

Invited Review

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

S. Cos and E.J. Sánchez-Barceló

Department of Physiology and Pharmacology, School of Medicine, University of Cantabria, Santander, Spain

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 etiology 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; Reiter, 1980; Sánchez-Barceló et al., 1985), or reducing their daily food intake (underfeeding) (Sorrentino et al., 1971a; Reiter, 1980) or exposing them to low environmental temperature (10 °C) (Sánchez-Barceló et al., 1986). Suppression of pineal actions (SPA) was obtained by pinealectomy. DMBA was given to the animals 1 month after their being treated to either enhance or suppress pineal actions. We conclude that in animals in which pineal actions had been enhanced, the tumoral latency significantly enlarged and the tumoral incidence decreased, these effects being prevented by pinealectomy (Fig. 1). Furthermore, in animals with enhanced pineal actions, most mammary tumors

Offprint requests to: Emilio J. Sánchez-Barceló, Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Cantabria, Cardenal Herrera Oria s/n, 39011 Santander, Spain. Fax: 34-942-201903. e-mail: barcelo@medi.unican.es

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).

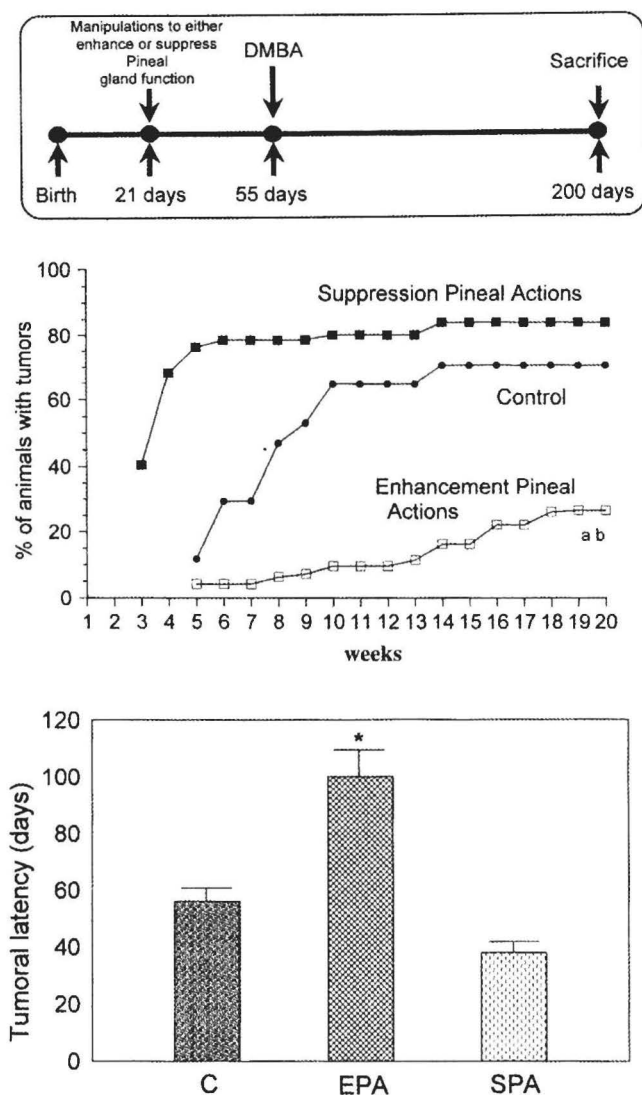


Fig. 1. Carcinogenic effects of DMBA in rats in which pineal actions (induced by light deprivation) had been previously either enhanced or suppressed. **Top.** Experimental design: the treatments to enhance light-deprivation induced pineal actions (EPA) consisted in rendering the animals anosmic, underfeeding 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).

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 TDLU, 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 TDLU 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

(Olcese and Reuss, 1986; Olcese, 1990; Reiter and Richardson, 1992; Kato et al., 1993; Reiter, 1993a). Recently, some epidemiological observations suggest that the exposure of humans to low EMF, the sources of which are ubiquitous in modern societies, induce a higher than normal incidence of breast cancer (Loomis et al., 1994; Tynes et al., 1996). Experimental studies have shown that 50/60-Hz EMF promotes or co-promotes mammary cancer (Baum et al., 1995; Löschner et al., 1994, 1997; Mavissen et al., 1998). Since non-ionizing radiations have no mutagenic effect, the EMF-induced 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 recopiates 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 (Welsch 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 (Vaticon 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., 1981), 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 1nM (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

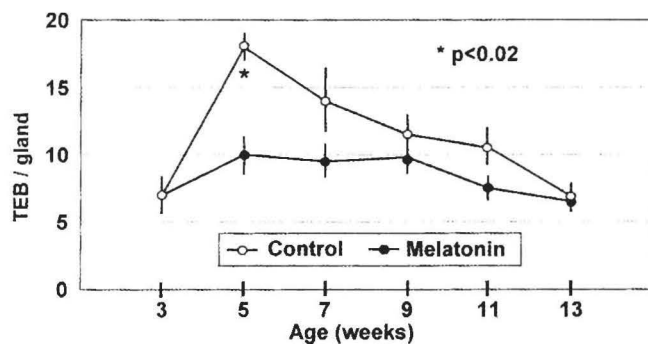


Fig. 2. Time-course changes in the number of Terminal End Buds (TEB) in thoracic mammary glands of female mice receiving melatonin (200µg/day, sc) or the diluent from 21 days of age. It can be appreciated how melatonin reduces the number of TEB, the most undifferentiated structures present in the mammary gland, which are the sites where chemical-induced tumors originate. Modified from Mediavilla et al. (1992).

*Melatonin and breast cancer***Table 1.** Effects of pineal gland and melatonin on mammary cancer in rodents models.

TUMORAL MODEL	PHOTOPERIOD LIGHT:DARK	TREATMENT	DURATION	RESULTS	REFERENCE
SD rat DMBA at 50 d 30 mg/rat i.g.	24:0 or 12:12 from 43 d old	±MEL 100 µg/d s.c. in a.m., from 43 d old for 8 mon after DMBA	DMBA + 8 mon	24:0 = ↑ tumor incidence (fibroadenomas) MEL= no effect 12:12, MEL= ↑ incidence ↑ % adenocarcinomas	Hamilton, 1969
C ₃ H mouse transplantable (RSM) tumor	?	±MEL 50 µg/d s.c., from 4 d prior transplantation for 10 d	Transpl. + 10 d	MEL= tumor growth	Anisimov et al., 1973
Fisher rat transplantable (R3230AC) tumor	?	±MEL 1mg/d	?	MEL= ↓ tumor weight and size	Karmali et al., 1978
Wistar rat DMBA at 3-3.5 mon, i.g., 50 mg/kg b.w., repeat twice at 10 d intervals	12:12	±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	DMBA + 400 d	Reserpine= ↑ tumor incidence, ↓ survival time of rats Greater effect in Px animals	Lapin, 1978
SD rat DMBA at 60 d 25 mg/rat p.o.	12:12	±MEL 200 µg twice/wk s.c. in p.m., from DMBA	DMBA + 140 d	MEL= ↓ tumor incidence ↓ tumor latency	Aubert et al., 1980
	12:12	±Px at 58 d old ±MEL 200 µg twice/wk s.c. in p.m., from DMBA		Px= ↑ latency Px+MEL= ↓ tumor incidence ↓ tumor latency	
	24:0	±MEL 200 µg twice/wk s.c. in p.m., from DMBA		no effects on tumors	
	24:0	±Px at 58 d old ±MEL 200 µg twice/wk s.c. in p.m., from DMBA		no effects on tumors	
	12:12	±MEL 200 µg twice/wk s.c. in p.m., from tumor appearance for 2 wk	Tumor + 2 wk	MEL= ↓ tumor number ↓ tumor size	
SD rat DMBA at 50 d 15 mg/rat i.g.	12:12	±MEL 500 µg/d i.p. in p.m., from DMBA for 90 d	DMBA + 140 d	MEL= ↓ tumor incidence	Tamarkin et al., 1981
SD rat DMBA at 50 d 7 or 10 mg, i.g.	12:12	±Px at 20 d old	DMBA + 240 d	Px= ↑ incidence greater effect at 7 DMBA	
SD rat DMBA at 52 d 15 mg/rat i.g.	12:12	±Px at 20 d old ±MEL 500 µg/d i.p. in a.m., for 92 days after DMBA	DMBA + 92 d	Px= no effect on incidence MEL= ↓ incidence, ↓ prolactin Px+MEL= no effect on tumor incidence, ↓ prolactin	
Holtzman rat DMBA at 55 d i.g., 20 mg per 200 g b.w.	10:14 or 24:0		DMBA + 6 mon	24:0= ↑ incidence, ↓ tumor latency	Kothari et al., 1982
Holtzman rat DMBA at 55 d i.g., 20 mg per 200 g b.w.	10:14 or 24:0	±Px at 1-2 d old	DMBA + 180 d	24:0= ↑ incidence, ↓ tumor latency than 10:14 24:0 + Px= ↑ incidence, ↓ tumor latency than intact and Px in 10:14	Kothari et al., 1984
Holtzman rat DMBA at 55 d i.g. 10 mg per 100 g b.w.	10:14 or 24:0	±Px at 1 d old ±MEL 500 µg/d i.p. in p.m., from 52 to 145 d of age	DMBA + 6 mon	24:0= ↑ incidence, ↓ latency, no Px effect, ↑ prolactin, ↑ DNA synthesis and morphological changes MEL= ↓ incidence in 10:14 and 24:0. Px + MEL= ↓ incidence in 24:0, no effect in 10:14	Shah et al., 1984
SD rat DMBA at 55 d 5 mg/rat i.g., repeat twice at 1 wk intervals	12:12	At 56 d old: ±Blindness + Anosmia (BA) ±SCGx	DMBA + 15 wk	BA= ↓ incidence, ↑ pineal HIOMT SCGx= ↓ incidence, ↓ tumor number, ↓ pineal HIOMT	Chang et al., 1985

(continued)

Melatonin and breast cancer

TUMORAL MODEL	PHOTOPERIOD LIGHT:DARK	TREATMENT	DURATION	RESULTS	REFERENCE
SD rat DMBA at 55 d 5 mg/rat i.g., repeat twice at 1 wk intervals	12:12	At 56 d old: ±Blindness + Anosmia (BA) ±SCGx ±BA + SCGx	DMBA + 15 wk	BA= ↓ body weight SCGx= ↓ tumor number, ↓ pineal HIOMT BA+SCGx= ↓ incidence, ↓ tumor mass, ↓ tumor number, ↓ pineal HIOMT	Chang et al., 1986
SD rat DMBA at 55 d 5 mg/rat i.v.	12:12	±Underfeeding (U) at DMBA + 3 wk for 15 wk ±MEL 250 µg/d s.c. in p.m. at DMBA + 3 wk for 15 wk	DMBA + 18 wk	U or MEL= ↓ tumor number and size, ↑ latency, ↓ incidence, MEL+U= ↓ incidence, ↓ tumor number (greater effect)	Blask et al., 1986
C ₃ CF ₁ mice Spontaneous tumor	12:12	±MEL 10 mg/kg/d staggered by 4 h, from 12 wk old and for 30 wk	42 wk	MEL= ↓ tumor incidence MEL effects are rhythm-stage dependent	Wrba et al., 1986
Holtzman rat DMBA at 55 d 20 mg/rat i.g.	10:14 or 24:0	±Px at 1-2 d old ±MEL 100 µg/d in drinking water from 20 to 140 d	DMBA + 180 d	24:0 + MEL= ↓ incidence, ↑ latency. Px ↓ MEL effect 14:10 + MEL= ↓ incidence	Kothari, 1987
SD rat DMBA at 55 d 20 mg/rat i.g.	12:12	At 28 d old: ±Blindness + Anosmia (BA) ±Blindness + Cold exposure (BC) ±Blindness + Underfeeding (BU) ±Px only and combined with each treatment (BAPx, BCPx, BUPx)	DMBA + 20 wk	BA or BC or BU= ↑ latency, ↓ incidence, ↓ tumor strogen receptor ↓ serum estradiol BAPx or BCPx or BUPx= ↓ latency, ↑ incidence,	Sánchez Barcelóet al., 1988
SD rat DMBA at 55 d 20 mg/rat i.g.	12:12	When tumor reached 1 cm: ±BC or BA or BU ±Px or BCPx or BAPx or BUPx	Tumor + 9 wk	BA or BC or BU= ↓ tumor growth, ↓ tumor estrogen receptors, ↓ serum estradiol Px counteracts effects	Sánchez- Barcelóet al., 1988
SD rat DMBA at 55 d 20 mg/rat i.g.		When tumor reached 1 cm: Ovx + Est 10 µg/d: ±BC or BU ±Px or BCPx or BUPx	Tumor + 9 wk	BC or BU= ↓ tumor growth, ↓ tumor estrogen receptors, Px counteracts effects	
SD rat DMBA at 55 d 20 mg/rat i.g.	12:12	At 28 d old: ±Blindness + Anosmia, ±Blindness + Cold exposure, ±Blindness + Underfeeding ±Px only and combined with each treatment	DMBA + 20 wk	Px= ↓ tumor regression, ↑ premalignant lesions	Cos et al., 1989
SD rat NMU at 50 and 60 d, 50 mg/kg b.w., i.v.	12:12	±MEL 500 µg/d s.c. in p.m. from 37 to 60 d of age	NMU + 20 wk	no effects on tumors	Blask et al., 1991
NMU at 50 and 57 d, 50 mg/kg b.w., i.v.	12:12	±MEL 500 µg/d s.c. in p.m. NMU + 4 wk and for 10 wk	NMU + 20 wk	MEL= ↓ tumor number	
NMU at 50 and 57 d, 50 mg/kg b.w., i.v.	12:12	±MEL 500 µg/d s.c. in p.m. from 57 d and for 19 wk ±Px at 47 d of age	NMU + 19 wk	MEL= ↓ incidence, ↓ tumor number Px= no effect on tumors	
NMU at 50 and 57 d, 50 mg/kg b.w., i.v.	12:12	When tumor reached 1 cm Ovx and: ±Est 12.5 µg/d s.c. in p.m. for 8 wk ±Est 12.5 µg/d s.c. in p.m. + MEL 20, 100 or 500 µg/d s.c. in p.m. for 8 wk ±Est 12.5 µg/d s.c. in p.m. + Tam 20 µg/d s.c. in p.m. for 8 wk.	Ovx + 8 wk	MEL and Tam= inhibition of estrogen-stimulated tumor regrowth following ovariectomy-induced regression	
Holtzman rat DMBA at 55 d 10 mg/rat i.g.	14:10 or 24:0	±MEL 200 µg/d in drinking water from 48 to 62 d ±Px at 1 day old	DMBA + 27 wk	MEL= ↓ tumor incidence in 14:10 and 24:0 MEL +Px= no effects on tumors MEL or MEL+Px= ↓ tumor incidence in 14:10 and 24:0	Subramanian and Kothari, 1991a
	14:10 or 24:0	±MEL 200 µg/d in drinking water from 62 to 244 d ±Px at 1 day old			

(continued)

Melatonin and breast cancer

TUMORAL MODEL	PHOTOPERIOD LIGHT:DARK	TREATMENT	DURATION	RESULTS	REFERENCE
C ₃ H/Jax mouse Spontaneous tumors	14:10	±MEL 25 µg/d in drinking water from 21 to 44 d and 50 µg/d from 45 d to the end	12 mon	MEL= ↓ tumor incidence, ↓ serum estradiol, ↓ DNA synthesis, ↓ premalignant lesions, ↓ mammary growth	Subramanian and Kothari, 1991b
Holtzman rat DMBA at 55 d 10 mg/rat i.g.	14:10 or 24:0	±MEL 200 µg/d in drinking water from 48 to 58 d of age	58 d	MEL= in intact rats hepatic and mammary glutathione and glutathione S-transferase (GSH), hepatic cytochromes b ₅ and P450 in 14:10 and 24:0 DMBA + MEL= hepatic and mammary glutathione in 14:10, ↑ mammary GSH in 14:10, ↑ hepatic GSH in 24:0, ↓ hepatic cytochrome b ₅ in 24:0, ↓ hepatic cytochrome p450 in 24:0 and 14:10	Kothari and Subramanian, 1992
Fischer rat DMBA at 51 d 17.5 mg/rat i.g.	12:12	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	Transpl. + 16 wk	Slow-growing passage 6 = MEL ↓ tumor growth Fast-growing passage 12 = no MEL effect	Bartsch et al., 1994
SD rat NMU at 50 d 50 mg/kg b.w. i.p.	?	When tumor reached 1 cm, before 6 mon after NMU, was excised and: ±MEL 200 µg/d in drinking water for 180 d ±Tam 60 µg/wk, s.c., only or with MEL for 180 d	Excision + 180 d	MEL= ↑ latency of second generation tumors Tam= no effects on tumors MEL+Tam= no additive effects	Kothari et al., 1995
MMTV-LTR/N ras transgenic mice	14:10	±MEL 200 µg/d 5 times/wk s.c. in p.m. from 4 wk old for 5 mon	6 mon	MEL= ↓ density of hyperplastic lesions, ↓ expression of N-ras protein, ↓ the hyperplasia of the mammary lymphoid tissue	Mediavilla et al., 1997
SD rat NMU at 50 d 50 mg/kg b.w. i.p.	12:12	At NMU and for 300 d: ±MEL 200 µg/d in drinking water ±Tam 60 µg/wk, s.c., only or with MEL ±Tam 180 µg/wk, s.c., only or with MEL	NMU + 300 d	MEL or Tam 60 µg/wk= ↑ tumor latency, ↓ mammary DNA synthesis, ↓ ovary weight Tam 180 µg/wk = ↓ incidence, ↓ mammary DNA synthesis, ↓ uterus and ovary weight MEL+Tam 60 or 180 µg/wk = ↓ incidence (greater effect), ↑ latency, ↓ mammary DNA synthesis, ↓ uterus and ovary weight Tam 60 µg/wk= ↓ uterus weight	Kothari et al., 1997

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.

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 β₁ 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

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 β_1 expression (Eck et al., 1998).

The oncostatic actions of melatonin on breast cancer

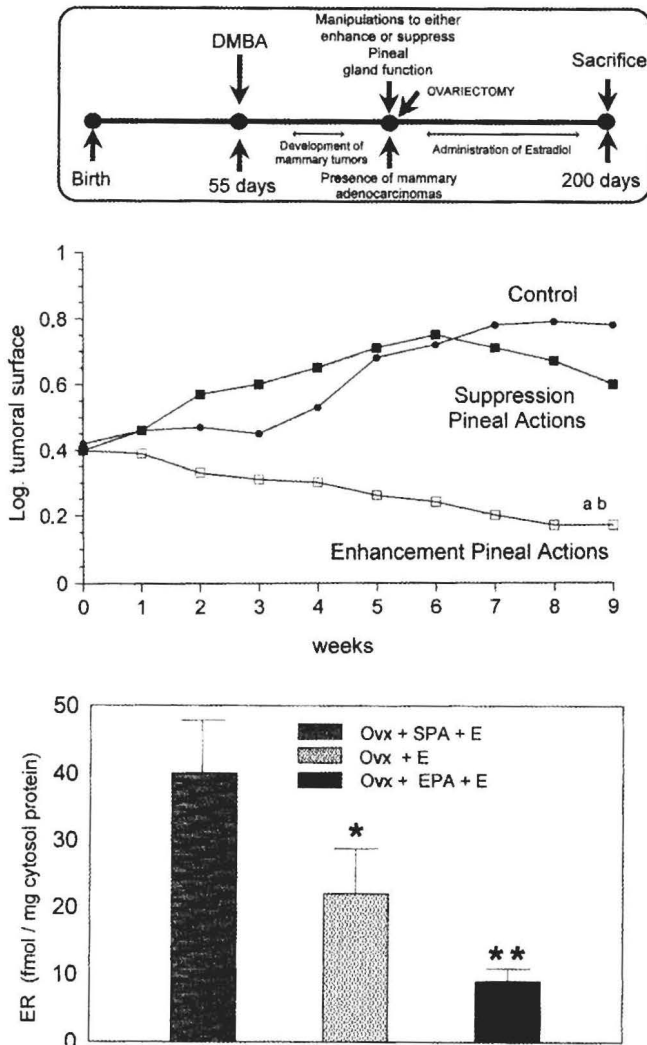


Fig. 3. Evaluation of whether the antitumoral effect obtained by enhancing the light-deprivation-induced pineal actions (EPA) are secondary to the reduction of endogenous steroids dependent on pineal gland activity. **Top.** Experimental design: the treatments to enhance light deprivation-induced pineal 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) (10 μ g). **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).

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 RZR α nuclear receptors, which have been considered as receptors to melatonin (Steinhilber et al., 1995).

In addition to estrogen, breast cancer cell growth is

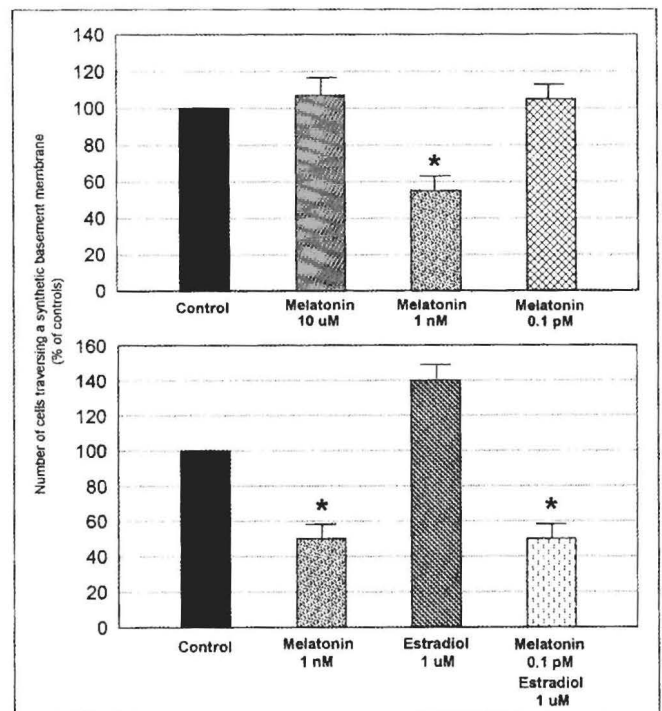


Fig. 4. Effects of melatonin on the metastatic behavior of MCF-7 human breast cancer cells *in vitro* evaluated by using modified Boyden's invasion chambers in which the number of cells which traverse a synthetic basal membrane (Matrigel) were counted. **Top.** Melatonin at physiological doses (1nM), 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 TGF β) (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; Poeggeler 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 disfunction 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.

Acknowledgements. Supported by grants from the Spanish General Direction of Scientific and Technological Investigation (PM97-0042) and the "Marqués de Valdecilla" Foundation (23/99).

References

- Anisimov V.N., Morozov V.G., Khabinson V.K.H. and Dilman V.M. (1973). Comparison of the anti-tumor activity of extracts of the epiphysis and hypothalamus melatonin and sygethin in mice with transplanted mammary gland cancer. *Vopr. Onkol.* 19, 99-101.
- Aubert C., Janiaud P. and Lecalvez J. (1980). Effect of pinealectomy and melatonin on mammary tumor growth in Sprague-Dawley rats under conditions of lighting. *J. Neural Transm.* 47, 121-130.
- Baldwin W.S. and Barrett J.C. (1988). Melatonin: Receptor-mediated events that may affect breast and other steroid hormone-dependent cancers. *Mol. Carcinog.* 21, 149-155.
- Bartsch C., Bartsch H. and Lippert T.H. (1992). The pineal gland and cancer: facts, hypotheses and perspectives. *Cancer J.* 5, 194-199.
- Bartsch C., Bartsch H., Buchberger A., Stieglitz D. and Lippert T.H. (1994). Serial transplants of DMBA-induced mammary tumors in Fischer rats as model system for human breast cancer: II Analysis of pineal melatonin biosynthesis and secretion. In: *Advances in pineal research*: 8. Moller M. and Pévet P. (eds). John T. Libbey. London. pp 479-484.
- Baum A., Mevissen M., Kamino K., Mohr U. and Löscher W. (1995). A histopathological study on alterations in DMBA-induced mammary carcinogenesis in rats with 50 Hz muT magnetic field exposure. *Carcinogenesis* 16, 119-125.
- Blask D.E. (1993). Melatonin in oncology. In: *Melatonin. Biosynthesis, physiological effects, and clinical applications*. Yu H.-S. and Reiter R.J. (eds). CRC Press. Boca Raton. pp 447-475.
- Blask D.E. and Hill S.M. (1986). Effects of melatonin on cancer: studies on MCF-7 human breast cancer cells in culture. *J. Neural Transm.* (Suppl) 21, 433-449.
- Blask D.E. and Wilson S.T. (1994). Melatonin and oncostatic signal transduction: evidence for a novel mechanism involving glutathione and nitric oxide. In: *Advances in pineal research*: 8. Moller M. and Pévet P. (eds). John Libbey & Company Ltd. London. pp 465-471.

- Blask D.E., Hill S.M., Orstead K.M. and Massa J.S. (1986). Inhibitory effects of the pineal hormone melatonin and underfeeding during the promotional phase of 7,12-dimethyl-benz(a)anthracene (DMBA)-induced mammary tumorigenesis. *J. Neural Transm.* 67, 125-138.
- Blask D.E., Pelletier D.B., Hill S.M., Lemus-Wilson A., Grosso D.S., Wilson S.T. and Wise M.E. (1991). Pineal melatonin inhibition of tumor promotion in the N-nitroso-N-methylurea model of mammary carcinogenesis: Potential involvement of antiestrogenic mechanism in vivo. *J. Cancer Res. Clin. Oncol.* 117, 526-532.
- Blask D.E., Lemus-Wilson A.M., Wilson S.T. and Cos S. (1993). Neurohormonal modulation of cancer growth by pineal melatonin. In: *Melatonin and the pineal gland. From basic science to clinical application.* Touitou Y., Arendt J. and Pevet P. (eds). Elsevier Science Publishers. Holland. pp 303-310.
- Brainard G.C., Kavet R. and Kheifets L.I. (1999). The relationship between electromagnetic field and light exposures to melatonin and breast cancer risk: A review of the relevant literature. *J. Pineal Res.* 26, 65-100.
- Brooks S.C., Locke E.R. and Soule H.D. (1973). Estrogen receptor in a human breast tumor cell line (MCF-7) from breast carcinoma. *J. Biol. Chem.* 248, 6251-6256.
- Chang N., Spaulding T.S. and Tseng M.T. (1985). Inhibitory effects of superior cervical ganglionectomy on dimethyl-benz(a)anthracene-induced mammary tumors in the rat. *J. Pineal Res.* 2, 331-340.
- Chang N., Tseng M.T. and Spaulding T.S. (1986). Induction and growth of mammary tumors after superior cervical ganglionectomy in sighted and blinded-anosmic rats. *Life Sci.* 38, 1821-1826.
- Cohen M., Lippman M. and Chabner B. (1978). Role of the pineal gland in aetiology and treatment of breast cancer. *Lancet* 2, 814-816.
- Cos S. and Blask D.E. (1994). Melatonin modulates growth factor activity in MCF-7 human breast cancer cells. *J. Pineal Res.* 17, 25-32.
- Cos S. and Sánchez-Barceló E.J. (1994). Differences between pulsatile or continuous exposure to melatonin on MCF-7 human breast cancer cell proliferation. *Cancer Lett.* 84, 105-109.
- Cos S. and Sánchez-Barceló E.J. (1995). Melatonin inhibition of MCF-7 human breast-cancer cells growth: influence of cell proliferation rate. *Cancer Lett.* 93, 207-212.
- Cos S., Garjón F., Corral J., Mediavilla M.D. and Sánchez-Barceló E.J. (1989). Histopathologic features of DMBA-induced mammary tumors in blind-underfed, blind-anosmic, and blind cold exposed rats: influence of the pineal gland. *J. Pineal Res.* 6, 221-231.
- Cos S., Blask D.E., Lemus-Wilson A. and Hill A.B. (1991). Effects of melatonin on the cell cycle kinetics and estrogen rescue of MCF-7 human breast cancer cells in culture. *J. Pineal Res.* 10, 36-42.
- Cos S., Recio J. and Sánchez-Barceló E.J. (1996a). Modulation of the length of the cell cycle time of MCF-7 human breast cancer cells by melatonin. *Life Sci.* 58, 811-816.
- Cos S., Fernández F. and Sánchez-Barceló E.J. (1996b). Melatonin inhibits DNA synthesis in MCF-7 human breast cancer cells *in vitro*. *Life Sci.* 58, 2447-2453.
- Cos S., Fernández R., Güézmé A. and Sánchez-Barceló E.J. (1998). Influence of melatonin on invasive and metastatic properties of MCF-7 human breast cancer cells. *Cancer Res.* 58, 4383-4390.
- Eck M.C., Yuan L., Duffy L., Ram P.T., Ayettey S., Chen I., Cohn C.S., Reed J.C. and Hill S.M. (1998). A sequential treatment regimen with melatonin and all trans retinoic acid induces apoptosis in MCF-7 tumour cells. *Br. J. Cancer* 77, 2129-2137.
- Frenkel K. (1992). Carcinogen mediated oxidant formation and oxidative DNA damage. *Pharmacol. Ther.* 53, 127-166.
- Hamilton T. (1969). Influence of environmental light and melatonin upon mammary tumor induction. *Br. J. Surg.* 56, 764-766.
- Harland J.D. and Liburdy R.P. (1997). Environmental magnetic fields inhibit the antiproliferative action of tamoxifen and melatonin in a human breast cancer cell line. *Bioelectromagnetics* 18, 555-562.
- Hill S.M. and Blask D.E. (1988). Effects of the pineal hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF-7) in culture. *Cancer Res.* 48, 6121-6126.
- Hill S.M., Spriggs L.L., Simon M.A., Muraoka H. and Blask D.E. (1992). The growth inhibitory action of melatonin on human breast cancer cells is linked to the estrogen response system. *Cancer Lett.* 64, 249-256.
- Horwitz K.B. and McGuire W.L. (1978). Estrogen control of progesterone receptor in human breast cancer: correlations with nuclear processing of estrogen receptors. *J. Biol. Chem.* 253, 2223-2228.
- Ianas O., Olivescu R. and Badescu I. (1991). Melatonin involvement in oxidative processes. *Rom. J. Endocr.* 29, 117-123.
- Karmali R.A., Horrobin D.F. and Ghayur T. (1978). Role of pineal gland in the aetiology and treatment of breast cancer. *Lancet* 2, 1002.
- Kato M., Honma K.I., Shigemitsu T. and Shiga Y. (1993). Effects of exposure to circulatory to a circulatory polarized 50-Hz magnetic field on plasma and pineal melatonin levels in rats. *Bioelectromagnetics* 14, 97-106.
- Kojima T., Mochizuki C., Mitaka T. and Mochizuki Y. (1997). Effects of melatonin on proliferation, oxidative stress and Cx32 gap junction protein expression in primary cultures of adult rat hepatocytes. *Cell Struct. Funct.* 22, 347-356.
- Kothari L.S. (1987). Influence of chronic melatonin on 9,10-dimethyl-1,2-benzanthracene induced mammary tumors in female Holtzman rats exposed to continuous light. *Oncology* 44, 64-66.
- Kothari L. and Subramanian A. (1992). A possible modulatory influence of melatonin on representative phase I and phase II drug metabolizing enzymes in 9,10-dimethyl-1,2-benzanthracene induced rat mammary tumorigenesis. *Anti-Cancer Drugs* 3, 623-628.
- Kothari L.S., Shah P.N. and Mhatre M.C. (1982). Effect of continuous light on the incidence of 9,10 dimethyl-1,2-benzanthracene induced mammary tumors in female Holtzman rats. *Cancer Lett.* 16, 313-317.
- Kothari L.S., Shah P.N. and Mhatre M.C. (1984). Pineal ablation in varying photoperiods and the incidence of 9,10-dimethyl-1,2-benzanthracene induced mammary cancer in rats. *Cancer Lett.* 22, 99-102.
- Kothari A., Borges A. and Kothari L. (1995). Chemoprevention by melatonin and combined melatonin-tamoxifen therapy of second generation nitroso-methylurea-induced mammary tumors in rats. *Eur. J. Cancer Prev.* 4, 497-500.
- Kothari A., Borges A., Ingle A. and Kothari L. (1997). Combination of melatonin and tamoxifen as a chemoprophylaxis against N-nitroso-N-methylurea-induced rat mammary tumors. *Cancer Lett.* 111, 59-66.
- L'Hermite-Balériaux M. and Launoit Y. (1992). Is melatonin really an *in vitro* inhibitor of human breast cancer cell proliferation. *In Vitro Cell Dev. Biol.* 28A, 583-584.
- Lapin V. (1978). Effects of reserpine on the incidence of 9,10-dimethyl-1,2-benzanthracene induced tumors in pinealectomized and thymectomized rats. *Oncology* 35, 132-135.
- Leadem C.A. and Blask D.E. (1981). Evidence for an inhibitory influence

- of the pineal on prolactin in female rat. *Neuroendocrinology* 33, 268-275.
- Lemus-Wilson A., Kelly P.A. and Blask D.E. (1995). Melatonin blocks the stimulatory effects of prolactin on human breast cancer cell growth in culture. *Br. J. Cancer* 72, 1435-1440.
- Liburdy R.P., Sloma T.R., Sokolic R. and Yaswen P. (1993). ELF magnetic fields, breast cancer, and melatonin: 60 Hz fields block melatonin's oncostatic action on ER+ breast cancer cell proliferation. *J. Pineal Res.* 14, 89-97.
- Loomis D.P., Savtz D.A. and Ananth C.V. (1994). Breast cancer mortality among female electrical workers in the United States. *J. Natl. Cancer Inst.* 86, 921-925.
- Löscher W., Wahnschaffe U., Mevissen M., Lerchl A. and Stamm A. (1994). Effects of weak alternating magnetic fields on nocturnal melatonin production and mammary carcinogenesis in rats. *Oncology* 51, 288-295.
- Löscher W., Mevissen M. and Häussler B. (1997). Seasonal influence on 7,12 dimethylbenz[a]anthracene-induced mammary carcinogenesis in Sprague-Dawley rats under controlled laboratory conditions. *Pharmacol. Toxicol.* 81, 265-270.
- Maestroni G.J.M. (1995). T-helper-2 lymphocytes as a peripheral target of melatonin. *J. Pineal Res.* 18, 84-89.
- Mavissen M., Häussler M., Lerchl A. and Löscher W. (1998). Acceleration of mammary tumorigenesis by exposure of 7,12-dimethylbenz[a]anthracene-treated female rats in 50-Hz, 100-microT magnetic field: replication study. *J. Toxicol. Environ. Health* 53, 401-418.
- Mediavilla M.D., San Martín M. and Sánchez-Barceló E.J. (1992). Melatonin inhibits mammary gland development in female mice. *J. Pineal Res.* 13, 13-19.
- Mediavilla M.D., Gúezmes A., Ramos S., Kothari L., Garíjo F. and Sánchez-Barceló E.J. (1997). Effects of melatonin on mammary gland lesions in transgenic mice overexpressing N-ras proto oncogene. *J. Pineal Res.* 22, 86-94.
- Mediavilla M.D., Cos S. and Sánchez-Barceló E.J. (1999). Melatonin increases p53 and p21waf1 expression in MCF-7 human breast cancer cells *in vitro*. *Life Sci.* 65, 415-420.
- Meites J. (1980). Relation of the neuroendocrine system to the development and growth of experimental mammary tumors. *J. Neural Transm.* 48, 25-42.
- Menéndez-Pelaez A., Poeggeler B., Reiter R.J., Barlow-Walden L.R., Pablos M.I. and Tan D.X. (1993). Nuclear localization of melatonin in different mammalian tissues: immunocytochemical and radioimmunoassay evidence. *J. Cell Biochem.* 53, 372-382.
- Mhatre M., Shah P. and Juneja H. (1984). Effect of varying photoperiods on mammary morphology, DNA synthesis and hormonal profile in female rats. *J. Natl. Cancer Inst.* 72, 1411-1416.
- Molis T.M., Walters M.R. and Hill S.M. (1993). Melatonin modulation of estrogen receptor expression in MCF-7 human breast cancer cells. *Int. J. Oncol.* 3, 687-694.
- Molis T.M., Spriggs L.L., Jupiter Y. and Hill S.M. (1994). Modulation of estrogen receptor mRNA expression by melatonin in MCF-7 human breast cancer cells. *Mol. Endocrinol.* 8, 1681-1690.
- Molis T.M., Spriggs L.L., Jupiter Y. and Hill S.M. (1995). Melatonin modulation of estrogen-regulated proteins, growth factors, and proto-oncogenes in human breast cancer. *J. Pineal Res.* 18, 93-103.
- Nandi S., Guzman R.C. and Yang J. (1995). Hormones and mammary carcinogenesis in mice, rats and humans: a unifying hypothesis. *Proc. Natl. Acad. Sci. USA* 92, 3650-3657.
- Olcese J. (1990). The neurobiology of magnetic field detection in rodents. *Prog. Neurobiol.* 35, 325-330.
- Olcese J. and Reuss S. (1986). Magnetic field effects on pineal gland melatonin synthesis: comparative studies on albino and pigmented rodents. *Brain Res.* 369, 365-368.
- Osborne C.K., Hobbs K. and Trent J.M. (1987). Biological differences among MCF-7 human breast cancer cell lines from different laboratories. *Breast Cancer Res. Treat.* 9, 111-121.
- Panzer A., Lottering M.-L., Bianchi P., Glencross D.K., Stark J.H. and Seegers C. (1998). Melatonin has no effect on the growth, morphology or cell cycle of human breast cancer (MCF 7), cervical cancer (HeLa), osteosarcoma (MG-63) or lymphoblastoid (TK6) cells. *Cancer Lett.* 122, 17-23.
- Papazisis K.T., Kouretas D., Geromichalos G.D., Sivridis E., Tsekrelli O.K., Dimitriadis K.A. and Kortsaris A.H. (1998). Effects of melatonin on proliferation of cancer cell lines. *J. Pineal Res.* 25, 211-218.
- Poeggeler B., Reiter R.J., Tan D.X., Chen L.D. and Manchester L.C. (1993). Melatonin, hydroxyl radical mediated oxidative damage and aging: a hypothesis. *J. Pineal Res.* 14, 151-168.
- Recio J., Cardinali D.P. and Sánchez-Barceló E.J. (1994a). 2-[¹²⁵I]iodomelatonin binding sites in murine mammary tissue. *Biol. Signals* 3, 85-90.
- Recio J., Mediavilla M.D., Cardinali D.P. and Sánchez-Barceló E.J. (1994b). Pharmacological profile and diurnal rhythmicity of 2-[¹²⁵I]iodomelatonin binding sites in murine mammary tissue. *J. Pineal Res.* 16, 10-17.
- Reiter R.J. (1980). The pineal gland and its hormones in the control of reproduction in mammals. *Endocrine Rev.* 1, 109-131.
- Reiter R.J. (1988). Pineal gland, cellular proliferation and neoplastic growth: An historical account. In: *The pineal gland and cancer*. Gupta D., Attanasio A. and Reiter R.J. (eds). Brain Research Promotion. Tübingen, Germany. pp. 41-64.
- Reiter R.J. (1993a). Electromagnetic fields and melatonin production. *Biomed. Pharmacother.* 47, 439-444.
- Reiter R.J. (1993b). Interactions of the pineal hormone melatonin with oxygen-centered free radicals: a brief review. *Braz. J. Med. Biol. Res.* 26, 1141-1155.
- Reiter R.J. (1995). Functional pleiotropy of the neurohormone melatonin: antioxidant protection and neuroendocrine regulation. *Front. Neuroendocrin.* 16, 383-415.
- Reiter R.J. and Klein D.C. (1969). Preliminary observations on the reproductive effects of the pineal gland in blinded anosmic male rats. *J. Reprod. Fertil.* 19, 563-565.
- Reiter R.J. and Richardson B.A. (1992). Magnetic field effects on pineal indoleamine metabolism and possible biological consequences. *FASEB J.* 6, 2283-2287.
- Reiter R.J., Poeggeler B., Tan D.X., Chen L.D., Manchester L.C. and Guerrero J.M. (1993). Antioxidant capacity of melatonin: a novel action not requiring a receptor. *Neuroendocrinol. Lett.* 15, 103-116.
- Russo I.H. and Russo J. (1978). Developmental stage of the rat mammary gland as determinant of its susceptibility to 7,12-dimethylbenz(a)anthracene. *J. Natl. Cancer Inst.* 61, 1439-1449.
- Russo I.H. and Russo J. (1996). Mammary gland neoplasia in long-term rodent studies. *Environ. Health Persp.* 104, 938-967.
- Russo I.H. and Russo J. (1998). Role of hormones in mammary cancer initiation and progression. *J. Mam. Gland Biol. Neoplasia* 3, 49-61.
- Russo J., Gusterson B.A., Rogers A.E., Russo I.H., Wellings S.R. and Van Zwieten M.J. (1990). Comparative study of human and rat mammary tumorigenesis. *Lab. Invest.* 62, 1-32.

- Sánchez-Barceló E.J., Mediavilla M.D., Sánchez-Criado J.E., Cos S. and Cortines M.D.G. (1985). Antigonadal actions of olfactory and light deprivation. I. Effects of blindness combined with olfactory bulb deafferentation, transection of vomeronasal nerves, or bulbectomy. *J. Pineal Res.* 2, 177-190.
- Sánchez-Barceló E.J., Cos S. and Mediavilla M.D. (1986). Cold exposure as a potentiating factor of pineal actions in nonhibernating mammals. *Cryobiology* 23, 72-80.
- Sánchez-Barceló E.J., Cos S. and Mediavilla M.D. (1988). Influence of pineal gland function on the initiation and growth of hormone-dependent breast tumors. Possible mechanisms. In: *The pineal gland and cancer*. Gupta D., Attanasio A. and Reiter R.J. (eds). Brain Research Promotion. Tübingen, Germany. pp 221-232.
- Sánchez-Barceló E.J., Mediavilla M.D. and Tucker H.A. (1990). Influence of melatonin on mammary growth: In vivo and in vitro studies. *Proc. Soc. Exp. Biol. Med.* 194, 103-107.
- Seshadri R., Subramanian A. and Kothari L.S. (1992). Effect of neonatal pineal ablation on estradiol receptors in mammary glands of rats housed under varying photoperiods. *Ind. J. Exp. Biol.* 30, 162-164.
- Shah P.N., Mhatre M.C. and Kothari L.S. (1984). Effect of melatonin on mammary carcinogenesis in intact and pinealectomized rats in varying photoperiods. *Cancer Res.* 44, 3403-3407.
- Shellard S.A., Whelan R.D.H. and Hill B.T. (1989). Growth inhibitory and cytotoxic effects of melatonin and its metabolites on human tumour cell lines *in vitro*. *Br. J. Cancer* 60, 288-290.
- Sorrentino S., Reiter R.J., Schalch D.S. and Donofrio R.J. (1971a). Interactions of the pineal gland, blinding, and underfeeding on reproductive organ size and radioimmunoassayable growth hormone. *Neuroendocrinology* 7, 105-115.
- Sorrentino S., Reiter R.J., Schalch D.S. and Donofrio R.J. (1971b). Role of the pineal gland in growth restraint of adult male rats by light and smell deprivation. *Neuroendocrinology* 8, 116-124.
- Soule H.D., Vazquez J., Long A., Albert S. and Brennan M.J. (1973). A human cell line from a pleural effusion derived from a breast carcinoma. *J. Natl. Cancer Inst.* 51, 1409-1412.
- Stankov B., Lucini V., Scaglione F., Cozzi B., Righi M., Canti G., Demartini G. and Fraschini F. (1991). ¹²⁵I-iodomelatonin binding in normal and neoplastic tissues. In: *Role of melatonin and pineal peptides in neuroimmunomodulation*. Fraschini F. and Reiter R.J. (eds). Plenum Press. New York. pp 117-127.
- Steinhilber D., Brungs M., Werz O., Wiesenberger I., Danielsson C., Kahlen J.P., Nayeri S., Schrader M. and Carlberg C. (1995). The nuclear receptor for melatonin represses 5-lipoxygenase gene expression in human B lymphocytes. *J. Biol. Chem.* 270, 7037-7040.
- Subramanian A. and Kothari L.S. (1991a). Suppressive effect by melatonin on different phases of 9,10 dimethyl-1,2-benzanthracene (DMBA)-induced rat mammary gland carcinogenesis. *Anticancer Drugs* 2, 297-303.
- Subramanian A. and Kothari L.S. (1991b). Melatonin, a suppressor of spontaneous murine mammary tumors. *J. Pineal Res.* 10, 136-140.
- Tamarkin L., Cohen M., Roselle D., Reichert C., Lippman M. and Chabner B. (1981). Melatonin inhibition and pinealectomy enhancement of 7,12-dimethyl-benz(a)anthracene-induced mammary tumors in the rat. *Cancer Res.* 41, 4432-4436.
- Tamarkin L., Danforth D.N., Lichter A., DeMoss E., Cohen M., Chabner B. and Lippman M. (1982). Decreased nocturnal plasma melatonin peak in patients with estrogen receptor positive breast cancer. *Science* 216, 1003-1005.
- Tan D.X., Poeggeler B., Reiter R.J., Chen L.D., Chen S., Manchester L.C. and Barlow-Walden L.R. (1993). The pineal hormone melatonin inhibits DNA adduct formation induced by the chemical carcinogen safrole *in vivo*. *Cancer Lett.* 70, 65-71.
- Trosko J.E., Chang C.C., Madhukar B.V. and Klaunig J.E. (1990). Chemical, oncogene and growth factor inhibition gap junctional intercellular communication: An integrative hypothesis of carcinogenesis. *Pathobiology* 58, 265-278.
- Tynes T., Hannevik M., Andersen A., Vistnes A.I. and Haldorsen T. (1996). Incidence of breast cancer in Norwegian female radio and telegraph operators. *Cancer Causes Control* 7, 197-204.
- Ubeda A., Trillo M.A., House D.E. and Blackman C.F. (1995). Melatonin enhances junctional transfer in normal C3H/10T1/1 cells. *Cancer Lett.* 91, 241-245.
- Vaticon D., Fernández-Galaz C., Esquifino A., Tejero A. and Aguilar E. (1980). Effect of constant light on prolactin secretion in adult female rats. *Horm. Res.* 12, 277-288.
- Welsch C.W. and Nagasawa H. (1977). Prolactin and murine mammary tumorigenesis: a review. *Cancer Res.* 37, 951-963.
- Wrba H., Halberg F. and Dutter A. (1986). Melatonin circadian-stage-dependently delays breast tumor development in mice injected daily for several months. *Chronobiology* 13, 123-128.

Accepted November 30, 1999