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Mini Review

Melatonin and Colon Carcinogenesis: New Perspective?

Vladimir N Anisimov*

Department of Carcinogenesis and Oncogerontology, N. N. Petrov National Medical Research Center of Oncology, St. Petersburg, Russia

***Corresponding Author:** Vladimir N Anisimov, Professor, Department of Carcinogenesis and Oncogerontology, N. N. Petrov National Medical Research Center of Oncology, St. Petersburg, Russia.

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Abstract

The available data on effects of pineal indole hormone melatonin (MLT) on development and growth of chemically induced colon carcinomas in rodents, human colon cancer cells *in vitro*, transplanted syngeneic and xenograft tumors *in vivo*, and results of therapy of colon cancer in clinical practice are briefly reviewed. It was observed that prevention of colon cancer with MLT in rodents mediated via its effect on early and late stages of carcinogenesis. We believe that melatonin could be potent additional means for prevention and treatment of colorectal cancer in humans.

Keywords: *Colon Cancer; 1,2-Dimethylhydrazine; Cancer Prevention; Melatonin; Rodents; Human*

Abbreviations

AOM: Azoxymethane; CRC: Colorectal Cancer; DMH: 1,2-Dimethylhydrazine; LL: Constantly Illuminated

Introduction

Colorectal cancer (CRC) is the third most worldwide occurring malignancy in humans. There are more than one million new cases annually and it is followed by 700,000 deaths in 2012 in the world [1,2]. More than 90% of CRC cases occur in people aged over 50, and about 75% occur in people older than 65. It is worth of note that in men and women over 75 CRC represents the first and the third site for cancer mortality, correspondingly. Thus, age is a leading risk factor for CRC and it is the particular interest of geriatric oncology [3,4]. Risk starting at the age of 40 years increased sharply at 50 years of age, doubling each decade until age 80 [5]. There are epidemiological and experimental evidences of the role of "night" hormone MLT (MLT) in development of many cancers, in particular, CRC [4-7]. It worthy to note that data on level of melatonin in CRC patients are rather contradictory [8].

Effect of melatonin on chemically-induced carcinogenesis in rodents

Colon tumors induced by 1,2-dimethylhydrazine (DMH) and its carcinogenic metabolite azoxymethane (AOM) are commonly accepted model in studies of various aspects of the morphology, pathogenesis, prevention and treatment of CRC [9,10].

In 1997 it was firstly shown that nocturnal MLT treatment inhibits colon carcinogenesis induced by 1,2-dimethylhydrazine (DMH) in rats [11]. Female rats were exposed to 5 or 15 weeklies s.c. injections of DMH (21 mg/kg of body weight). From the day of the 1st injection of the carcinogen a part of rats from both series of the experiment was given MLT dissolved in tap water, 20 mg/L for five days a week during the night time (from 18.00 h to 8.00 h). The experiment was finalized 6 months after the first injection of the carcinogen. An inhibitory effect of MLT on DMH-induced intestinal carcinogenesis in rats was manifested by a decrease of the incidence and multiplicity

of colon tumors, by decreasing the invasion rate and dimensions of colon carcinomas and by increasing its differentiation. The level of immunohistochemically detected MLT in epithelium of the intestinal tract of rats exposed to DMH was significantly reduced as compared to intact rats, while in rats treated with DMH + MLT it was in control ranges. In the serum of rats exposed to DMH alone the levels of diene conjugates (DC) and Schiff's bases (SB) were significantly increased as compared with controls. In colon tissue of DMH-treated rats the levels of DC, SB, carbonyl derivatives of amino acids NO-synthase activity were significantly increased and total antioxidative activity was decreased as compared to controls. In rats exposed to DMH + MLT a normalization of free radical processes in serum and colon has been observed. MLT also inhibits the mutagenic effect of DMH *in vivo* (chromosome aberration and sperm head animalities tests) and *in vitro* (Ames' test). MLT also exerts some normalizing influence on glucose and lipid metabolism in rats exposed to DMH and inhibits proliferation and stimulates apoptosis in DMH-induced colon tumors. Thus, our data have shown that MLT affect initiating, promoting and progression stages of colon carcinogenesis.

Weisburger., *et al.* (2003) [12] exposed male rats to 2 i.p. injections of AOM (15 mg/kg) at the age of 50 and 57 days and starting at the age of 47 days a part of rats were i.p. injected with MLT (0.5 mg x 5 times a week). Foci and aberrant crypts numbers were decreased in MLT treated rats as was evaluated in 8 weeks after start of treatment.

Male rats were exposed to DMH (21 mg/kg s.c. x 5) at the time of 10:00 or 22:00 hours. Incidence, multiplicity and size of colon carcinomas were much higher when animals were exposed to the carcinogen in the morning time as compared with those at the evening times. When DMH-exposed to the same dose rats were maintained in the room constantly illuminated (LL: 24h light) number of colon tumors was much higher in comparison to those kept at standard light/dark regime (LD: 12h light/12h dark) or at light deprivation (DD: 24h dark/0 light) condition. When animals exposed to DMH and kept at LL illumination were treated with MLT (20 mg/L daily at night time) number, multiplicity and size of colon cancers were significantly reduced [13,14].

Male rats were treated with a single dose of DMH (125 mg/kg i.p.) and kept at LL regime (300 lx) or LL + MLT (10 mg/kg i.p.). All rats were sacrificed 2 weeks after DMH injection [15]. It was shown that LL induces aberrant crypt focuses in much more number then in LD condition. Thus, MLT supplementation prevent preneoplastic changes in colon.

When MLT (0.4; 2 or 10 ppm in drinking water) was given to male F344 rats initiated with a single i.p. injection of AOM (20 mg/kg) and promoted by dextran sodium sulphate (DSS) the parameters of colon carcinogenesis were significant supressed [16]. MLT treatment modulated mitotic and apoptotic indices in the colon tumors and suppressed expression of nuclear factor kappa B, TNF α, interleukin-1β and STAT3 in CRC.

Trivedi., *et al.* [17] studied effect of MLT using a mouse model of colitis-associated colon carcinogenesis induced by DMH (20 mg, i.p., x1) followed by 3 cycles of dextran sulphate sodium and drinking water. MLT (1 mg/kg per os) treatment started in 1 week after DMH injection was given for 8 or 18 weeks. Experiment was stopped 10 or 20 weeks later. Supplementation with MLT significantly supressed colon carcinogenesis: inhibit number of aberrant crypt; decreased morphological abnormalities in crypt structure; alleviate the signs of inflammation and oxidative stress, as well as autophagy in colon carcinomas.

DMH is an alkylating agent that is significant for its carcinogenic effect [9]. There is some evidence on the role of free radicals in DMH-induced carcinogenesis [18]. It was found that MLT is a potent scavenger of free radicals [7] which could be one of the mechanisms of its possible antitumor effect.

It was suggested that the anticarcinogenic effect of MLT is realized, at least in part, by its interaction with active metabolites of DMH and/or its adducts with DMH in the bowel lumen. The effect of MLT on free radical processes involved in the realization of the carcinogenic capacity of DMH also must be taken into consideration. In our experiments, MLT was given to rats from the day of the 1st injection of DMH, during the whole period of the exposure to the carcinogen and also after the injections of DMH were stopped. Thus, it is reasonable to suggest that the anticarcinogenic effect of MLT could be realized both on early and late stages of colon carcinogenesis (Table 1).

Species, strain	Sex	Carcinogenic agent	Doses of MT	Effect	References
LIO rats	F	DMH, 21 mg/kg x5, s.c., weekly	20 mg/L with nocturnal d.w.	↓	[11]
LIO rats	F	DMH, 21 mg/kg x15, s.c., weekly	20 mg/L with nocturnal d.w.	↓	[11]
F344 rats	M	AOM, 15 mg/kg, x2, s.c.	0.5mg x5 times/week, i.p.	↓	[12]
Outbred rats	M	DMH, 21 mg/kg x5, weekly + LL	20 mg/L with nocturnal d.w.	↓	[13,14]
Outbred rats	M	DMH, 21 mg/kg x5, weekly + LL	DD (light deprivation)	↓	[13,14]
F344 rats	M	AOM, 20 mg/kg+DSS,1%, in d.w.	0.4, 2 or 10 ppm in nocturnal d.w.	↓	[16]
Wistar rats	M	DMH, 125 mg/kg, x1, i.p. + LL	10 mg/kg/day, i.p.	↓	[15]
Wistar rats	M	DMH, 125 mg/kg, x1, i.p. + LL	10 mg/kg/day, i.p.	↓	[15]
Swiss albino mice	M	20 mg/kg ip + DSS	1 mg/kg/day, i.p.	↓	[17]

Table 1: *Effect of melatonin on colon carcinogenesis in rodents.*

Abbreviations *M: Males; F: Females; Dw: Drinking Water; i.p: Inter peritoneally; ppm: Parts Per Million; AOM: Azoxymethane; DMH: 1,2-Dimethylhydrazine; DSS: Dextran Sodium Sulfate; LL: Light at Night (L: D - 24:00h); DD: Light Deprivation↓ -Inhibition; = No Effect.*

Stages of DMH metabolism leading to the initiation of carcinogenesis included: formation of "active" metabolites (methylazoxymethanol or methyldiazohydrate) mainly in the liver; binding of the metabolites to glucuronic acid; delivery of conjugates to the intestine via blood flow; liberation of "active" metabolites due to enzyme activity of intestinal flora (β-glucuronidase); formation of carbonium ion (CH_3^+); specific ethylation of enterocyte macromolecules (mainly DNA at O^6-position of guanine); miscoding effect [9]. These events result in mutations followed by the activation of the oncogene Ki-ras and inactivation of the antioncogene p53 [18]. There is evidence of an important role of free radical processes in the realization of DMH-induced carcinogenesis [19,20]. MLT has been found to inhibit X-ray induced mutagenesis in human lymphocytes *in vitro,* to reduce Cis-platinum-induced genetic damage in the bone marrow of mice, to decrease hepatic DNA adduct formation caused by safrole in rats and to protect rat hepatocytes from chromium(VI)-induced DNA single-strand breaks *in vitro* [20]. MLT failed increase both chromosome aberration (ChA) and sperm head anomalies (SHA) incidence in mice in comparison to those treated with normal saline but significantly decreased the incidence of DMH-induced ChA and of SHA [21].

Effect of melatonin on colon tumor growth *in vitro* and *in vivo*

The results of studies on effect of MLT on human colon cancer cell *in vitro* are summarised in table 2. Different cancer cell lines were susceptible to MLT supplemented to cultural media in rather wide diapason from physiological to supraphysiological doses (10^{-9} - 10^{-2}). Effects of MLT were described as antiproliferative, inhibitory, decreasing the viability of cancer cells, inducing apoptosis. Only one human colon cancer cell line – DLD-1 was insensitive to high concentrations of MLT (10^{-4} M) [26].

Tumor line	Dose of melatonin	Effect of melatonin	References
CaCo - 2	10^{-6} - 10^{-1}	Inhibitory effect in doses > 10^{-3}	[22]
Caco - 2	0.1 - 1 mM	Inhibition tumor growth and progression	[23]
Caco - 2	0.78;1.56μg/mL	Antiproliferative effect	[24]
Colon 38	10^{-7},10^{-9} M	Decreased the viability of cancer cells	[25]
DLD - 1	10^{-4} M	No effect	[26]
HCT116	10 mM	Inducing apoptosis	[27]
HCT116	10^{-6} M	Inhibition cellular viability	[28]
HT29	10^{-6} - 10^{-2} M	Antiproliferative effect	[29]
HT - 29	1 mM	Inducing apoptosis	[30]
LoVo	10 - 2000 pg/mL	Inhibits tumor growth	[31]
LoVo	10^{-4} - 10^{-1} - 2 mM	Antiproliferative effect, inducing apoptosis	[32]
LoVo	0.1 mM; 1.0 mM	Potentiates cytostatic effect of doxorubicin	[33]
LOVO	0.125 - 1 mM	Antiproliferative effect	[34]
$LoVo_{DX}$	0,1 mM; 1.0 mM	Potentiated cytostatic effect of doxorubicin	[33]
RKO	2.5 mM	Antiproliferative effect	[35]
RKO	2.5 mM	Inhibition of cellular migration	[36]
SW620	0.125 - 1 mM	Antiproliferative effect	[34]
TP4	0.1 - 1 mM	Inhibition tumor growth and progression	[23]

Table 2: *Effect of melatonin on growth of human colon tumors in vitro.*

In our experiments Balb/c female mice were transplanted with AKATOL mouse colon carcinoma cells and starting from the next day after grafting were treated with saline or with MLT (100 mg/kg, s.c. at 10.00 - 11.00 hours x 10 days). There was no effect of MLT on tumor growth or survival of tumor-bearing animals [20]. Some other tumor cell lines inoculated into mice were more sensitive to antiproliferative effect of MLT and induction apoptosis (Table 3). It is worthy to note that MLT increased susceptibility of various rodent transplantable and xenograft tumors to cytostatics [34,39].

Species, strain	Tumor strain	Dose of melatonin	Effect	References
Balb/c mice	AKATOL	100mg/kg, s.c., x10 days	No effect	[20]
B6D2F1 mice	Colon 38	10-100 mkg/day	Inhibits cell proliferation, induces apoptosis	[37]
Balb/c mice	CL26		Reducing growth and metastazing	[38]
Nude Balb/c mice	CL26	10 mg/kg s.c.	Inhibits tumor growth	[39]
Athymic Nude mice	LOVO	MLT 25 mg/kg. s.c.	Inhibits tumor growth, and potentiated effect of 5-FU	[34]

Table 3: *Effect of melatonin on growth of transplantable colon tumors in rodents in vivo.*

Effect of melatonin on colorectal cancer in humans

Barni., *et al.* [40] reported that 9 out of 25 patients treated with MLT plus interleukin-2 (IL-2) survived for 1 year while among 25 patients of the control group only 3 patients survived for 1 year. Analysis of the reported trials with treatment of 112 colorectal cancer patients with MLT combined with IL-2 has shown zero cases with complete response, 11 cases with partial or minor response, 35 cases of stable disease and 66 (50%) cases of progressive disease [41]. A report of Neri., *et al.* [42] failed to show a significant effect of MLT treatment in 31 cancer patients, including 9 enteric cancers. Ermachenkov., *et al.* [43] have studied excretion of 6-sulfatoxyMLT (a6-MTs) in colorectal cancer patients and effect of MLT on clinical characteristics of cancer process. It was shown decrease in level of excretion of a6MTs in CRC patients in direct proportion to the rate of tumour process spread. Nocturnal excretion of a6MTs was 1047 ± 202 ng/hrs in T1 patients and 499±70 in patients with distant metastases. Low content of enterochromaffin cells in colon mucosa was negative prognostic predictor studied [41]. Also, it was observed 14% increase of relapse–free period in 86 CRC patients who received orally 3 mg of MLT before go to bed during autumn and spring months as compared with CRC patients not given MLT.

Thus, MLT has some inhibitory effect on growth of colon cancer both *in vitro* and *in vivo*. The results of treatment of colon cancer patients with MLT are not very impressive. We believe that MLT could be effective as supplement therapy for treatment CRC patients.

Many carcinogens influence the metabolism of carbohydrates and lipids at the early stages of carcinogenesis [44,45]. It was suggested that these disturbances contribute to the promotion of initiated cells. In our experiments it was shown that the serum level of cholesterol was increased by 43.5% and the level of glucose and triglycerides was unchanged in rats exposed to DMH as compared to controls [46].

[^{3}H]-DMH injected into rats accumulated in high concentration in the hypothalamus as compared in brainstem, cerebral cortex or liver [47]. DMH produced an antigonadotropic effect both in male and female rats and increased the threshold of sensitivity of the hypothalamus to inhibition by estrogens [48]. The data on a pronounced influence of DMH on the level of biogenic amines in hypothalamus of rats correspond to the above-stated observation [47]. DMH does not influence the level of biogenic amines in the brain stem and hemispheres of rats. It was shown that the exposure to constant light which inhibits MLT synthesis and secretion also increases the threshold of sensitivity of hypothalamo-pituitary system to feedback regulation by estrogens [49].

Thus, MLT was able to influence several steps in initiating the effect of DMH. Our experiments failed to elucidate the exact mechanism of the anti//mutagenic effect of the hormone. MLT was shown to be a scavenger of hydroxyl radicals. Likewise, MLT can stimulate glutathione peroxidase activity in different tissues thereby reducing the generation of the $OH^{\cdot}$ by neutralizing its precursor H_2O_2. These properties allow MLT to preserve macromolecules including DNA, protein and lipid from oxidative damage resulting from ionizing radiation exposure and chemical carcinogen administration. Being both lipophilic and hydrophilic, MLT may prove to play an important role in the antioxidant defense system in all cells and tissues of the body [7]. Some early events induced by DMH treatment in the neuroendocrine system as well as in carbohydrate and lipid metabolism could be factors which create the macro- and microenvironment facilitating survival of carcinogen-damaged target cell(s) [44,50]. These disturbances could be alleviated, at least in part, by MLT treatment.

We observed a significant increase in proliferative activity of enterocytes in the colon mucosa in rats exposed to DMH as compared with intact controls. The treatment with MLT reduced the mitotic index significantly in colon tumors [51]. The number of Ki-67 positive cells was much higher in colon tumor than in colon mucosa from the same rats. Treatment with MLT failed to change the relative number of Ki-67 positive epithelial cells in these tissues. The apoptotic index (AI) was similar in the colon mucosa of intact rats and in the colon mucosa of rats treated with DMH and with DMH + MLT. The value of AI significantly increased in colon adenocarcinomas as compared to the colon mucosa. Treatment with MLT was followed by an increase of AI in colon tumors.

Our findings are in agreement with observations on the inhibitory effect of MLT on cell proliferation in the rodent colon [52], in mammary tumor and hepatoma cells [26]. It was shown also that pinealectomy was followed by the increase of the crypt cell proliferation in rat bowels (colon including) persisting at least 6 months after the operation [53]. The presence of specific binding sites for MLT in the mouse colon has been demonstrated as well [54]. MLT enhances cell-to-cell junction contacts [55]. Detailed study of AOM-induced colon carcinogenesis in rats revealed a decrease of the expression of the apoptotic repressor bcl-2 and the increase of the expression of the apoptosis accelerator bax protein in colon tumors as compared to colon mucosa from tumor-free rats treated with a carcinogen and to the saline-treated colon mucosa [56].

Serum MLT level was estimated in rats with DMH-induced colon tumors in day-time and at night [51]. There is a significant (by 2.7 times, $p < 0.005$) elevation of the night level of MLT as compared to the morning level in control rats. In rats with DMH-induced colon tumors the morning level of MLT was increased ($p < 0.005$) as compared to time-matched controls. However, there was no significant elevation of night levels of MLT in comparison to the morning levels in colon tumor-bearing animals. Our data have shown that in rats with DMH-induced colon tumors the morning levels of serum MLT are increased in comparison to the controls. These findings coincide with clinical observations that there were disturbances of the circadian rhythms of MLT excretion in such patients [57,58].

In all tissues of rats with DMH-induced colon tumors the number of M-cells was decreased in comparison to corresponding controls [51]. In ileum and colon of rats treated with DMH + MLT the number of M-cells was similar to control level whereas in stomach and duodenum this number was significantly higher than that in rats treated with DMH alone, but less than in corresponding controls. It has been shown that the mammalian gastrointestinal tract contains much more MLT than the pineal gland and enterochromaffin cells are the main source of MLT in the organism [54,59]. In spite of data demonstrating an active participation of MLT in adaptive responses, the normal function of extrapineal MLT as well as the feedback mechanisms between pineal and gastrointestinal MLT production are largely unknown.

It is possible to suggest that the decrease of number of M-cells and their functional activity in colon of rats exposed to DMH could play an important role in its carcinogenic effect. The treatment with MLT prevents the decrease in the content of M-cells in gastrointestinal tract of rats exposed to DMH that correlated with inhibitory effect of the hormone on colon tumor development [20].

We have studied the changes of glucose and lipid levels during carcinogenesis induced by DMH in rats [46]. Administration of DMH did not cause changes in the basal blood levels of glucose, however a decrease in the tolerance to glucose loading was observed. The levels of insulin in serum of rats exposed to DMH for 6 months was observed to be higher than in the control group being evaluated both after 18-hr starvation and 20 minutes. after i.v. glucose loading [46]. In rats subjected to long-term exposure to DMH triglyceride levels in serum were decreased by 18% in the 16^{th} and 24^{th} week of the experiment and after 48 weeks, when animals had developed colon tumors, they were increased by 33% [60].

Thus, significant disturbances of carbohydrate and lipid metabolism developed during DMH-induced carcinogenesis. These changes are involved in the mechanism of metabolic immunodepression [61] and are factors promoting tumor growth and progression [44,45].

MLT treatment was followed by some decrease in serum cholesterol and triglyceride levels in colon tumor-bearing rats as compared to animals exposed to the carcinogen alone [51]. Thus, MLT exerts some normalizing influence on glucose and lipid metabolism in rats exposed to DMH.

In our experiments significant immunodepression was observed in rats one week after exposure to DMH [61]. At this time only few morphologically detected colon tumors could be observed. Long-term treatment with MLT was followed by a decrease in the square of lymphoid infiltrates in the colon mucosa. This parameter was practically similar between the colon mucosa in intact rats and colon tumors in rats treated with DMH and MLT [51].

Conclusion

Thus, the inhibitory effect of MLT on DMH-induced colon carcinogenesis in rats has been shown in many experiments. This effect was expressed by the decrease of the incidence and multiplicity of colon tumors, by decrease in tumor size and invasiveness and by an increase in tumor differentiation. These observations suggest an influence of MLT on both early and late stages of DMH-induced colon carcinogenesis. It seems that effects of MLT on late stages of carcinogenesis have less significance for its anticarcinogenic effect than its effects on early stages. Data on the absence of an inhibitory effect of MLT on the growth of colon tumors *in vivo* and *in vitro* are in accordance with this suggestion. These data allowed to suggest that melatonin could be used both for prevention and treatment of CRC in humans as additional means to standard therapy.

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Conflict of Interest

Author have no any conflicts of interests.

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Volume 5 Issue 8 August 2018