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# THE EFFECT OF MELATONIN TREATMENT REGIMEN ON MAMMARY ADENOCARCINOMA DEVELOPMENT IN HER-2/NEU TRANSGENIC MICE

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**The effect of various regimens of treatment with melatonin on the development of mammary tumors in HER2/neu transgenic mice was investigated. Female HER-2/neu mice starting from the age of 2 months were kept under standard light/dark regimen and as given melatonin with tap water (20 mg/l) during the night time 5 times monthly (interrupted treatments) or constantly to natural death. Intact mice served as controls. Treatment with melatonin slowed down age-related disturbances in estrous function most in the group exposed to interrupted treatment with the hormone. Constant treatment with melatonin decreased incidence and size of mammary adenocarcinomas, and incidence of lung metastases, compared to controls. The number of mice bearing 4 and more tumors was reduced in the group with constant melatonin treatment. Interrupted treatment with melatonin promote mammary carcinogenesis in HER-2/neu transgenic mice. The data demonstrate the regimen-dependent inhibitory effect of melatonin on the development of spontaneous mammary tumors in HER-2/neu mice but not on overall survival with implication about the likely cause of the effect. Polycystic kidney disease is common in this transgenic line. Adverse effect of melatonin on the life span in our study may be unique to the transgenic model used and may not be relevant to the suppressive effect of melatonin in delay of mammary cancer.**


**Key words:** *melatonin; HER-2/neu, transgenic mice; mammary cancer*

Breast cancer is one of the most common cancers and is a leading cause of mortality in women.[1,2] The *HER-2/neu* oncogene encodes a 185 kDa (p 185) receptor protein that belongs to the epidermal growth factor receptor family involved in organogenesis and epithelial differentiation.[3] Amplification and mutation of *HER-2/neu* plays a pathogenetic role in several malignancies, including carcinoma of the breast, ovary and uterus.[4,5] Overexpression of *ErbB-2/HER-2/neu* occurs in 15–40% of human breast cancers.[6] Its appearance is correlated with poor prognosis and is therefore an important target for physiologic investigation and therapeutic intervention.[5] It was shown that treatment with pineal indole hormone melatonin inhibits the development of carcinogen-induced or transplanted tumors in the mammary gland, uterine cervix and colon in various animal models.[7–10] It is worthy of note that in the majority of *in vivo* studies only 1 dose and regimen of treatment with melatonin has been investigated; however *in vitro* studies have shown the dose-dependent effect of the hormone on tumor growth.[9,10] At present, several mechanisms for the effect of melatonin on mammary cancer tumorigenesis have been proposed: an endocrine hypothesis,[9,10] based on the effects of melatonin on pituitary or sex hormones controlling mammary gland development, and a direct action on tumor cells through melatonin-mediated antiproliferative, antioxidant or immunoenhancing effects.[10]

Recently by using the HER-2/neu transgenic mice as a model of mammary carcinogenesis, we showed an inhibitory effect of melatonin dependent on modulation of HER-2/neu gene expression.[11] In our study melatonin was given with drinking water on an interrupted schedule for 5 days a week. We believe constant treatment with the hormone will be more effective in inhibiting mammary carcinogenesis. In this article, a test of this hypothesis is presented.

## MATERIAL AND METHODS

### *Animals*

Homozygous *HER-2/neu* transgenic mice obtained from Charles River (Hollister, CA) by the Italian National Research Center for Aging were housed and breed in the Laboratory of Carcinogenesis and Aging. The mice were kept 5–7days in polypropylene cages (30 × 21 × 10 cm) under standard light/dark regimen (12 hr light:12 hr darkness) at a temperature of 22 ± 2°C and received standard laboratory chow[12] and tap water *ad libitum*. Granular diet was produced by Agricultural Company "Volosovo" (Leningrad Region) and contained natural ingredients [wheat (30%), corn (22.7%), powdered milk (4.3%), soya (12%); meat meal (20%), yeast (5%), fat (3%), melassa (2%) and premix (1%)]. The total amount of the proteins in the food was 25.66%; fat, 7.0%; methionine, 0.7%; lysine, 1.44%; calcium, 2.04%; phosphor, 1.38%; natrium chloride, 0.37%; vitamins (mg/kg): A, 5000 i.e.; D, 500 i.e.; $B_1$, 1 mg; $B_2$, 2.5 mg; $B_3$, 2.5 mg; $B_4$, 120 mg; $B_5$, 12 mg; $B_6$, 1.5 mg; E, 50 mg; K, 10 mg; $B_{12}$, 0.03 mg; H, 0.2 mg; Cu, 8 mg; Fe, 60 mg; Co, 2.4 mg; Mg, 6 mg; Zn, 5 mg; iodine and 1.5 mg. Total calories was 305 kilocalories per 100 g.

### *Experimental design*

Seventy-nine female FVB/N *HER-2/neu* mice at the age of 2 months were randomly divided into 3 groups. Mice from group 1 were not treated and served as controls. Mice from groups 2 and 3 were given melatonin (Sigma Chemical Co., St. Louis, MO) dissolved in tap water (20 mg/l) during the night time (from 18.00 to 09.00 hr) 5 times monthly (interrupted treatment, group 2) or constantly (constant treatment, group 3) until natural death. Melatonin was dissolved in several drops of 96% ethanol and diluted with sterile tap water to a relevant concentration. A fresh melatonin solution, which is stable in water solution for 6 months, was prepared 3 times a week. Melatonin was used at a dose that has proved effective for life span extension[13] and anti-carcinogenic effect.[7,8,10] Once a week, all mice were palpated to detect mammary tumors. Localization and size of tumors were registered on special charts. Once a month, all mice were weighed and simul-

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taneously the amount of drinking water and food consumed was measured, and the rate of consumed tap water (mL) and food (g) per mouse and per body weight unit were calculated. Once every 3 month, 5 times daily for 2 weeks, vaginal smears were cytologically examined to determine the estrus function. The time of appearance of mammary tumors was evaluated by palpation, and neoplastic masses were measured with calipers in the 2 perpendicular diameters. Progressively growing masses of >3 mm in mean diameter were regarded as tumors. Because some treated mice did not display carcinomas in all mammary glands, the mean number of palpable mammary carcinomas/mouse was calculated as the cumulative number of incident tumors/number of tumor-bearing mice.

*Pathomorphological examination*

All animals were autopsied. Site, number and size of mammary tumors and metastases to lungs were checked. All tumors, as well as tissues and organs with suspected tumor development, were excised and fixed in 10% neutral formalin. After fixation the number of metastases in each lung lobe as well as size of metastases were estimated as recommended by the International Agency for Research on Cancer.[14] After routine histological processing, tissues were embedded in paraffin; 5–7 μm thin histological sections were stained with haematoxylin and eosin and were microscopically examined. Tumors were classified according to the International Agency for Research on Cancer recommendations[14] and Annapolis Consensus Report.[15]

*Statistics*

Experimental results were statistically processed using STATGRAPH. The significance of discrepancies was defined according to the Student *t*-criterion, Fischer's exact method, $\chi^2$ and nonparametric Wilkoxon-Mann-Whitney. Student-Newman-Keuls Method was used for all pairwise comparisons. For survival analysis, Cox's method[16] was used for testing 2 groups. Taron's life table test[17] was used. All reported test values for survival analyses are 2 sided.

*Mathematical models and estimations*

The mathematical model used to describe survival is the Gompertz model with the survival function,

$$S(x) = \exp\left\{-\frac{\beta}{\alpha}[\exp(\alpha x) - 1]\right\}$$

where parameters α and β are associated with the aging and initial mortality rate, respectively. Parameters for the model were estimated from data using the maximum likelihood method implemented in the Gauss statistical system.[18]

## RESULTS

The body weight of control mice increased with age. Treatment with melatonin in interrupted or constant schedule slowed down weight gain most in group 3 compared to controls. No significant weight difference was observed in mice treated with melatonin in both regimens (Table I). Food consumption was slightly decreased at the age of 3 months in group 2 and at 5 months in group 3 (Table II). The amount of drinking water was stable during the entire period of observation, that is 5.0 ± 1.2 ml/mouse per 24 hr and 3.4 ± 1.0 ml/mouse per night. There were no differences in this parameter between the control and melatonin-treated animals. A calculation has shown that the daily dose of melatonin was approximately 0.076 mg/mouse or 2.5–3.1 mg/kg of the body weight.

There was no significant difference in the length, or regularity, of estrus cycles between groups exposed, or not exposed, to melatonin (Table III). In controls, the age-related decrease of the incidence of short estrous cycles (<5 days) and increase of long cycles (more 7 days) as well as increase in the rate of irregular estrous cycles have been observed (Table III). Treatment with melatonin reduced age-related disturbances in estrous function in group 2.

Survival in mice treated with melatonin is presented at the Figure 1*a*. In the figure, survival was similar in all groups to age 5–7 months. Afterward, an increase in mortality was observed in the group constantly exposed to melatonin. No mice survived to the age of 12 months in group 3, whereas in the controls 13% of mice survived. No mice from group 2 survived to 13 months. The maximum longevity was for the controls. Survival curves for both groups that were exposed to melatonin were shifted to the left compared to the controls (Fig. 1*a*). The mean life span of mice constantly treated with melatonin was decreased compared to controls (−13.2%, $p<0.05$, the Student's *t* test). The mean life span in the last 10% of the mice of group 3 decreased 15.6% ($p<0.05$). Maximum life span was reduced 16.4%. MRTD was also reduced 17.6 %. The analogous parameters for group 2 were insignificantly decreased compared to controls (Table IV).

Mammary adenocarcinomas developed in 76.7% of controls. Its incidence was only slightly reduced in mice exposed to melatonin ($p> 0.05$; the exact Fischer test). Interrupted treatment increased multiplicity of mammary tumors (by 1.6 fold, $p<0.05$) compared to controls (Table V). It is clear in Figure 1*c* that the cumulative number of tumors sharply increased in group 2 after the age of 7 months, whereas in group 3 (constant treatment) the cumulative increase slowed. There were no significant differences in tumor yield curves between control and exposed groups (Fig. 1*b*). Nevertheless, the mean sizes of mammary adenocarcinomas in group 3 were smaller than in the controls and group 2. The incidence of mammary adenocarcinomas metastasizing into the lung, and mean size of metastases, were significantly decreased in group 3 compared to controls. At the same time, the mean life time of tumor-bearing mice in group 3 was shorter than in the controls. The number of mice without a tumor was a maximum, and a number of mice bearing 4 tumors was a minimum in group 3. The number of mice bearing 4 and more tumors was a maximum in group 2 (Fig. 2).

The kidney polycystosis of different grades was observed in the majority of mice of the control group (63.3%). The interrupted and constant treatment with melatonin failed significantly to influence this parameter.

## DISCUSSION

There is considerable evidence to show that pineal hormone melatonin exerts a protective effect against tumor development in animals and humans.[7–10] In our study, we demonstrate that a) treatment with melatonin slows down age-related disturbances in estrous function; b) the effect of melatonin on the incidence, multiplicity and the size of mammary carcinomas and the incidence of lung metastasis in transgenic *HER-2/neu* mice depends on

**TABLE I** – BODY WEIGHT GAIN DYNAMICS IN FEMALE HER-2/NEU TRANSGENIC MICE TREATED WITH MELATONIN[1]

| Group and treatment | Body weight (g) | | | |
|---|---|---|---|---|
| | 3 months | 5 months | 7 months | 9 months |
| 1. Control | 24.0 ± 0.28 | 26.8 ± 0.67 | 28.4 ± 0.60 | 35.2 ± 1.26 |
| 2. Melatonin, interrupted | 23.3 ± 0.48 | 26.0 ± 0.59 | 29.1 ± 0.65 | 29.0 ± 0.73[a] |
| 3. Melatonin, constant | 22.3 ± 0.35[b] | 25.0 ± 0.44[a] | 27.8 ± 0.65 | 27.3 ± 0.69[b] |

[1]The difference with the controls is significant,–[a]$p < 0.05$;–[b]$p < 0.001$, the Student's *t* test.

**TABLE II** – FOOD CONSUMPTION DYNAMICS IN FEMALE HER-2/NEU TRANSGENIC MICE TREATED AND NOT TREATED WITH MELATONIN

| Group and treatment | Daily food consumption (g) | | | |
|---|---|---|---|---|
| | 3 months | 5 months | 7 months | 9 months |
| 1. Control | 5.10 ± 0.28 | 5.50 ± 0.19 | 5.40 ± 0.91 | 5.00 ± 0.35 |
| 2. Melatonin, interrupted | 3.80 ± 0.50[1] | 4.60 ± 0.47 | 4.80 ± 0.18 | 4.80 ± 0.30 |
| 3. Melatonin, constant | 4.70 ± 0.72 | 4.40 ± 0.33[a] | 4.70 ± 0.90 | 5.00 ± 0.35 |

[1]The difference with the controls is significant, $p < 0.05$; the Student's $t$ test.

**TABLE III** – AGE-RELATED DYNAMICS OF ESTROUS PARAMETERS IN HER-2/NEU TRANSGENIC MICE-TREATED AND NOT TREATED WITH MELATONIN

| Age (month) | Number of mice | Length of estrous cycle (days) | Rate on separate phases of estrous cycle (%) | | | Rate of estrous cycles (%) | | | Number of mice with regular cycles (%) | Number of mice with irregular cycles (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | E | D | P + M | <5 days | 5–7 days | >7 days | | |
| Group 1 (Controls) | | | | | | | | | | |
| 3 | 30 | 5.5 ± 0.31 | 45.3 | 46.4 | 8.3 | 32 | 54 | 14 | 83.3 | 16.7 |
| 6 | 30 | 6.26 ± 0.45 | 52.3 | 39.7 | 8.0 | 22 | 48 | 30 | 90.5 | 9.5 |
| 9 | 19 | 6.30 ± 0.35 | 50 | 50 | 0 | 8** | 30* | 62*** | 50 | 50 |
| Group 2 (Melatonin, interrupted) | | | | | | | | | | |
| 3 | 27 | 6.58 ± 0.69 | 45.7 | 39.0 | 15.3 | 16.7 | 41.7 | 41.6 | 100 | 0 |
| 6 | 27 | 6.17 ± 0.27 | 45.5 | 45.5 | 9.0 | 17.4 | 58.7 | 23.9 | 100 | 0 |
| 9 | 13 | 6.40 ± 0.43 | 42.2 | 51.1 | 6.7 | 20.0 | 53.3[1] | 26.7[1] | 88.9[1] | 11.1[1] |
| Group 3 (Melatonin, constant) | | | | | | | | | | |
| 3 | 22 | 6.38 ± 0.25 | 51.5 | 38.6 | 9.9 | — | 90.5 | 9.5 | 96.7 | 3.3 |
| 6 | 20 | 6.04 ± 0.27 | 53.4 | 37.3 | 9.3 | 13.9 | 46.5 | 39.6 | 95.5 | 5.0 |
| 9 | 5 | 5.62 ± 0.82 | 50 | 50 | 0 | 33.3**[1] | 47.6* | 19.1* | 50 | 50 |

Note: E, estrus; D, diestrus; P, proestrus; M, metaestrus. The difference with the age of 3 months is significant:*$p < 0.05$;–**$p < 0.01$; –***$p < 0.001$; the Student's $t$ test.–[1]The difference with the age of 9 months in control group is significant: *$p < 0.05$; the Student's $t$ test.

treatment regimen; c) interrupted treatment (smaller total dose) has no protective effect and increases the multiplicity of mammary adenocarcinomas in mice; and d) constant treatment with melatonin (larger total dose) inhibits mammary carcinogenesis in transgenic *HER-2/neu* mice but decreased survival of mice as compared to the control group.

Mediavilla *et al.*[19] observed an inhibitory effect of melatonin (200 μg/mouse/day at 5 times a week s.c. late in the evening) on the development of hyperplastic alveolar nodules and mammary carcinomas in transgenic mice carrying the N-*ras* proto-oncogene under the control of the MMTV-LTR. Melatonin (50 mg/kg, orally for 30 weeks) delayed appearance of palpable mammary tumors and their growth in TG.NK transgenic mice expressing the c-neu oncogene under control of a MMTV promoter.[20] Our observation is in agreement with other reports on the inhibitory effect of melatonin on mammary tumor development.[8,9,10,21] With regards to the mechanisms in the inhibitory effect of melatonin on mammary carcinogenesis, anti-proliferative, anti-estrogen capacity or inhibition of prolactin level, antioxidative potential and immunostimulating activity of the pineal hormone[9,10,22] have been reported.

It is worthy of note that the treatment with melatonin led to decrease in the body weight in transgenic *HER-2/neu* mice more than expressed in constantly treated animals (Table I). It is well known that excess of body weight is associated with increased breast cancer risk in women and mammary cancer in rodents.[23–25] On the other hand, daily melatonin administration suppressed body weight, plasma leptin and insulin in rats.[26] The tumorigenesis of the mammary gland depends on insulin for expression.[25] Studies have shown that insulin plays an important role in regulating tumor estrogen receptors.[27] Thus, the inhibitory effect of melatonin in our *HER-2/neu* mice may be related to the lower body weight in the mice and also to the decrease in insulin level.

Using the same model of mammary carcinogenesis in *HER-2/neu* transgenic mice, we recently showed that under constant exposure to melatonin its anticancer action down-regulates *HER-2/neu* gene transcription.[11] Interrupted treatment with melatonin at the same dose (20 mg/l) and schedule (5 times a week) increased incidence of lung adenocarcinomas and leukemais in CBA mice.[13]

To identify early molecular events regulated by melatonin, gene expression profiles were studied in hearts melatonin-treated CBA mice in comparison to the control that used cDNA gene expression arrays (15,247 cDNA clone set, NIA, USA).[28] The dose and schedule of the treatment were similar to the used in the study.[13] Comparative analysis of cDNA gene expression arrays hybridized with heart RNA samples from control and melatonin-treated mice has shown that primary effectors are the genes that control the cell cycle, cell/organism defense, protein expression and transport. A significant effect of melatonin on expression of some oncogenesis-related genes was detected. While expression of myeloblastosis oncogene-like 1 (Mybl1) was down-regulated by melatonin (exceeding a 2-fold confidence level), melatonin up-regulated an expression of *RAS p21* protein activator 1, Enigma homolog 2 and myeloid/lymphoid or mixed-lineage leukemia [trithorax (Drosophila) homolog], translocated to 3 (MLLT3). Of a great interest is an effect of melatonin onto a large number of genes related to calcium exchange, such as cullins, Kcnn4 and Dcamkl1, calmodulin, calbindin, Kcnn2 and Kcnn4. Whereas the expression of cullin-1 in the mouse heart is down-regulated, that of cullin-5 is highly up-regulated, and expression of cullins-2 and -3 is not altered significantly. The cullin family, comprising at least 6 members, is involved in ubiquinone-mediated protein degradation required for cell-cycle progress through the G1 and S phases. Nevertheless, cullin-1, but not other members of the cullin family, is generally thought to be implicated in SCFs (Skp1-cullin-F-box protein ligase complexes), that control the ubiquinone-dependent degradation of G1 cyclins and inhibitors of cyclin-dependent kinases, thus playing an important role in cell proliferation and differentiation.[29–31] Like the effects of other proteins of this family, the effect of cullin-5 is mediated by a SKP1/F-box-independent mechanism. It is believed that melatonin may influence tumor growth by interfering with calcium binding and blocking the formation of the MAPs/calmodulin and tubulin/calmodulin complexes to prevent cytoskeletal degradation.[22] Four serine/threonine kinases (Pctk3, FUSED, TOPK and Stk11) with expression increased by both peptides can be found in the same functional category (cell signaling/communication).[28] At least one of these, Stk11 kinase with an unclear function, has anticarcinogenic effects, and mutations in it lead to

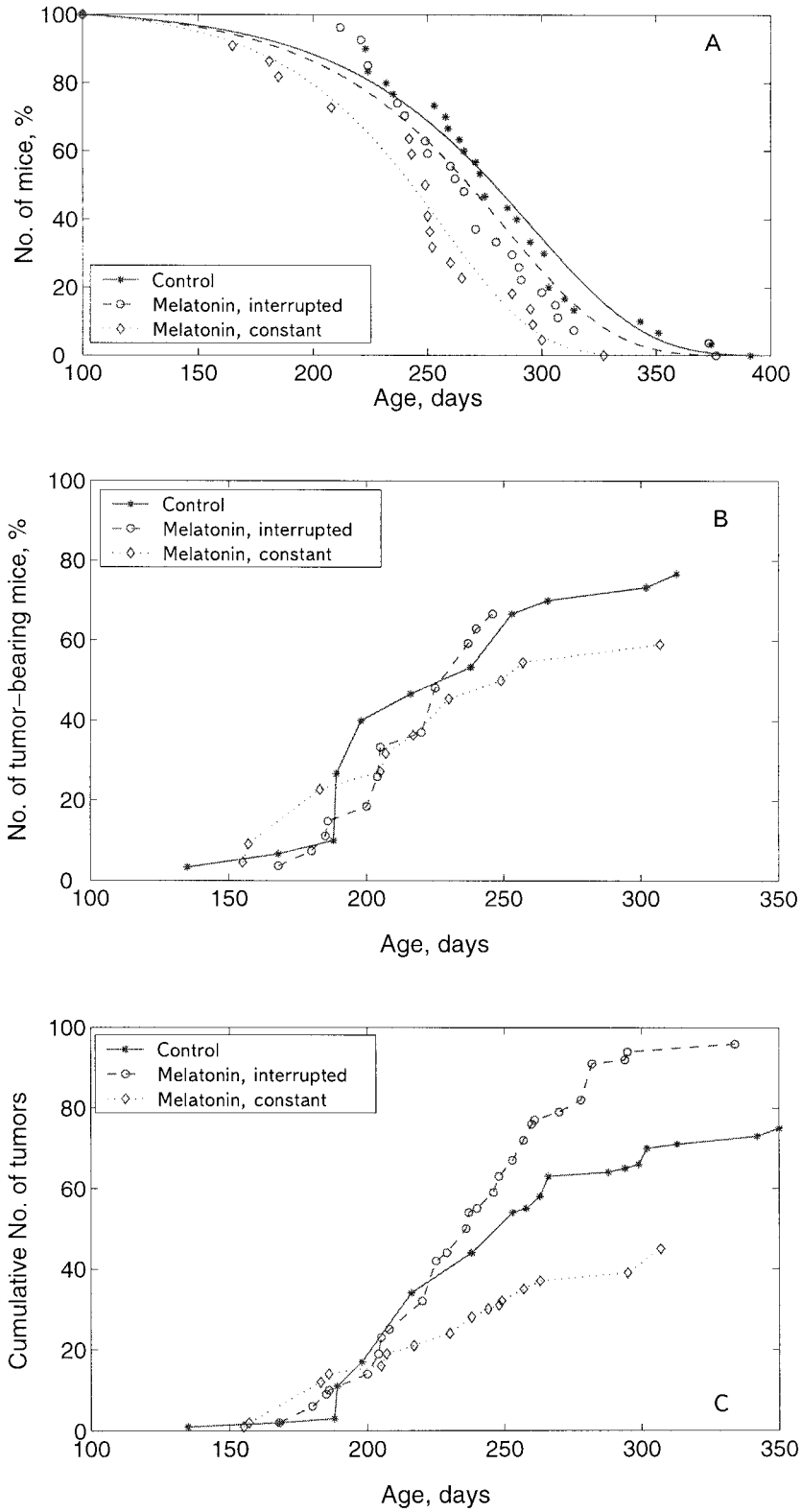


**FIGURE 1** – Effect of treatment with various regimens of melatonin on survival and tumorigenesis in HER-2/neu transgenic mice. Abscissa, age, days. (*a*) Survival. Ordinate, number of mice, %. The difference in the survival between the control group and mice exposed to constant melatonin treatment was significant ($p < 0.01$) at the age of 240–300 days. (*b*) Age-dependent tumor rate curves. Ordinate, number of tumor-bearing mice, %. (*c*) Cumulative tumor number gain. Ordinate, cumulative number of tumors.

the Peutz-Jeghers syndrome, which is associated with a high risk of tumor development in multiple localizations.[32]

Mechanisms of promotion of interrupted treatment with melatonin on mammary carcinogenesis in *HER-2/neu* mice is unclear. A disregulation of hormonal balance and cell proliferation in target tissues is possible. This question is under investigation in our laboratory. Constant treatment in our experiment inhibited mammary carcinogenesis in *HER-2/neu* mice. It was expressed by the decrease both in tumor and in metastases sizes, and by the decrease in the number of mice with lung metastases of MAC (Table V) and the slow down of cumulative number of mammary tumor gain (Fig. 1*c*). At the same time, this treatment significantly reduced the mean life span of the

**TABLE IV** – PARAMETERS OF LIFE SPAN IN FEMALE HER-2/NEU TRANSGENIC MICE TREATED AND NON TREATED WITH MELATONIN

| Parameters | Group 1 Control | Group 2 Melatonin, interrupted | Group 3 Melatonin, constant |
|---|---|---|---|
| Number of mice | 30 | 27 | 22 |
| Mean life span, days (mean ± SE) | 281 ± 8.1 | 271 ± 7.9 | 244 ± 9.4* |
| Median | 275 | 266 | 250 |
| Mean life span of last 10% survivors | 372 ± 11.6 | 354 ± 20.2 | 314 ± 13.5* |
| Maximum life span | 391 | 376 | 327 |
| α (days$^{-1}$) | 0.0204 | 0.0214 | 0.0244 |
| MRDT, days | 34 | 32 | 28 |

Constant α in the Gompertz equation: $R = R_0$ (exp) αt, where $R_0$ = mortality at t = 0. MRDT, mortality rate doubling time, days. The difference with parameter in the control group is significant, $*p < 0.01$; the Student's *t* test.

**TABLE V** – MAMMARY ADENOCARCINOMA (MAC) AND KIDNEY LESION DEVELOPMENT IN FEMALE HER-2/NEU TRANSGENIC MICE TREATED AND NOT TREATED WITH MELATONIN[1]

| Parameters | Group 1 Controls | Group 2 Melatonin, interrupted | Group 3 Melatonin, constant |
|---|---|---|---|
| Number of mice | 30 | 27 | 22 |
| Number of tumor-bearing mice | 23 (76.7%) | 18 (66.7%) | 13 (59.1%) |
| Time of the 1st MAC detection, d | 135 | 168 | 155 |
| Total number of MAC | 75 | 95 | 45 |
| Number of MAC per tumor-bearing mouse | 3.26 ± 0.20 | 5.3 ± 0.12*[a] | 3.5 ± 0.13 |
| Maximal tumor size, cm | 1.88 ± 0.15 | 2.0 ± 0.22 | 1.35 ± 0.18*[2] |
| Number of mice with lung metastases of MAC | 10 (33.3%) | 6 (22,2%) | 3 (13.6%)**[3] |
| Maximal metastasis size, cm | 0.38 ± 0.04 | 0.7 ± 0.35 | 0.26 ± 0.03**[2] |
| Mean latency of the 1st MAC, days | 218 ± 45.6 | 213 ± 50,1 | 212 ± 58,9 |
| Mean latency of all MAC, days | 239 ± 27.6 | 237 ± 24.1 | 230 ± 34.2. |
| Mean life span of tumor-free mice, days | 236 ± 11.2 | 265 ± 15.4 | 224 ± 16.8 |
| Mean life span of tumor-bearing mice, days | 295 ± 8.1 | 274 ± 9.4 | 258 ± 9.6*[1] |
| Mean survival time of tumor-bearing mice after the 1st MAC detection, days | 76 ± 15.9 | 61 ± 14.3 | 46 ± 12.6 |
| Number of mice with kidney polycystosis | 19 (63.3%) | 12 (44.4%) | 13 (59.1%) |

[1]The difference with parameter in the control group is significant, $*p < 0.05$; $**p < 0.01$.–[2]The Student's *t* test.–[3]The exact Fischer test.

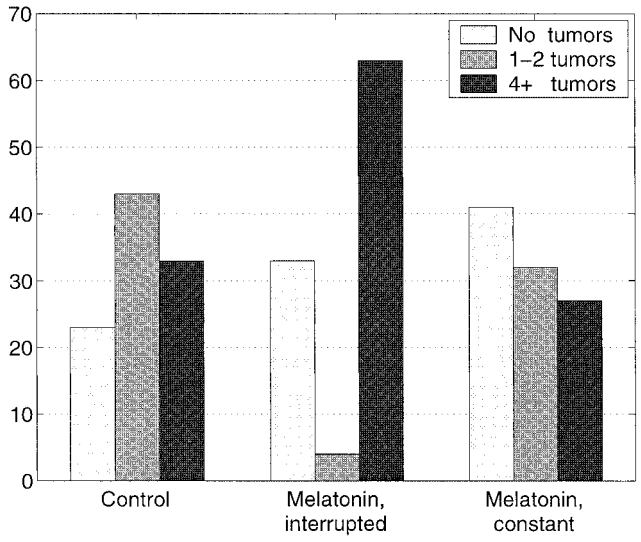


**FIGURE 2** – Number of mammary adenocarcinomas in *HER-2/neu* transgenic mice exposed to interrupted or constant treatment with melatonin. Ordinate, number of mice with mammary tumors, %. Total number of tumor-bearing mice was regarded as 100% in each group.

animals (Fig. 1*a*, Table IV). Because the number of tumors per mouse in this group was not differ from the control group and the size of MAC was reduced, it seems that the observed effect is not related to the tumor progression. The doses of melatonin in our study (2.5μ3.1 mg/kg) and several-fold higher than 3 mg were not reported to be toxic to rodents.[9,21] A possible reason for accelerated mortality of constant administration of melatonin could related to its diuretic effect.[33,34] In the majority of the transgenic *HER-2/neu* FVB/N mice, kidney lesions and the constant treatment with melatonin could lead to the decompensation of kidney function and to premature death not from tumor growth but from kidney insufficiency. The kidney polycystosis was not described in non-transgenic FVB/N.[35] It is worthy to note that the presence of this lesion, which may contribute to mortality, complicates the interpretation of the chemopreventive effect of melatonin. The adverse effect of melatonin on life span in our study may be unique to the transgenic model used and may not be relevant to the suppressive effect of melatonin on mammary cancer.

We have observed that the exposure to the interrupted courses of melatonin was followed by a slowdown of age-related changes in estrous function. A similar effect was observed in female CBA mice[13] and rats.[36] This phenomenon was less expressed in mice that were treated with melatonin constantly (Table III); however the antitumor effect of melatonin was more expressed in group 3 than that in group 2 (Table V). There is no contradiction here because the antigonadotropic effect of the treatment with melatonin can explain more effective inhibition of mammary tumorigenesis[9,10] and less effective restoration of the normal cyclicity of the estrus when it was administered constantly. These observations also suggest the estrogen dependency of mammary tumors in *HER-2/neu* transgenic mice. Recently we found that MAC have estrogen receptors and an ovariectomy inhibits mammary tumorigenesis in *HER-2/neu* transgenic mice (unpublished data).

In conclusion, the data in this article demonstrates melatonin may have an important role in mammary tumor development, and it may be modulated by treatment regimen. At the same time, the adverse effect of melatonin on life span of transgenic *HER-/neu* mice has been observed, which may not be relevant to the inhibitory effect of melatonin on mammary carcinogenesis.

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