchez-Barceló et al., 1990; Mediavilla et al., 1992). The effects of melatonin consist of a reduction in the number of TEB at the 5th week of life, when it reaches its highest value and when susceptibility to chemical carcinogens is highest (Fig. 2).

In this way, melatonin could exert its protective effects against the chemical carcinogens by reducing the target tissues of these agents. Studies carried out in rats also found that both PRL levels and number of TEB at day 55 of age (when carcinogenic effects of DMBA are highest) are significantly higher in animals in which melatonin has been suppressed by functional pinealectomy (exposure to LL) than in control animals (under LD 10/14) (Shah et al., 1984).

More arguments in support of the role of melatonin in the prevention of the development of breast cancer can be found in the relationship between the exposure to magnetic fields (EMF) and the incidence of breast cancer. Earlier studies demonstrated that exposure to low EMF decreased nocturnal melatonin levels in plasma.

**Fig. 1.** Carcinogenic effects of DMBA in rats in which pineal actions (induced by light deprivation) had been previously either enhanced or suppressed. **Top.** Experimental design: the treatments to enhance light-deprivation induced pineal actions (EPA) consisted in rendering the animals anosmic, underfeeding them, or exposing them to low environmental temperature (10 °C); suppression of pineal actions (SPA) was obtained by associating pinealectomy to the above mentioned treatments. **Center.** Tumoral incidence controlled until 20 weeks after the administration of the chemical carcinogen. **Bottom.** Tumoral latency: time elapsed between the administration of DMBA and the appearance of palpable mammary tumors. Figure constructed with data from Sánchez-Barceló et al. (1988).
Melatonin and breast cancer

Enhance MCF-7 breast cancer cell proliferation by well as growth of mammary gland tumors. Table 1 recopilates the increased rates of breast cancer among people reduction of melatonin synthesis could be the cause of the increased rates of breast cancer among people exposed to these radiations (Baldwin and Barret, 1998). In vitro, low EMF can act at the cellular levels to enhance MCF-7 breast cancer cell proliferation by blocking melatonin oncostatic action (Liburdy et al., 1993; Harland and Liburdy, 1997). The possible implications of electric power on breast cancer have been recently revised in depth by Brainard et al. (1999).

Effects of melatonin on growth of mammary tumors

Numerous experimental studies carried out in vivo as well as in vitro, have demonstrated the ability of melatonin to inhibit not only the initiation but also the growth of mammary gland tumors. Table 1 recopilates most of the published evidence of in vivo effects of melatonin administration on murine models of mammary carcinogenesis. Despite the great differences among the experimental approaches, with a few exceptions, most results reported in the articles referred to in Table 1, support the theory that melatonin increases the tumoral latency and decreases the incidence and size of mammary adenocarcinomas.

Since estrogens and PRL are considered to be the main hormonal factors involved in the promotion and proliferation of mammary adenocarcinomas (Welsh and Nagasawa, 1977; Meites, 1980; Nandi et al., 1995; Russo and Russo, 1998), the antitumoral effects of melatonin have been related to its ability to down-regulate the circulating levels of these mammotrophic hormones (Blask et al., 1991). Thus, the fact that pinealectomy or exposure to constant light increases serum PRL levels (Vaticcn et al., 1980; Leadem and Blask, 1981; Shah et al., 1984; Blask et al., 1991), whereas melatonin treatment decreases the plasmatic concentration of PRL (Tamarkin et al., 1991), could explain the pineal influence on mammary carcinogenesis. While the chronic activation of the pineal gland inhibits synthesis, storage and secretion of PRL in rats, changes in plasma estradiol after melatonin treatment are not frequently found (Shah et al., 1984; Blask et al., 1991). Indeed, on the contrary, melatonin-dependent changes in the concentration of estradiol receptors (ER) have been described. We observed that DMBA-induced tumors in rats subjected to experimental manipulations which enhance pineal actions showed significantly lower concentrations of cytosolic ER than in tumors of rats with the same treatment plus pinealectomy, even if animals had been gonadectomized and injected with the same doses of estradiol (Sánchez-Barceló et al., 1988) (Fig. 3). Also, in normal mammary gland rats, melatonin downregulates and pinealectomy accelerates the appearance of estradiol receptors (Seshadri et al., 1992). These results agree with the earlier studies in humans demonstrating an inverse correlation between nocturnal levels of melatonin and the ER concentration in breast tumors (Tamarkin et al., 1982).

The direct effects of melatonin on mammary cancer have been studied in vitro, basically by using, as a model, the MCF-7 human breast cancer cell line (Soule et al., 1973), which contains both estrogen and progesterone receptors (Brooks et al., 1973; Horwitz and McGuire, 1978). Melatonin, at concentrations within the physiological range, and especially InM (corresponding to night peak levels), inhibits the in vitro proliferation of MCF-7 cells (Blask and Hill, 1986; Hill and Blask, 1988). Interestingly, the highest antiproliferative effect of melatonin is achieved when its concentration in culture media is changed every 12h from 1nM to 10pM, thus mimicking the night/day rhythm of serum melatonin in most mammals (Cos and Sánchez-Barceló, 1994). Melatonin blocks the mitogenic effect of estradiol (Blask and Hill, 1986) as well as the stimulatory effects of prolactin (Blask et al., 1993; Lemus-Wilson et al., 1995) and epidermal growth factor (Cos and Blask, 1994) on MCF-7 cell proliferation. The growth-inhibitory actions of melatonin show a good correlation with the cell proliferation rate of MCF-7 cells; thus, it decreases the proliferation of cells which grow fast, but is not effective when cells proliferate slowly (Cos and Sánchez-Barceló, 1995). Other effects of melatonin on MCF-7 cells are the increases in the cell-cycle duration (Cos et al., 1996a), with the fraction of cells in G1 phase increasing as the proportion of cells in S phase drops (Cos et al., 1991), and a decrease in DNA synthesis (Cos et al., 1996b). These inhibitory effects of melatonin on
Table 1. Effects of pineal gland and melatonin on mammary cancer in rodents models.

<table>
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<tr>
<th>TUMORAL MODEL</th>
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<th>REFERENCE</th>
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<tbody>
<tr>
<td>SD rat DMBA at 50 d 30 mg/rat i.g.</td>
<td>24:0 or 12:12 from 43 d old</td>
<td>±MEL 100 µg/d s.c. in a.m., from 43 d old for 8 mon after DMBA</td>
<td>DMBA + 8 mon</td>
<td>24:0 = ↑ tumor incidence (fibroadenomas) MEL= no effect 12:12, MEL= ↑ incidence ↑ % adenocarcinomas</td>
<td>Hamilton, 1969</td>
</tr>
<tr>
<td>C3H mouse transplantable (RSM) tumor Fisher rat transplantable (R3230AC) tumor</td>
<td>12:12</td>
<td>±MEL 50 µg/d s.c. from 4 d prior transplantation for 10 d</td>
<td>Tranpl. + 10 d</td>
<td>MEL= tumor growth</td>
<td>Anisimov et al., 1973</td>
</tr>
<tr>
<td>Wistar rat DMBA at 3-3.5 mon, i.g., 50 µg/kg b.w., repeat twice at 10 d intervals</td>
<td>12:12</td>
<td>±Reserpine 100 mg/kg b.w., s.c., from the first DMBA and for 40 d ±Px at 1 d old ±Thymectomy (Tx) at 1 d old ±Px + Tx at 1 d old</td>
<td>DMBA + 400 d</td>
<td>Reserpine= ↑ tumor incidence, ↓ survival time of rats Greater effect in Px animals</td>
<td>Lapin, 1978</td>
</tr>
<tr>
<td>SO rat DMBA at 60 d 25 mg/rat p.o.</td>
<td>12:12</td>
<td>±MEL 200 µg twice/wk s.c. in p.m., from DMBA</td>
<td>DMBA + 140 d</td>
<td>MEL= ↑ tumor incidence ↓ tumor latency Px= ↑ latency Px+MEL= ↓ tumor latency no effects on tumors</td>
<td>Aubert et al., 1980</td>
</tr>
<tr>
<td>SD rat DMBA at 50 d 15 mg/rat i.g.</td>
<td>12:12</td>
<td>±MEL 500 µg/d i.p. in p.m., from DMBA for 90 d</td>
<td>DMBA + 140 d</td>
<td>MEL= ↑ tumor incidence Px= ↑ incidence greater effect at 7 DMBA Px=MEL= no effect on incidence MEL+MEL= no effect on tumor incidence, ↓ prolactin</td>
<td>Tamarkin et al., 1981</td>
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<tr>
<td>SO rat DMBA at 50 d 7 or 10 mg, i.g.</td>
<td>12:12</td>
<td>±Px at 20 d old</td>
<td>DMBA + 240 d</td>
<td>MEL= ↑ tumor latency, ↓ prolactin Px+MEL= no effect on tumor appearance for 2 wk</td>
<td>Shah et al., 1984</td>
</tr>
<tr>
<td>SD rat DMBA at 50 d 15 mg/rat i.g.</td>
<td>12:12</td>
<td>±MEL 500 µg/d i.p. in p.m., from DMBA for 92 d after DMBA</td>
<td>DMBA + 92 d</td>
<td>MEL= ↑ tumor incidence Px=MEL= no effect on tumor appearance for 2 wk</td>
<td>Shah et al., 1984</td>
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<tr>
<td>Holtzman rat DMBA at 55 d i.g., 20 mg per 200 g b.w.</td>
<td>10:14 or 24:0</td>
<td>±MEL 500 µg/d i.p. in p.m., from DMBA for 92 d after DMBA</td>
<td>DMBA + 92 d</td>
<td>MEL= ↑ tumor incidence Px=MEL= no effect on tumor appearance for 2 wk</td>
<td>Shah et al., 1984</td>
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<td>Holtzman rat DMBA at 55 d i.g., 20 mg per 200 g b.w.</td>
<td>10:14 or 24:0</td>
<td>±Px at 1-2 d old</td>
<td>DMBA + 180 d</td>
<td>MEL= ↑ tumor latency, ↓ prolactin Px=MEL= no effect on tumor appearance for 2 wk</td>
<td>Shah et al., 1984</td>
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<tr>
<td>Holtzman rat DMBA at 55 d i.g., 10 mg per 100 g b.w.</td>
<td>10:14 or 24:0</td>
<td>±MEL 500 µg/d i.p. in p.m., from 55 to 145 d of age</td>
<td>DMBA + 6 mon</td>
<td>24:0 = ↑ incidence, ↓ tumor latency no effect on prolactin</td>
<td>Shah et al., 1984</td>
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<tr>
<td>SD rat DMBA at 55 d 5 mg/rat i.g., repeat twice at 1 wk intervals</td>
<td>12:12</td>
<td>At 56 d old: ±Blindness + Anosmia (BA) ±SCGx</td>
<td>DMBA + 15 wk</td>
<td>MEL= ↑ tumor latency, ↓ prolactin BA= ↑ incidence, ↑ pineal HIOMT SCGx= ↑ incidence, ↓ tumor number, ↓ pineal HIOMT</td>
<td>Chang et al., 1985</td>
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<table>
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<tr>
<td>SD rat</td>
<td>12:12</td>
<td>At 56 d old:</td>
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<tr>
<td>DMBA at 55 d</td>
<td>5 mg/rat i.g., repeat twice at 1 wk intervals</td>
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<td></td>
<td>DMBA + 3 wk for 15 wk</td>
<td>DMBA</td>
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<td>BA= ↓ body weight</td>
<td>Chang et al., 1986</td>
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<td></td>
<td>MEL 250 µg/d s.c. in p.m.</td>
<td>DMBA</td>
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<td>SCGx= ↓ tumor number, ↓ pineal HIOMT</td>
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<td></td>
<td>at DMBA + 3 wk for 15 wk</td>
<td>DMBA</td>
<td></td>
<td>BA+SCGx= ↓ incidence, ↓ tumor mass, ↓ tumor number, ↓ pineal HIOMT</td>
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<tr>
<td>SD rat</td>
<td>12:12</td>
<td>↓ Underfeeding (U) at</td>
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<tr>
<td>DMBA at 55 d</td>
<td>5 mg/rat i.g.</td>
<td>DMBA</td>
<td></td>
<td>U or MEL= ↓ tumor number and size, ↑ latency, ↓ incidence, MEL+U= ↓ incidence, ↓ tumor number (greater effect)</td>
<td>Blask et al., 1986</td>
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<tr>
<td>C3Hf, mice</td>
<td>12:12</td>
<td>↓ MEL 10 mg/kg/d staggered by 4 h, from 12 wk old and for 30 wk</td>
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<td>Spontaneous tumor</td>
<td></td>
<td>DMBA</td>
<td></td>
<td>42 wk MEL= ↓ tumor incidence, MEL effects are rhythm-stage dependent</td>
<td>Wrba et al., 1986</td>
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<td>Holtzman rat</td>
<td>10:14 or 24:0</td>
<td>↓ MEL 100 µg/d in drinking water from 20 to 140 d</td>
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<td>DMBA at 55 d</td>
<td>20 mg/rat i.g.</td>
<td>DMBA</td>
<td></td>
<td>24:0 + MEL= ↓ incidence, ↑ latency, Px+ MEL= ↓ incidence</td>
<td>Kothari, 1987</td>
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<tr>
<td>SD rat</td>
<td>12:12</td>
<td>At 28 d old:</td>
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<tr>
<td>DMBA at 55 d</td>
<td>20 mg/rat i.g.</td>
<td>DMBA</td>
<td></td>
<td>↑ latency, ↓ incidence, ↓ tumor strogen receptor, ↓ serum estradiol, PX only and combined with each treatment (BAPx, BCPx, BUPx)= ↓ latency, ↓ incidence,</td>
<td>Sánchez, 1988</td>
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<tr>
<td>SD rat</td>
<td>12:12</td>
<td>When tumor reached 1 cm:</td>
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<tr>
<td>DMBA at 55 d</td>
<td>20 mg/rat i.g.</td>
<td>DMBA</td>
<td></td>
<td>↓ tumor growth, ↓ tumor estrogen receptors, ↓ serum estradiol, PX counteracts effects</td>
<td>Sánchez-Barceló et al., 1988</td>
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<td>20 mg/rat i.g.</td>
<td>DMBA</td>
<td></td>
<td>↓ tumor growth, ↓ tumor estrogen receptors, ↓ serum estradiol, PX counteracts effects</td>
<td>Cos et al., 1999</td>
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<tr>
<td>SD rat</td>
<td>12:12</td>
<td>When tumor reached 1 cm:</td>
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<td>NMu at 50 and</td>
<td>60 d, 50 mg/kg b.w., i.v.</td>
<td>DMBA</td>
<td></td>
<td>↓ tumor number, NMU+Px= no effect on tumors</td>
<td>Blask et al., 1991</td>
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<tr>
<td>NMu at 50 and</td>
<td>57 d, 50 mg/kg b.w., i.v.</td>
<td>DMBA</td>
<td></td>
<td>↓ tumor growth, ↓ tumor estrogen receptors, ↓ serum estradiol, PX counteracts effects</td>
<td>Subramanian and Kothari, 1991a</td>
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<tr>
<td>NMu at 50 and</td>
<td>20 and 12:12</td>
<td>DMBA</td>
<td></td>
<td>↓ tumor number, NMU+Px= no effect on tumors</td>
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<tr>
<td>Holtzman rat</td>
<td>14:10 or 24:0</td>
<td>↓ MEL 200 µg/d in drinking water from 48 to 62 d</td>
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<tr>
<td>DMBA at 55 d</td>
<td>10 mg/rat i.g.</td>
<td>DMBA</td>
<td></td>
<td>14:10 and 24:0 MEL +Px= no effects on tumors</td>
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<td></td>
<td></td>
<td>DMBA</td>
<td></td>
<td>MEL or MEL+Px= ↓ tumor incidence in 14:10 and 24:0</td>
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Melatonin and breast cancer

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<td>C3H/HeJax mouse Spontaneous tumors</td>
<td>14:10</td>
<td>±MEL 25 µg/d in drinking water from 21 to 44 d and 50µg/d from 45 d to the end</td>
<td>12 mon</td>
<td>MEL: ( \downarrow ) tumor incidence, ( \downarrow ) serum estradiol, ( \downarrow ) DNA synthesis, ( \downarrow ) premalignant lesions, ( \downarrow ) mammary growth</td>
<td>Subramanian and Kothari, 1991b</td>
</tr>
<tr>
<td>Holtzman rat DMBA at 55 d 10 mg/rat i.g.</td>
<td>14:10 or 24:0</td>
<td>±MEL 200 µg/d in drinking water from 48 to 58 d of age</td>
<td>58 d</td>
<td>MEL: in intact rats hepatic and mammary glutathione and glutathione S-transferase (GSH), hepatic cytochromes b5 and P450 in 14:10 and 24:0</td>
<td>Kothari and Subramanian, 1992</td>
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<td>Fischer rat DMBA at 51 d 17.5 mg/rat i.g.</td>
<td>12:12</td>
<td>DMBA-tumor cells were transplanted at passages 2 and 12, at 70 d old and: ±MEL 100 µg/d in drinking water from 36 d to the end</td>
<td>Transpl. + 16 wk</td>
<td>Slow-growing passage 6 = MEL ( \downarrow ) tumor growth</td>
<td>Bartsch et al., 1994</td>
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<td>SD rat NMU at 50 d 50 mg/kg b.w. i.p.</td>
<td>?</td>
<td>When tumor reached 1 cm, before 6 mon after NMU, was excised and: ±MEL 200 µg/d in drinking water for 180 d ( \uparrow ) Tam 60 µg/wk, s.c., only or with MEL for 180 d</td>
<td>Excision + 180 d</td>
<td>MEL: ( \uparrow ) latency of second generation tumors</td>
<td>Kothari et al., 1995</td>
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<td>MMTV-LTR/N ras transgenic mice</td>
<td>14:10</td>
<td>±MEL 200 µg/d 5 times/wk s.c. in p.m. from 4 wk old for 5 mon</td>
<td>6 mon</td>
<td>MEL: ( \downarrow ) density of hyperplastic lesions, ( \downarrow ) expression of N-ras protein, ( \downarrow ) the hyperplasia of the mammary lymphoid tissue</td>
<td>Mediavilla et al., 1997</td>
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<tr>
<td>SD rat NMU at 50 d 50 mg/kg b.w. i.p.</td>
<td>12:12</td>
<td>At NMU and for 300 d: ±MEL 200 µg/d in drinking water ( \uparrow ) Tam 60 µg/wk, s.c., only or with MEL</td>
<td>NMU + 300 d</td>
<td>MEL or Tam 60µg/wk = ( \uparrow ) tumor latency, ( \downarrow ) mammary DNA synthesis, ( \downarrow ) ovary weight</td>
<td>Kothari et al., 1997</td>
</tr>
</tbody>
</table>
| MCF-7 cell growth however have not been confirmed by other researchers (Shellard et al., 1989; Bartsch et al., 1992; L'Hermite-Balériaux and Launoit, 1992; Panzer et al., 1998; Papazisis et al., 1998). Different sources of MCF-7 cells (Osborne et al., 1987) and different experimental culture conditions, especially an inappropriate rate of cell growth, could be responsible for these discrepancies. Recent findings on melatonin action on MCF-7 human breast cancer cells include: - The demonstration of its ability to reduce (at physiological doses) the invasiveness of these cells, and also to counteract the estradiol-induced increase of its metastatic capacity (Cos et al., 1998) (Fig. 4). These actions are mediated by a melatonin-induced increase in the expression of B1 integrin (a subunit of integrins), receptors that regulate interaction between cells and the extracellular matrix and E cadherin (a calcium-dependent membrane protein responsible for cell-cell contact) (Cos et al., 1998). - The observation of increased expression of both p53 and p21WAF1 proteins in MCF-7 cells treated with 1nM melatonin (Mediavilla et al., 1999). Sequential treatment

SD: Sprague-Dawley; DMBA: 7,12-dimethylbenzanthracene; NMU: N-methyl-N-nitrosourea; i.g.: intragastric; b.w.: body weight; s.c.: subcutaneous; p.o.: per os; i.v.: intravenous; i.p.: intraperitoneal; Px: pinealectomy; SCGX: superior cervical ganglionectomy; Ovx: ovariectomy; MEL: melatonin; Tam: tamoxifen; Est: estradiol; mon: month; wk: week; d: day.
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of MCF-7 cells with melatonin (1nM) followed by all trans retinoic acid (1nM) induces cell death by activating pathways leading to apoptosis, as evidenced by decreased Bcl-2 and increased Bax and transforming growth factor β1 expression (Eck et al., 1998).

The oncostatic actions of melatonin on breast cancer cells in vitro suggest the existence of direct actions of melatonin at cellular level. Since only ER-positive breast cancer cells have been found to be responsive to the antimitogenic effects of melatonin, the current hypothesis is that the oncostatic actions of melatonin are mediated via their effects on the estrogen-response pathway of tumoral cells (Hill and Blask, 1988; Cos et al., 1991; Hill et al., 1992; Molis et al., 1995), as it behaves as a naturally occurring antiestrogen. The effects of melatonin on estrogen receptors (ER) are controversial. Melatonin appears not to bind directly to ER (Molis et al., 1994), although it could modulate ER expression through an intermediary factor, or by interacting with its own receptors, to start the events which induce the down-regulation of the ER expression (Molis et al., 1993). The attempts to characterize melatonin receptors in membranes from MCF-7 cells or normal mammary tissue have been unsuccessful, since only low affinity binding sites have been found (Stankov et al., 1991; Recio et al., 1994a,b). Alternatively, MCF-7 cells endogenously express RZRα nuclear receptors, which have been considered as receptors to melatonin (Steinhilber et al., 1995).

In addition to estrogen, breast cancer cell growth is

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**Fig. 3.** Evaluation of whether the antitumoral effect obtained by enhancing the light-deprivation-induced pineal actions (EPA) are secondary to the reduction of endogenous steroids dependent on pineal gland activity. **Top.** Experimental design: the treatments to enhance light deprivation-induced pineal actions (EPA) or to suppress pineal actions (SPA) are the same as those described in Figure 1, and were carried out in rats bearing mammary adenocarcinomas (1 cm diameter) previously induced by DMBA administration. All animals were also ovariectomized (Ovx) and injected daily with estradiol (E) (10µg). **Center.** Rate of tumoral growth, significantly decreases in rats with EPA, in comparison with control or those with SPA, although in all cases the circulating levels of estradiol is similar. **Bottom.** Concentration of estrogen receptors (ER) in mammary tumors of rats with EPA is significantly lower than in their pinealectomized counterparts; this fact demonstrates that pineal influence on tumoral growth could be mediated by interaction with ER. Figure constructed with data from Sánchez-Barceló et al. (1988).

**Fig. 4.** Effects of melatonin on the metastatic behavior of MCF-7 human breast cancer cells in vitro evaluated by using modified Boyden’s invasion chambers in which the number of cells which traverse a synthetic basal membrane (Matrigel) were counted. **Top.** Melatonin at physiological doses (1nM), but not at sub or supraphysiological ones, significantly reduces the number of cells traversing the basal membrane. **Bottom.** Melatonin (1nM) also counteracts the estradiol-induced increase in the invasiveness of MCF-7 cells through out the Matrigel membrane. Modified from Cos et al. (1998).
under the complex regulatory influence of estrogen-inducible polypeptide hormones such as pituitary PRL as well as autocrine growth factors. Melatonin can block the in vitro mitogenic effects of PRL or PRL-receptor antibodies (Lemus-Wilson et al., 1995). It has been demonstrated that melatonin may not only inhibit the action and/or release of growth stimulatory factors, but also stimulate the production or release of growth inhibitory factors (probably TGFβ) (Cos and Blask, 1994; Molis et al., 1995).

The oncostatic actions of melatonin have also been explained by a mechanism not directly related to its modulatory effects on the circulating levels of the hormones of the neuroendocrine reproductive axis. These hypotheses include:

a) The melatonin properties as a potent radical scavenger (Ianas et al., 1991; Poepp格尔 et al., 1993; Reiter, 1993b, 1995; Reiter et al., 1993). Because of its high lipophilicity, melatonin enters all cells of the body and every subcellular compartment (Menéndez-Pelaez et al., 1993). Within the nucleus, melatonin may exert anti cancer effects by scavenging reactive oxygen radicals and electrophilic intermediates responsible for DNA damage (Frenkel, 1992). Since the radical-scavenging function of melatonin is strictly dose-dependent (Tan et al., 1993), a decreased melatonin concentration may be directly connected with a diminished protection of DNA, leading to a higher risk of cancer.

b) The possible role of nitric oxide (NO) in the melatonin inhibition of human breast cancer growth. In the presence of NMMA (an inhibitor of the enzyme NO synthetase), melatonin lacked its antiproliferative activity (Blask and Wilson, 1994).

c) The immunoenhancing properties of melatonin (Maestroni, 1995). A reduction in melatonin concentration could perturb the immune function, thus enhancing tumor proliferation and reducing tumor surveillance.

d) The possible ability of melatonin to increase, at physiological concentrations, gap-junctional intercellular communication, as has been demonstrated in mouse embryo fibroblasts (Ubeda et al., 1995) and primary cultures of adult rat hepatocytes (Kojima et al., 1997). Most cancer cells have some dysfunction in gap-junction-mediated intercellular communication and, in addition, most, if not all, tumor-promoting chemicals and conditions down-regulate gap-junction function, whereas antitumor-promoting chemicals usually up-regulate gap-junctional communication (Trosko et al., 1990). If melatonin is proved to increase gap-junctional communication in breast cancer cells it may suppress tumor growth by allowing the transfer of other molecules with the ability to suppress tumor growth.

Concluding remarks

In this article we have reviewed some evidence that as a whole, supports the possible usefulness of melatonin in prevention and treatment of breast tumors. Thus, from experiments on rodents, it has been reported that suppression of pineal function, either surgically or with appropriate light exposure, enhances tumor incidence, number and size, and reduces tumor latency. These antitumoral actions of pineal gland may be related to its ability, via the secretion of melatonin, to exert a modulatory effect on pituitary and gonadal hormones which control the normal and neoplastic mammary gland growth. Studies in vitro, carried out on the ER-positive MCF-7 human breast cancer cells, have consistently demonstrated antiproliferative and anti-invasive effects of melatonin at doses within the physiological range. These effects could be dependent on the interaction of melatonin with the estrogen response pathway. In summary, the data from the in vivo and in vitro studies on the pineal gland and melatonin influence on mammary tumorigenesis, reveals, despite some discrepancies, the role of melatonin as an interesting oncostatic agent with potential clinical application.

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References


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