Effect of Melatonin on the Induction of Foci of Aberrant Crypts in the Colon by Azoxymethane in Rats¹

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Abstract

Objective: A program of research was established on the question whether melatonin played a chemopreventive role in the development of foci of aberrant crypts in the intestinal tract of male rats.

Male F344 rats were injected i.p. with an aqueous solution of 15 mg/kg azoxymethane (AOM) on day 50 and day 57, and a group was also injected i.p. with 0.5 mg melatonin in 0.5 ml of 10% ethanol solution 5 times per week beginning at age 47 days. Foci and multiplicity of aberrant crypts were determined after 8 weeks. These groups of animals were kept in light daily from 4:30 to 16:30.

Results: In the group receiving AOM and the melatonin injections, there were fewer foci of aberrant crypts in the colon and the average number of crypts was lower after 8 weeks, compared to the group on AOM alone.

Conclusions: Melatonin inhibited the formation of foci of aberrant crypts in the descending colon of rats. Also, it reduced the number of aberrant crypts per focus from foci with 3 and more aberrant crypts.

Key words: melatonin, foci of aberrant crypts in the colon, rats, inhibition

Introduction

Melatonin is an interesting natural product elaborated mainly by the pineal gland in animals and in humans. The pineal gland generates melatonin during the dark hours at night. The gland is connected to receptors in the eyes and neural pathways. The production and evolution of melatonin assumes low levels during the day or in bright light. There are results indicating that blind people do produce melatonin fairly continuously (1,2). Blind women display a lower breast cancer rate, relative to women, in general (3). Thus, melatonin seems to have an inhibiting effect on breast cancer formation in humans (4).

The role of melatonin in biomedical research and especially in cancer research came to the attention of the field when there were reports that the electric power lines, including those in people's homes emit waves harmful to health (5-8). Initially, the emphasis was on childhood leukemia, and leukemia in

workmen, but subsequently the view was expressed that other types of cancer, especially cancer of the breast might be increased, consequent to residence in areas with high electric or magnetic fields (9-11). Some interpretations were based on the hypothesis that exposure to electric and magnetic field may increase oxidative stress. The main target of such electromagnetic fields were thought to be the pineal gland, an important organ that biosynthesizes melatonin (12-15).

Melatonin has been demonstrated to have powerful antioxidant properties, more so than the vitamins with antioxidant protection, such as vitamin C or E (16), and it is of the same order of magnitude compared to green or black tea that we have studied previously (17) that inhibit the oxidation of LDL-cholesterol through the action of reactive oxygen species. Also, tea decreases cancer initiation and development by blocking the effect of reactive oxygen species (19-23).

Modulation of melatonin levels by short intermittent light cycling affected the melatonin levels moderately after 10 weeks, but had minimal action on the formation of mammary tumors in female F344/N rats (24). Melatonin was shown to inhibit the formation of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in female SD rats (25). This research, showed that melatonin inhibited tumor growth. Therefore, it seemed interesting to study the potential of melatonin in affecting the formation of

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foci of aberrant crypts in the colon of F344 rats injected with azoxymethane (AOM). Male F344 rats are the favorite, sensitive laboratory animals for investigations involving the colon. Bird (26) introduced research on foci of aberrant crypts to determine factors related to colon carcinogenesis. Many investigations have validated this rapid model of tumor induction in the colon, much faster than endpoints such as adenoma or carcinoma formation. A recent paper describes the application to research on mechanism associated with development of colon neoplasia (27).

Methods and Materials

Melatonin was obtained from Regis Technologies, Inc., Morton Grove, IL showed the correct mass and NMR spectra, without any peaks ascribable to impurities. Melatonin is not very soluble in water so the necessary amount was dissolved in 100% ethanol, and diluted with distilled water to make solution of melatonin in 10 percent ethanol. Male F344 rats were procured from Taconic Laboratory Animals, Germantown, NY and held in the Quarantine Facility of the Research Animal Facility in this Institute. Research in the facility is reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). For these experiments the automatic lighting system was set to go on at 4:30 and off at 16:30 to support the physiologic effects of melatonin. The rats received the i.p. dose about 16:00 5 days a week, Monday to Friday.

Induction of Foci Aberrant Crypts of the Colon

Groups of male F344 rats were fed an AIN-76A diet (Dyets, Bethlehem, PA No. 112204). When they were 50 and 57 days old, groups of 10 rats were given 50 mg/kg azoxymethane, AOM (Ash Stevens, Inc., Detroit, MI) in a solution of deionized water by i.p. injection, a standard method. Another group of 10 rats were injected i.p. twice with AOM, and they received 0.5 mg of melatonin as 0.5 ml of the 10% ethanol solution 5 days per week by i.p. injection, beginning at 48 days of age. After 8 weeks, they were sacrificed in a CO₂ atmosphere. The descending colon was obtained and foci of aberrant crypts in 4 each 2 cm segments of colon, 8 cm from the anus, were visualized after fixation in 4% neutral formalin, and staining with a 0.2% solution of methylene blue, using a 40-fold magnification with a dissecting microscope (26, 27). There were groups of 10 rats on melatonin only, 10 on vehicle only, and 10 untreated controls. The observation on the location of the foci were made identically in all AOM-treated groups. The number of aberrant

crypts in the foci were studied by the procedures developed by Bird (26,27).

Biostatistical methods

Continuous measures (foci, crypts) were compared among the groups using analysis of variance (ANOVA) followed by Tukey's multiple comparisons procedures, as well as the classic Student's t-test. All tests were considered significant at $p < 0.05$ (28).

Results and Discussion

Injections of melatonin solutions lowered the induction of foci, as well as the average number of crypts (Table 1), with emphasis on foci with more than 3 aberrant crypts (Table 2).

In the sets of experiments, we found that doses of 0.5 mg or 2.0 mg/kg) of melatonin injected 5 times a week inhibited the production of AOM generated foci of aberrant crypts in the distal colon of male rats. Also, it significantly decreased the multiplicity of such crypts with more than 3 crypts per focus. The human daily dose is 100 mg melatonin equivalent of 1.67 mg/kg for a 60 kg human.

Carcinogenesis in humans involves a series of steps in an overall complex process (29). One step is the metabolic activation of genotoxic carcinogens, such as AOM we used here, to reactive metabolites that yield mutational events at a level of the gene, such as oncogenes or tumor suppressor genes (30, 31). Further studies are warranted to address the mechanisms under-

Table 1 Role of melatonin on induction of foci of aberrant crypts in the colon of male F344 rats given 2 sc doses of 15 mg/kg azoxymethane (AOM), and 5 doses per week of melatonin for 8 weeks

Group	Mean Foci per Rats	Mean Crypts per Rat
Group 1 AOM ^a	146.932.0 ^b	373.7107.8 ^b
Group 2 AOM+Melatonin ^c	96.829.4	244.777.2

^aThe azoxymethane injections were on days 50 and 57, and the experiment was terminated after 8 weeks. The descending colon was flushed with saline solution and 2 cm segments were fixed in 4% formalin solution for 2 hrs, rinsed with saline, and stained with a 2% methylene blue solution for 15 minutes. The tissue was read at a magnification of 40, to determine the number of foci of aberrant crypts and the mean number of crypts per rat.

^bStandard deviation, SD, $p < 0.05$ compared to group 2.

^cMethods described in text.

Table 2 Multiplicity of aberrant crypts in groups of rats receiving azoxymethane (AOM) only, or with melatonin

Group	AC 1		AC 2		AC 3		AC 4		AC 5, 6, or 7	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Group 1 AOM	7.00	3.89	14.39	7.25	11.97	4.80	5.12	3.16	3.91	3.26
Group 2 AOM+Melatonin	5.90	4.35	11.20	4.65	9.43	4.24	3.23	2.46	1.50	1.76
p-value	0.29		0.04		0.03		0.01		0.005	

Analyses of foci of aberrant crypts through the methods of Bird (26, 27).

AC1 is 1 aberrant crypt per focus up to AC4 or AC5 or more.

Note that 2 aberrant crypts were almost significant and 3 or more were significant, using Student's t-test in all evaluations.

lying the chemoprevention effects of melatonin in rat colon carcinogenesis. In terms of the processes whereby tea operates in chemoprevention, the polyphenols in tea have been shown to reduce the reactive oxygen species involved in cancer causation and also incidentally in heart disease causation, through the effect of reactive oxygen on LDL cholesterol (18, 23). Another mechanism involves control of the growth of cells containing mutated gene (32). Tea polyphenols have been documented to inhibit the growth of tumor cells and raised the level of apoptosis (18, 31, 32).

It seems likely that melatonin operates via such mechanisms (33). In the context of the results obtained in our studies, it is possible that growth control and increased apoptosis may account for the findings. A number of papers indicate that melatonin lowers the risk of Alzheimer's disease, possibly through an effect of melatonin on reactive oxygen species, associated with this chronic aging-related condition (34-38).

The biosynthesis of melatonin involves conversion of tryptophan to the 5-hydroxy derivative, that in turn was subjected to decarboxylation to yield serotonin, and upon methylation of the phenolic OH group and acetylation of the amino group formed melatonin (39). It was concluded that enzymes concerned with methylation and acetylation might play key roles. In addition, it was found that melatonin had a sleep evoking effect, just as did tryptophan (40, 41). The latter compound had been used frequently for the clinical management of insomnia, but came into disuse when a product on the market was contaminated with 1,1'-ethylidene-bis- α -tryptophan, that seemed to be responsible for the eosinophilia-myalgia syndrome and related diseases (34, 42, 43). It now seems clear that the reason

tryptophan was found beneficial in inducing sleep because it was converted biochemically to melatonin (41). In a healthy normal individual the pineal gland generates melatonin in the dark and provides only minimal levels during the day. The mechanism is neural transfer of light signals from the eyes. Because of the circadian rhythm of melatonin generation, we reinforced this natural rhythm by administering melatonin in the late afternoon, at 16:00, in the experiments described.

Melatonin has many interesting physiological functions. It acts as an antioxidant. It decreases proliferation of abnormal cells, it inhibits the expression of mutagenicity of carcinogens. It has been studied in aging research. As we describe here, it controls cancer development of neoplasia in the mammary gland (see ref 25) and of foci of aberrant crypts in the intestinal tract.

In conclusion, the natural hormone-like effect of melatonin in modifying carcinogenesis induced by genotoxic carcinogens may be accounted for by a number of specific mechanisms that might be subject to future research. The known, established mechanisms underlying the beneficial health effects of the tea polyphenols might provide realistic leads (16, 18, 23)

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