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Melatonin prevents the estrogenic effects of sub-chronic administration of cadmium on mice mammary glands and uterus

Abstract: Cadmium (Cd) is a heavy metal classified as a human carcinogen. Occupational exposure, dietary consumption and cigarette smoking are sources of Cd contamination. Cd-induced carcinogenicity depends on its oxidative and estrogenic actions. A possible role of Cd in breast cancer etiology has been recently suggested. Melatonin, because of its antioxidant and antiestrogenic properties could counteract the toxic effects of this metalloestrogen. Our aim was both to determine the effects of relevant doses of Cd on mice mammary glands and uterus and to test whether melatonin would counteract its effects. Female mice of different ages and estrogenic status (prepuberal, adult intact, adult ovariectomized) were treated with $CdCl_2$ (2–3 mg/kg, i.p.), melatonin (10 μg/mL in drinking water), $CdCl_2$ + melatonin, or diluents. Whereas in prepuberal animals Cd disturbs mammary ductal growth and reduces the number of terminal end buds, in adults, regardless of the steroidal milieu, Cd exerts estrogenic effects on mammary glands, increasing lobuloalveolar development and ductal branching. Uterine weight also increased as a result of Cd treatment. The effects of Cd are partially inhibited by melatonin. In adult ovariectomized mice, Cd concentration in blood of animals treated with $CdCl_2$ + melatonin was lower than in mice receiving only Cd; the opposite effects were found in non-castrated animals. As Cd mimics the effect of estrogens, the high incidence of breast cancer in tobacco smokers and women working in industries related with Cd could be explained because of the properties of this metal. The effects of melatonin point to a possible role of this indoleamine as a preventive agent for environmental or occupational Cd contamination.

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Introduction

Cadmium (Cd), a heavy metal, is a widespread environmental pollutant recently classified as a human carcinogen by the International Agency for Research on Cancer [1]. Workers involved in activities such as smelting and refining of metal ores, electroplating and welding, Ni-Cd battery manufacturing, or manipulation of paints and plastic stabilizers, are exposed to Cd. Among the general population, cigarette smoking and contaminated water, food or air, are the main sources of exposure [2]. Because of its long biological half-life, Cd accumulates in the body, thereby increasing the risk of toxicity [3]. Because of its selective binding to heavy metals such as Cd, metallothioneins, intracellular low-molecular-weight proteins ubiquitous in eukaryotes, have been considered to be involved in the metal detoxification mechanisms [4, 5].

The possible mechanisms of Cd-induced carcinogenicity include oxidative effects [6] as well as the activation of responses mediated by estrogen receptors (ERα) [7–10]. This latter property helps qualify Cd as a metalloestrogen [10]. The estrogenic activity of Cd is more potent than that of phytoestrogens [7]. In female rats, an initial study demonstrated the estrogenic effects of Cd on uterus and mammary glands [8]. From this study it is possible to conclude that endocrine disruptors, such as Cd, could play an important role in the etiology of breast cancer [11]. However, the number of studies focusing on the specific effects of Cd on mammary tissue is still small and mostly centered on the mammary gland of lactating animals in relation with the transfer of the metal to milk [12]. The state of the art can be summarized as follows: (a) the estrogen-like activity of Cd on MCF-7 breast cancer cells is known, the effects being dependent on the binding and activation by Cd of the ERα [7, 13, 14]; (b) our group has recently demonstrated that Cd not only binds to and activates ERα, but also to ERβ and AP1 sites in both ERα and ERβ [9]; and (c) in vivo, the estrogenic effects of Cd in mammary glands of ovariectomized rats have been described [8].

Melatonin has antioxidant [15, 16] as well as antiestrogenic [17] properties. The melatonin–estrogen interactions give this indoleamine oncostatic properties against hormone-dependent tumors, such as most mammary adenocarcinomas [17, 18] as well as a role in the development of normal mammary tissue [19]. Because of its antioxidant and antiestrogenic actions, melatonin could counteract the toxic

effects of xenoestrogens such as Cd, which acts as an oxidant and estrogenic agent [6–10]. In line with this, the antioxidant effects of melatonin have been demonstrated to provide protection against Cd-mediated free-radical damage [20–24]. Concerning the mammary gland, as far as we are aware, the only studies of the melatonin–Cd interactions have been conducted only by us, and with the finding that, in vitro, melatonin counteracts the Cd-induced proliferation in MCF-7 human breast cancer cells [9]. The aim of this work was to determine the effects of the exposure to certain doses of Cd on mice mammary glands at different steps of their postnatal development as well as whether the antiestrogenic actions of melatonin can counteract the effects of this metalloestrogen.

Material and methods

Animals

The experiments were carried out in female CD-1 Swiss Albino mice provided by Harlan Interfauna Ibérica (Barcelona, Spain). The animals were housed under conditions of constant temperature (22 ± 2°C) and humidity (50–60%), under a 10-h light/14-h dark photoperiod in the vivarium of the University. Water and standard laboratory food (PANLAB, Barcelona, Spain) were provided ad libitum. All the experimental procedures were carried out in accordance with the guidelines for ethics in animal research of the European Union, under the control of the Committee of Ethics of the University of Cantabria.

Chemicals

Unless otherwise indicated, all chemicals used in this work were purchased from Sigma-Aldrich (Madrid, Spain).

Experiment 1

To study the effects of Cd and melatonin on the prepuberal development of the mammary glands and uterus, 3-week-old female mice were assigned to one of the following groups and treatments (10–12 mice per group): Cd, mice receiving i.p. injections of $CdCl_2$ [3 mg/kg body weight (BW), 5 days per week, 2 weeks]; melatonin, mice with drinking water containing melatonin (10 μg/mL); Cd + melatonin, animals injected with Cd and receiving melatonin in drinking water; C (control), mice receiving injections of saline (Cd diluent) and drinking water with 0.01% ethanol (melatonin diluent). Melatonin was prepared from a stock solution (1 mg/mL, in absolute ethanol) and added to drinking water. The bottles were covered with foil alum to protect them from light. Water (with melatonin or ethanol 0.01% as its diluent) was changed every 2 days. The presence of melatonin in drinking water does not modify the amount of water drunk nor the drinking rhythm of rodents (predominantly nocturnal) [25]. After 2 weeks of treatment, the mice were killed by decapitation under anesthesia with diethyl ether, and the inguinal mammary glands dissected and whole-mounts prepared for analysis as previously described [19]. The uteri were also dissected, weighed, and fixed for histological study.

Experiment 2

To study the effects of Cd and melatonin on mammary gland development in adult mice, 4-month-old female animals were assigned to experimental groups (10–12 mice per group) and treated in a similar way to that in experiment 1 (Cd, 2 mg/kg BW; melatonin 10 μg/mL) for 7 weeks. Then, animals were killed, the mammary glands and uteri collected and analyzed as in experiment 1.

Experiment 3

This experiment was carried out to study the effects of Cd and melatonin on mammary gland regression after estrogen deprivation. Four-month-old female mice were ovariectomized (Ovx) and assigned to one of the following groups and treatments (10–12 mice per group): Ovx + Cd, mice were given i.p. injections of $CdCl_2$ (2 mg/kg BW, 5 days per week) for 7 weeks from the same day of their being ovariectomized; Ovx + Mel, animals receiving melatonin (10 μg/mL) in drinking water; Ovx + Cd + Mel, mice treated with $CdCl_2$ and melatonin as in the preceding groups; OvxC (control), animals injected daily with saline (Cd diluent) and drinking water with 0.01% ethanol (melatonin diluent). Sampling procedures were also the same as in experiment 1.

Experiment 4

In this experiment we studied the effects of Cd and melatonin on atrophic mammary glands which were regressed because of the estrogen deprivation subsequent to ovariectomy. Four-month-old female mice were ovariectomized. After 7 weeks, in order to allow a complete regression of the mammary glands, mice were assigned to four groups (10 mice per group) as in experiment 3. The treatments ($CdCl_2$ or $CdCl_2$ + melatonin) were administered for 3 weeks (5 days per week). Sampling procedures were also the same as in previous experiments.

Mammary gland whole mounts for the evaluation of gland development

After the mice were killed, the fourth inguinal mammary glands were removed, spread onto glass slices, fixed in ethanol–glacial acetic acid (3:1, vol/vol), stained overnight with carmine alum, dehydrated in graded solutions of ethanol, cleared with xylene, and mounted in glass slices with permount. Mammary whole mounts were photographed (6 or 12×) with a video camera (Motican 2000, Motic Spain SL, Barcelona) adapted to a stereoscope (Wild M5A, Wild Heerbrugg AG, Aarau, Switzerland). The degree of mammary gland development was evaluated on whole-mount gland preparations by comparison with a visual scale (Fig. 1). This is a standard approach widely used because it provides global information about the number and spatial arrangement of the different structures of the gland [26, 27].

In experiment 1, the degree of mammary gland development was established by comparing (five different researchers in a double-blind study) the digitalized pictures of the mammary glands with the visual scale of Fig. 1.

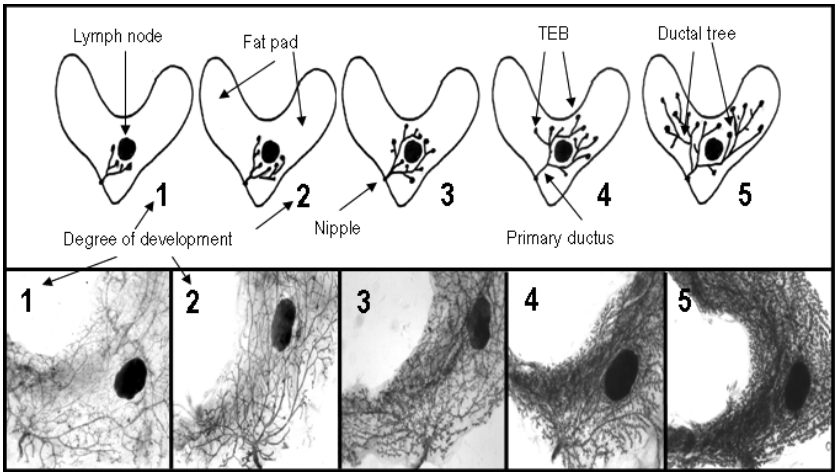

Fig. 1. (Upper panel). Schematic drawing showing the five degrees of ductal tree growth: 1, the ductal tree reaches the lower limb of the lymph node; 2, the ductal branches surpass the lower limb of the lymph node which is partially surrounded; 3, the ductal tree completely surrounds the lymph node; 4, the ductal branches exceed the upper limb of the lymph node and progress toward the limits of the mammary fat pad; 5, the ductal tree grows toward the limits of the fat pad, reaching its borders. (Lower panel). Examples of the five degrees of mammary gland development used for the evaluation of glands from adult mice. Mammary glands from adult ovariectomized mice were evaluated in accordance with the same visual scale but considering only fourth degrees of development (1 to 4).

Furthermore, the terminal end buds (TEB) in each mammary gland were also counted.

In experiments 2, 3 and 4, as the development of the ductal branches was already complete in the adult animals, the above-mentioned method to evaluate mammary gland development, based on the extension of the ducts, cannot be used. In these cases, what we evaluated was the development of the lobuloalveolar structures. Five (four in the case of Ovx mice) different degrees of lobuloalveolar development were defined (see Fig. 1) and five different researchers assigned, on a double-blind assay, the degree corresponding to each gland.

Concentration of Cd in blood

Blood samples were collected in Eppendorf tubes with ethylenediaminetetraacetic acid (EDTA) (1 mg/mL) when the animal was killed, and stored at −20°C until assayed for Cd determinations. The concentration of total Cd in blood samples was measured by electrothermal atomic spectrometry (AAnalyst 600, Perkin Elmer Inc., Shelton, CT, USA), with Zeeman background correction (ETAAS-Z) and palladium nitrate-magnesium as modifier [28].

Statistical analysis

The differences in the degree ductal or lobuloalveolar mammary gland development among experimental groups were assessed by nonparametric ANOVA (Kruskal–Wallis test) followed by Dunn's test for comparisons between selected pairs of groups. Differences in body weight, uterine weight, total number of mammary TEB or blood concentration of Cd, were analyzed by parametric one-way ANOVA followed by Student–Newman–Keuls' tests for comparisons between groups. Values of $P < 0.05$ were considered as significant.

Results

In prepuberal mice (experiment 1), the blood concentration of total Cd in those receiving the metal ranged between 70 and 80 μg/100 mL and there were no differences between Cd- and Cd–melatonin-treated animals (Table 1). As expected, control as well as melatonin-treated mice had almost undetectable levels of Cd in plasma (Table 1). Body weight of animals treated with Cd was significantly lower than that of controls or mice receiving only melatonin (Table 1). The uterine weight of Cd-treated animals was significantly lower than in controls (Table 1) and the uteri showed a thin endometrial lining, with a lower number of secretory glands (data not shown). The Cd-treated animals showed a lower degree of mammary gland development than controls, with significant differences in the length of the ductal tree as well as a lower number of TEB (Fig. 2). Melatonin counteracted the inhibitory effect of Cd on mammary gland development (Fig. 2).

In adult mice (experiment 2), interestingly, Cd concentration in blood of animals treated with Cd + Mel was significantly higher than in mice receiving only the metal (Table 1). No differences in body weight were found between control animals and those treated with Cd, Mel, or Cd + Mel (Table 1). Treatment with Cd induced a significant increase in the relative (but not in the absolute) weight of the uterus, an effect which is also partially prevented by melatonin (Table 1). Mammary glands of mice receiving Cd showed a high lobuloalveolar development, similar to that on the first days of pregnancy. This development was significantly higher than those in control animals (Fig. 3). Melatonin partially inhibited the stimulatory effects of Cd on mammary glands (Fig. 3).

In ovariectomized adult mice (experiment 3), Cd concentration in the blood of animals treated with Cd + Mel was significantly lower than in mice receiving only the metal

Table 1. Body and uterine weights, and Cd concentration in blood in mice from the four experiments

	Body weight (g)	Absolute uterine weight (mg)	Relative uterine weight (mg/100 g BW)	Total Cd concentration (μg/100 mL blood)
Experiment 1. Prepuberal mice treated with Cd and/or melatonin from 3rd to 5th week of age.				
Control (C)	21.74 ± 0.28 (11)	89.48 ± 12.64 (11)	410.30 ± 57.75 (11)	0.18 ± 0.04 (7)
Cd	18.96 ± 0.24[a] (9)	33.14 ± 4.43[a] (9)	172.80 ± 21.23[b] (9)	83.84 ± 3.17[a] (9)
Mel	21.28 ± 0.26 (9)	60.43 ± 16.80 (9)	291.71 ± 81.44 (9)	0.17 ± 0.05 (9)
Cd + Mel	19.75 ± 0.31[a] (8)	33.13 ± 2.99[a] (8)	193.1 ± 14.67[a] (8)	97.88 ± 11.58[a] (7)
	[a]$P < 0.001$ vs C and vs Mel	[a]$P < 0.01$ vs C	[a]$P < 0.01$ vs C; [b]$P < 0.05$ vs C	[a]$P < 0.001$ vs C and vs Mel
Experiment 2. Adult mice treated with Cd and/or melatonin from the 16th to the 23rd week of age.				
Control (C)	35.68 ± 0.73 (12)	175.30 ± 9.97 (12)	505.04 ± 27.57 (12)	0.25 ± 0.04 (12)
Cd	34.30 ± 0.55 (12)	200.81 ± 10.20 (12)	593.66 ± 28.05[a,b] (12)	86.73 ± 0.04[a,b] (12)
Mel	35.95 ± 0.88 (12)	170.40 ± 11.66 (11)	452.92 ± 41.06 (11)	0.33 ± 0.04 (12)
Cd + Mel	34.07 ± 0.91 (11)	183.11 ± 15.73 (10)	512.75 ± 38.84 (10)	106.10 ± 0.04[b] (11)
	Differences NS	Differences NS	[a]$P < 0.05$ vs C and Cd + Mel; [b]$P < 0.001$ vs Mel	[a]$P < 0.05$ vs Cd + Mel; [b]$P < 0.001$ vs C and Mel
Experiment 3. Ovariectomized (Ovx) mice treated with Cd and/or melatonin during 7 week period from ovariectomy.				
Ovx-Control (OvxC)	34.87 ± 1.50 (11)	54.68 ± 14.06 (11)	159.42 ± 42.01 (11)	0.14 ± 0.02 (11)
Ovx + Cd	28.93 ± 0.68[a] (10)	78.12 ± 21.87 (10)	265.65 ± 77.54 (10)	105.48 ± 6.18[a,b] (10)
Ovx + Mel	32.26 ± 0.81 (9)	56.25 ± 15.62 (9)	170.22 ± 48.53 (9)	0.09 ± 0.01 (9)
Ovx + Cd + Mel	27.50 ± 1.70[b] (8)	31.25 ± 9.37 (8)	112.61 ± 24.67 (8)	85.18 ± 6.32[b] (8)
	[a]$P < 0.01$ vs OvxC; [b]$P < 0.001$ vs OvxC	Differences NS	Differences NS	[a]$P < 0.01$ vs Ovx + Cd + Mel; [b]$P < 0.001$ vs OvxC and Ovx + Mel
Experiment 4. Ovariectomized (Ovx) mice treated with Cd and/or melatonin during 4 weeks period from the 7th week after ovariectomy.				
Ovx-Control (OvxC)	32.51 ± 0.99 (7)	51.03 ± 12.10 (7)	153.86 ± 56.36 (7)	0.03 ± 0.01 (6)
Ovx + Cd	30.66 ± 1.01[a] (8)	61.85 ± 18.11 (8)	199.13 ± 30.76 (8)	125.58 ± 6.01[a,b] (8)
Ovx + Mel	33.27 ± 1.02 (11)	55.63 ± 15.75 (11)	166.31 ± 25.62 (11)	0.12 ± 0.01 (6)
Ovx + Cd + Mel	29.67 ± 0.47[a] (10)	68.69 ± 19.91 (10)	230.83 ± 28.71 (10)	91.94 ± 4.99[b] (9)
	[a]$P < 0.01$ vs OvxC and Ovx + Mel	Differences NS	Differences NS	[a]$P < 0.01$ vs Ovx + Cd + Mel; [b]$P < 0.001$ vs OvxC and Ovx + Mel

See description in Material and methods.
Data are mean ± SEM. Number of samples are indicated in parentheses.

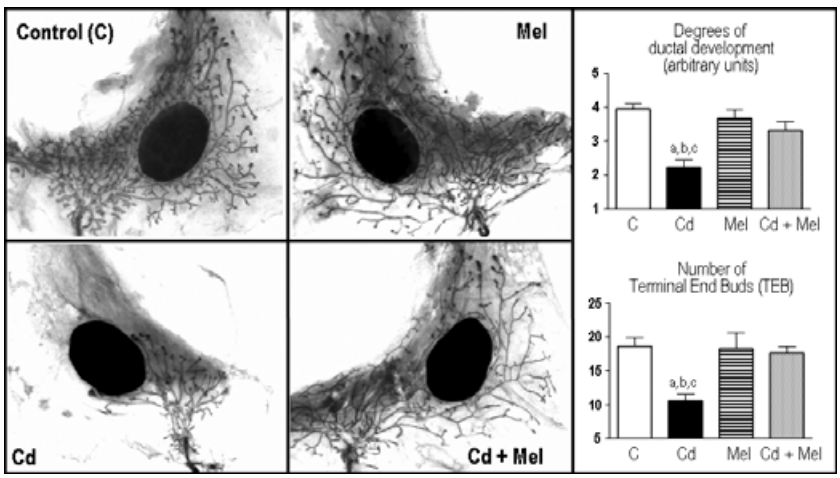

Fig. 2. Representative examples of whole mount mammary glands from 5-week-old prepuberal mice treated with cadmium (Cd), melatonin (Mel), Cd + Mel or the diluents of both (C). The upper right panel shows the degrees of ductal development of mammary glands evaluated according to the visual scale indicated in Fig. 1. Differences between groups are: [a]$P < 0.001$ versus C; [b]$P < 0.01$ versus Mel; [c]$P < 0.05$ versus Cd + Mel. The lower right panel shows the number of terminal end buds (TEB) in the mammary glands. Differences between groups are: [a]$P < 0.01$ versus C, versus Mel, and versus Cd + Mel.

(Table 1). Cd treatment, either alone or with melatonin, induced a significant decrease in body weight (Table 1). As expected, the uterus of the Ovx mice was atrophied because of the estrogenic deprivation, and no differences between the different treatments were found (Table 1). Seven weeks after Ovx, untreated animals (OvxC) showed a complete regression of the alveolar buds, although the ductal branching was completely developed and occupied the

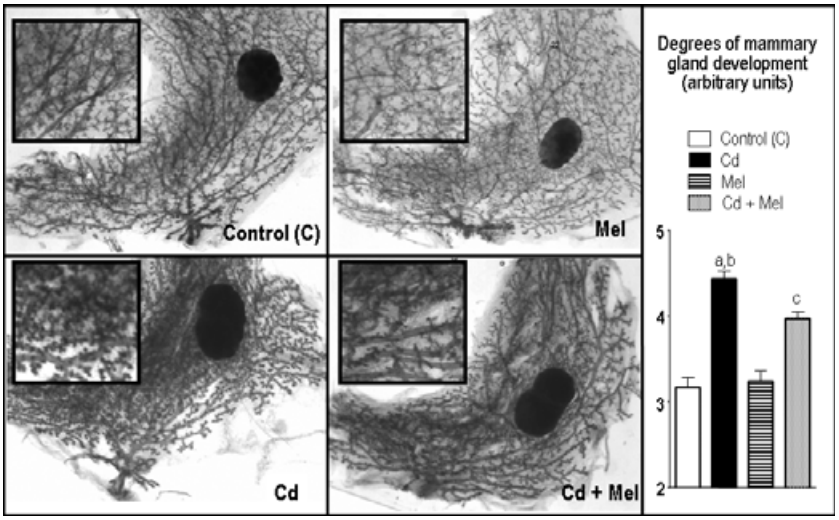

Fig. 3. (Left panels) Representative examples of whole mount mammary glands from 23-week-old adult virgin mice after 7 weeks of treatment with cadmium (Cd), melatonin (Mel), Cd + Mel or the diluents of both (C). Magnification: 6× and 12× (inserts). (Right panel) Analysis of the degrees of lobuloalveolar development evaluated by the visual scale shown in Fig. 1. Differences between groups: $^{a}P < 0.001$ versus C and versus M; $^{b}P < 0.05$ versus Cd + Mel; c, $P < 0.001$ versus C and versus Mel.

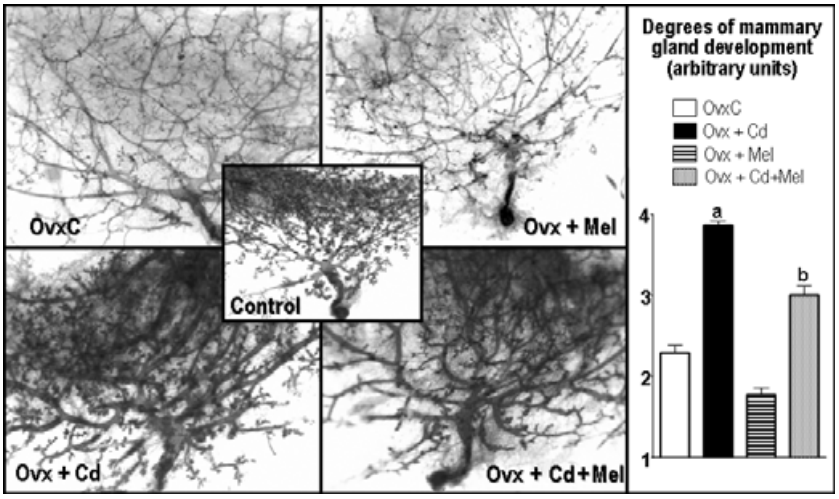

Fig. 4. 23-week-old adult ovariectomized mice after 7 weeks of treatment with cadmium (Ovx + Cd), melatonin (Ovx + Mel), Cd + Mel (Ovx + Cd + Mel) or the diluents of both (OvxC), administered from the same day of their being castrated. (Left panels) Representative examples of whole mount mammary glands (6×). The central insert shows the mammary gland from an age-matched uncastrated mouse. (Right panel) Analysis of the degrees of lobuloalveolar development following the visual scale shown in Fig. 1. Differences between groups: $^{a}P < 0.001$ versus OvxC, versus Ovx + Mel and versus Ovx + Cd + Mel; $^{b}P < 0.01$ versus OvxC; c, $P < 0.001$ versus Ovx + Mel.

entire fat pad (Fig. 4). The extent of mammary gland involution can be seen in Fig. 4, by comparing the level of lobuloalveolar development prior to Ovx (central insert) with a sample of a mammary gland from an OvxC mouse, 7 weeks after Ovx. The regression of ducts and alveolar structures that occurs following removal of the ovaries was prevented by treatment with Cd, which behaves as an estrogen (Fig. 4). Melatonin alone (Ovx + Mel) did not modify the mammary gland regression after estrogen deprivation although, when administered together with Cd, it diminished the estrogenic effects of this metal. Fig. 4 also shows the results of the semiquantitative analysis of the differences in the degrees of development. The highest degree of lobuloalveolar development was present in Cd-treated mice, whereas melatonin partially inhibited the estrogenic effects of Cd on mammary glands.

In ovariectomized mice treated with Cd and/or melatonin from the 7th week after castration (experiment 4), the concentration of Cd in the blood of mice treated with Cd + Mel was significantly lower than in those receiving only Cd (Table 1). The administration of Cd alone or with melatonin decreased the body weight of the Ovx mice (Table 1). Differences in the uterine weight among the animals of the four experimental groups were not found (Table 1). The mammary glands of Cd-treated animals showed a degree of lobuloalveolar development higher than

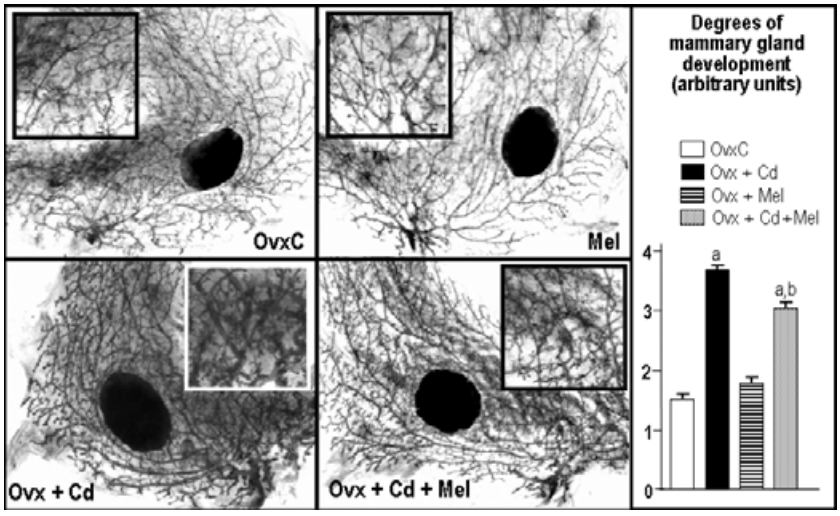

Fig. 5. 27-week-old adult ovariectomized mice. The animals were ovariectomized at 16 weeks of age and treated 7 weeks later, for 4 weeks, with cadmium (Ovx + Cd), melatonin (Ovx + Mel), Cd + Mel (Ovx + Cd + Mel) or the diluents of both (OvxC). (Left panels) Representative examples of whole mount mammary glands. Magnification: 6× and 12× (inserts). (Right panel) Analysis of the degrees of lobuloalveolar development following the visual scale shown in Fig. 1. Differences among groups: $^{a}P < 0.001$ versus OvxC and versus Ovx + Mel; $^{b}P < 0.05$ versus Ovx + Cd.

in melatonin-treated or control mice (Fig. 5). Melatonin, when co-administered with Cd, partially counteracted the stimulatory effects of Cd on mammary gland growth.

Discussion

The objective of this work was to assess the effects of subchronic administration of Cd on mammary glands and uterus of female mice as well as their possible modification by melatonin. Each experiment was designed to study the effects of Cd at a different stage of mammary gland development. Thus, the first experiment focused on mammary development at prepuberal age, when ductal elongation and development of TEB occurs under the control of estrogens, GH, growth factors (IGF-1) and corticoids [29, 30]. In the second experiment, we studied the effects of Cd on the mammary glands of adult virgin animals, when the ductal tree is already fully developed. The third and fourth experiments were designed to study the effects of Cd intoxication on mammary glands regressing or already atrophied after estrogen deprivation similar to what happens after menopause.

Our results in prepuberal animals suggest that, during this period, Cd has antiestrogenic effects. The mouse mammary gland began to show a proliferative response to estrogens from the third week of age [29]. Thus, in our animals, we expected that Cd would increase the development of mammary glands and uterus as previously reported [8]. However, our results are the contrary. This discrepancy could be due to the duration of Cd treatment (a single dose versus 2 weeks of treatment) or because our animals were intact whereas those of the referred experiment [8] had been ovariectomized. In prepuberal uncastrated mice, Cd, a xenoestrogen less potent than estradiol, could compete with the low concentrations of estradiol for binding to ERα, thus interfering with the development of estrogen-sensitive organs such as the mammary gland and uterus. Another explanation could be the direct effects of Cd on the hypothalamus and pituitary gland. Cd induces redox damage in the hypothalamic–pituitary axis and influences the secretion of pituitary hormones [31], some of which are involved in the prepuberal mammary gland development [30]. Recently, melatonin has been described as capable of protecting the hypothalamus and pituitary from Cd-induced oxidative damage [23]. These data could explain why the effects of Cd on mammary glands are counteracted by the simultaneous treatment with melatonin.

The results from our experiments on adult animals, despite their being ovariectomized or intact, show the estrogenic effects of Cd. Hence it can be seen that Cd increased lobuloalveolar development in mammary glands as well as uterine weight. The lobuloalveolar development observed in glands of adult (uncastrated) animals treated with Cd was similar to that reached in the initial days of pregnancy, which depends on the continuous stimulation by estradiol and progesterone [29–30]. The subchronic administration of Cd, because of its binding and activation of ERα [13], giving a continuous estrogenic stimulation and an increase in progesterone receptors [29, 30], could mimic the initial stages of pregnancy. Melatonin inhibition of the stimulatory effects of Cd on estrogen-dependent organs could be due to an interaction at the ER level. Our group recently demonstrated that, in MCF-7 cells, melatonin is a specific inhibitor of Cd-induced ERα-mediated transcription in estrogen response elements (ERE) as well as AP1-containing promoters [9].

To explain the different nature of Cd-actions in peri- and post-puberal animals, what must be taken into consideration are the differences between the control of mammary gland development in these stages. Whereas the post-puberal development basically depends on estrogens and progesterone, pre-puberal growth also implicates pituitary

and adrenal hormones as well as growth factors [29, 30]. Furthermore, the number of epithelial ERα-positive cells in mammary glands increases with age and, in adult female mice, ovariectomy leads to an increase of ERα gene expression by approximately 50% [32]. Thus, in young mice, with low levels of estrogens as well as a low proportion of ERα-positive cells [32], the oxidative effects of Cd on the hypothalamic–pituitary axis, with the subsequent decrease of pituitary hormone secretion [23, 31], could be responsible for the low mammary gland development. In the adult mice, with a higher proportion of mammary epithelial cells expressing ERα [32], the estrogenic effects of Cd could be more important than the oxidative ones. Interestingly, the Cd-induced expression of metallothionein (the protein which binds the metal and reduces its circulating levels) increases with age, it being higher in adult mice than in young [33]. In conclusion, the age-dependent balance of the oxidative [6] and estrogenic [7–9] actions of Cd together with the different hormonal control of mammary growth [29–30], explain the different effects of Cd on pre- and post-puberal mice. Similar differential effects of Cd on hypothalamic–pituitary–testicular axis of pre- and post-puberal rats have also been described [34].

Among ovariectomized animals those treated with Cd + melatonin had significantly lower blood concentration of Cd than those receiving Cd alone. The opposite results were found among uncastrated mice. This is the first description of such effects of melatonin on circulating levels of Cd. Recently, other authors [35] described how, in male bank voles, melatonin co-treatment brought about a significant increase in the tissue concentration of Cd when compared with those in the group treated with Cd alone. The changes in Cd deposits in tissue could explain changes in blood concentration. However, the mechanisms underlying these effects remain to be studied. One hypothesis is that Cd forms metal complexes with melatonin, thus decreasing the fraction of free Cd and contributing to metal detoxification [36]. The second hypothesis involves the analysis of the effects of Cd, melatonin and estradiol on the expression of metallothionein. In this concern, it is interesting to point out that Cd-induced expression of metallothionein is age- and estrogen-dependent [34, 37]. In female mice, ovariectomy increases (and administration of estradiol decreases) Cd-induced expression of metallothionein in animals not younger than 4 weeks or older than 46 weeks [37]. Whether melatonin also influences metallothionein expression, and the possible modulation of this effect by estrogens are key points to be clarified.

In conclusion, whereas in prepuberal animals Cd disturbs the normal growth of ducts and reduces the number of TEB, in adult animals and regardless of the steroidal milieu, Cd exerts estrogenic effects on mammary glands, increasing lobuloalveolar development and ductal branching. In all cases, the effects of Cd are partially inhibited by melatonin. As Cd mimics the effect of estrogens, the high incidence of breast cancer in tobacco smokers and women working in industries related with Cd could be explained by the properties of this metal. The effects of melatonin point to a possible role of this indoleamine as a preventive agent for environmental or occupational Cd contamination.

Acknowledgments

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