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Review

## Melatonin as antioxidant, geroprotector and anticarcinogen

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### Abstract

The effect of the pineal indole hormone melatonin on the life span of mice, rats and fruit flies has been studied using various approaches. It has been observed that in female CBA, SHR, SAM and transgenic HER-2/neu mice long-term administration of melatonin was followed by an increase in the mean life span. In rats, melatonin treatment increased survival of male and female rats. In *D. melanogaster*, supplementation of melatonin to nutrient medium during developmental stages produced contradictory results, but an increase in the longevity of fruit flies has been observed when melatonin was added to food throughout the life span. In mice and rats, melatonin is a potent antioxidant both in vitro and in vivo. Melatonin alone turned out neither toxic nor mutagenic in the Ames test and revealed clastogenic activity at high concentration in the COMET assay. Melatonin has inhibited mutagenesis and clastogenic effect of a number of indirect chemical mutagens. Melatonin inhibits the development of spontaneous and 7-12-dimethylbenz(a)anthracene (DMBA)- or N-nitrosomethylurea-induced mammary carcinogenesis in rodents; colon carcinogenesis induced by 1,2-dimethylhydrazine in rats, N-diethylnitrosamine-induced hepatocarcinogenesis in rats, DMBA-induced carcinogenesis of the uterine cervix and vagina in mice; benzo(a)pyrene-induced soft tissue carcinogenesis and lung carcinogenesis induced by urethan in mice. To identify molecular events regulated by melatonin, gene expression profiles were studied in the heart and brain of melatonin-treated CBA mice using cDNA gene expression arrays (15,247 and 16,897 cDNA clone sets, respectively). It was shown that genes controlling the cell cycle, cell/organism defense, protein expression and transport are the primary effectors for melatonin. Melatonin also increased the expression of some mitochondrial genes (16S, cytochrome *c* oxidases 1 and 3 (COX1 and COX3), and NADH dehydrogenases 1 and 4 (ND1 and ND4)), which agrees with its ability to inhibit free radical processes. Of great interest is the effect of melatonin upon the expression of a large number of genes related to calcium exchange, such as *Cul5*, *Dcamk11* and *Kcnn4*; a significant effect of melatonin on the expression of some oncogenesis-related genes was also detected. Thus, we believe that melatonin may be used for the prevention of premature aging and carcinogenesis.

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### 1. Introduction

According to the free radical theory of aging, various oxidative reactions occurring in the organism (mainly in mitochondria) generate free radicals as byproducts which cause multiple lesions in macromolecules (nucleic acids, proteins and lipids), leading to their damage and aging. This theory explains not only the mechanism of ageing per se but also a wide variety of age-associated pathology, cancer including. Recent evidence sug-

gests that key mechanisms of both aging and cancer are linked via endogenous stress-induced DNA damage caused by reactive oxygen species [1–5].

Melatonin (N-acetyl-5-methoxy-tryptamine) is the main pineal hormone synthesized from tryptophan, predominantly during the night [6]. Melatonin is critical for the regulation of circadian and seasonal changes in various aspects of physiology and neuroendocrine function [6,7]. As age advances, the nocturnal production of melatonin decreases in animals of various species, including humans [8,9]. The grafting of a pineal gland from young donors into the thymus of old syngeneic mice or in situ into pinealectomized old mice prolongs the life span of the

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recipients [10,11]. The capacity of melatonin to cause life span extension is a hot topic for discussion [12–17]. There are a number of reports showing the anticarcinogenic and antitumor potential of melatonin [16,17].

In this work, the results of studies on the effect of melatonin on reactive oxygen species generation, life span and tumorigenesis in experimental animals are reviewed.

## 2. Melatonin as antioxidant

Since 1993, when melatonin was firstly identified as a free radical scavenger [18] many papers have been published confirming the ability of melatonin to protect DNA from free radical damage (Table 1). There is evidence that melatonin in vitro directly scavenges  $\cdot\text{OH}$ ,  $\text{H}_2\text{O}_2$ , singlet oxygen ( $\uparrow\text{O}_2^-$ ), and inhibits lipid peroxidation. Melatonin stimulates a number of antioxidative enzymes including SOD, glutathione peroxidase, glutathione reductase, and catalase. It has been shown that melatonin enhances intracellular glutathione levels by stimulating the rate-limiting enzyme in its synthesis,  $\gamma$ -glutamylcysteine synthase, which inhibits the peroxidative enzymes nitric oxide synthase and lipoxygenase. There is evidence that melatonin stabilizes microsomal membranes, thereby probably helping them resist oxidative damage [19]. Melatonin has been shown to increase the efficiency of the electron transport chain and, as a consequence, to reduce electron leakage and the generation of free radicals [20]. It was shown that melatonin reduced the

formation of 8-hydroxy-2'-deoxyguanosine, a damaged DNA products, 60 to 70 times more effectively than some classic antioxidants (ascorbate and  $\alpha$ -tocopherol) [21]. Thus, melatonin acts as a direct scavenger of free radicals with the ability to detoxify both reactive oxygen and reactive nitrogen species, indirectly increasing the activity of the antioxidative defense systems [20–23]. However, although the majority of studies confirm the antioxidant potential of melatonin, in some conditions, it can be prooxidant [23–28].

## 3. Effect of melatonin on gene transcriptional activity

In addition to its primary roles (circadian rhythm transduction and free radical scavenging), melatonin also serves as a potent modulator of gene transcriptional activity. Clearly, the expression of melatonin receptors in tissues and cell types makes a major impact upon selectivity of biological action of melatonin. Spectrum of cell surface membrane receptors of melatonin (including  $\text{MT}_1$  ( $\text{Mel}_{1a}$ ),  $\text{MT}_2$  ( $\text{Mel}_{1b}$ ),  $\text{Mel}_{1c}$  (found in amphibians, avians and fishes)) demonstrates (i) spatial/tissue-specific, (ii) spatial/cell-specific and (iii) temporal (developmental) variations, reflecting the overall complexity of the melatonin-mediated signal transduction. Additionally, “melatonin-related” cell surface membrane receptor (similar to known membrane melatonin receptors, but unable to bind melatonin itself) and nuclear receptors of melatonin (namely, RZR/ROR $\alpha$  and NR1F2 (RZR/ROR $\beta$ )) are known to have diverse expression profiles (ranging from ubiquitous to restricted), adding to the complexity of signal transduction mediated by the pineal hormone. It is believed that the existence of multiple melatonin receptor isoforms ensures the selectivity to natural ligands, differential regulation of the receptor expression (both temporal (developmental) and special (in different tissues)), and selective pathways for an intracellular signal transduction [42].

Over the course of last decade, conventional approaches have allowed identification of a large number of genes, targeted by melatonin centrally (in brain structures, most importantly in suprachiasmatic nucleus (SCN) of the hypothalamus and in *pars tuberalis* (PT) of the hypophysis) or in peripheral tissues. Discovering the mechanisms of melatonin interaction with so-called clock-genes (Per, Clock, Bmal and others) could be considered as one of the major achievements of these studies. It has been hypothesized that melatonin mediate the photoperiodic control of a season physiology via the phasing of expression of clock-genes in the PT, with a length of the melatonin signal decoded in target tissues in a form of the clock-gene expression profile signatures (“internal coincidence model”; [43]). Being rather complex and involving a number of regulatory and autoregulatory loops, close interaction of melatonin with clock genes ensures the consistency of biological rhythms and precision of photoperiod affect upon gene expression in melatonin-sensitive (target) tissues.

Progress in a development of high-throughput technologies (most importantly of DNA microarrays) have substantially incremented the list of melatonin targets in peripheral tissues, and have significantly improved our understanding of the mechanisms of its biological action. Numerous technological advances

Table 1  
Effect of melatonin on free radical processes

Radical or reactive oxygen specimens, enzyme	Effect of melatonin	References
<i>Reactive oxygen specimens</i>		
Hydroxyl radical ( $\cdot\text{OH}$ )	↓	[18]
Singlet oxygen ( $\uparrow\text{O}_2$ )	↓	[21]
Hydrogen peroxide ( $\text{H}_2\text{O}_2$ )	↓	[29]
Nitric oxide (NO)	↓	[30]
Peroxy radical (LOO $\cdot$ )	↓	[31]
ONOO $^-$	↓	[32]
<i>Products of oxidation</i>		
Malone aldehyde	↓	[33]
Ketodiene	↓	[34]
Diene conjugate	↓	[35]
Schiff's bases	↓	[35]
CO-derivatives of aminoacids	↑	[36]
8-oxyguanine	↓	[33]
8-hydroxy-2'-deoxyguanosine	↓	[37]
<i>Enzymes of antioxidative defense system</i>		
Cu,Zn-superoxide dismutase (SOD)	↑	[38]
Catalase	↑	[29]
Glutathione peroxidase	↑	[39]
Glutathione reductase	↑	[40]
$\gamma$ -Glutamylcysteine synthase	↑	[41]
Glucoso-6-phosphate dehydrogenase	↑	[34]
Myeloperoxidase	↑	[34]
<i>Prooxidant enzymes</i>		
Nitric oxide synthase	↓	[30]

have led to the evolution of DNA microarrays, which may now contain tens to over hundred thousand of spots of DNA material, thus reaching a truly genomic scale [44]. The principle of this approach has been proved in a diversity of experiments. In one of the first screening-oriented studies, the effect of melatonin treatment upon gene expression in rat retina (retinal neurons and retinal pigment epithelium (RPE)) was addressed using cDNA microarray. Microarray-based screening of ~8000 rat cDNA clones have led to the identification of the limited group of genes with expression in rat neural retina and RPE being changed significantly by melatonin [45]. In neural retina, treatment with melatonin had stimulated an expression of 6 and repressed an expression of 8 genes, while in RPE cells 15 genes were up-regulated and 2 were down-regulated. Among these, genes with important physiological functions were present. For example, melatonin had down-regulated gene expression of integrin and integrin-associated protein-encoding genes in rat retina, while a cyclic AMP response element binding protein (CREB) gene was up-regulated in RPE. Importantly, many gene targets identified were expressed sequence tags (ESTs) matching novel genes, thus providing an invaluable hint for future insights into the mechanisms of biological action of melatonin in retina. It should be also mentioned that lists of melatonin targets in two closely related retinal cell types were mutually exclusive: this unexpected observation was further supported in further studies of our own.

In first of the latter, we have employed an avowed NIA 15 K cDNA microarray that contained over 15,000 mouse cDNA clones representing about 11,000 individual gene products [46]. We have hypothesized that a short-term course of melatonin treatment (for duration of 5 days) followed by RNA harvest would allow us identifying gene which determine the initial stages in the cascades of events resulting in carrying out vital biological effects of melatonin. CBA mice of experimental group were administered melatonin with drinking water ad libitum, and after 5-day treatment total RNA purified from cardiac tissue was used to synthesize isotope ( $^{33}\text{P}$ )-labeled probes which were subsequently hybridized to microarrays. Analysis of the microarray data allowed us to identify a limited group of transcripts (212, <1.4% of the clones screened) with significantly altered cardiac gene expression [47]. Among these, 146 genes were up-regulated and 66 down-regulated ( $Z_{\text{Ratio}}$  confidence level=2.0), including such genes as Cop1, Wnt4 and Stk11 (up-regulated) and Ubc2e and Spi4 (down-regulated), all of which have important biological roles. Using an established system for functional categorization of genes expressed in cardiovascular system [48], we have discovered that although melatonin indeed affects the expression of a wide spectrum of genes, its primary effectors are associated with the genes controlling the cell cycle, adhesion, and transport. This finding is in agreement with the established data on the effect of melatonin on cell proliferation, apoptosis, and adhesion [49], as well as with numerous earlier studies based on the low-throughput approaches. Notably, melatonin have also demonstrated a pronounced effect on the expression of genes related to oncogenesis (e.g., Myb11, Rasa1, Mlt3 and Enigma homolog 2) and calcium metabolism (Kcnn4 and Dcamk11).

Importantly, we have also detected a significant effect of melatonin upon expression of some mitochondrial genes: namely, the genes encoding 16S ribosomal RNA (mt-Rnr2), cytochrome c oxidase subunits I and III (mt-Co1, mt-Co3) and NADH dehydrogenase 1 (mt-Nd1) (all up-regulated) and ATP synthase subunit 6 (mt-Atp6; down-regulated). This finding supports previous observations of the direct effect of melatonin upon the expression of mitochondrial genome-encoded genes in the brown adipocytes of Siberian hamster. Although the approach used in that study have allowed identification of only one mitochondrial gene (cytochrome b, mt-Cytb) as a target of melatonin, it was further suggested in the same study that melatonin may act on the entire mitochondrial genome [50]. Justifiably, the expression of mitochondrial genome-encoded genes is very high in tissues with biological function directly associated with continuous contraction of muscle fibers and/or transmembrane ion transport [51,52]. Alterations in the amount of mitochondrial transcripts and, subsequently, in the volume of energy produced in mitochondria by oxidative phosphorylation (OXPHOS) are therefore critical for organ function.

To study the effect of melatonin upon gene expression in mouse brain, we have used an updated NIA 17K cDNA microarray, representing a reformatted combination of NIA 15K [46] and NIA 7,4K libraries [53] containing nearly 17,000 mouse cDNA clones representing more than 12,000 individual gene products. A limited group of transcripts (38, <0.3% of the clones screened) had demonstrated altered expression [54]. Among these, 5 genes were up-regulated and 33 down-regulated ( $Z_{\text{Ratio}}$  confidence level=1.5). Examples include such genes as Irf3 and Tlk2 (down-regulated); notably, biological function of the last is related to CREB protein. As it have been observed in the earlier study by Wiechmann [45], nearly independent set of melatonin gene targets was identified in the tissue constituted by the diverse cell types. Importantly, we were able to identify only one transcript with expression changed by melatonin in both heart and brain: notably, it was encoded by mitochondrial genome (NADH dehydrogenase 4; mt-Nd4). Comparative analysis of melatonin effect upon gene expression in mouse heart and brain thus supports observations on some tissue-specific biological effects of the melatonin signaling system [55] and further points out the potential importance of mitochondrial genes in biological actions of melatonin in target tissues.

#### 4. Effects of melatonin on mutagenesis

There is evidence of an age-related accumulation of spontaneous mutations in somatic and germ cells [56,57]. The accumulation with age of some spontaneous mutations or mutations evoked by endogenous mutagens can induce genome instability and, hence, increases the sensitivity to carcinogens and/or tumor promoters. It has been shown that clonally expanded mtDNA mutations accumulate with age in normal human tissues [58]. The finding that deleted mtDNA accumulated in human muscle tissue as well as evidence for partially duplicated mtDNA in aged human tissues [59] allow us to suggest the important role of clonal expansion of mutant mtDNA in the age-related increase of systemic oxidative stress in the

whole organism [60]. A significant trend toward increasing *p53* mutations frequency with advancing age was found in some normal and malignant tissues [61,62]. Simpson [63] suggests that the aging human body accumulates enough mutations to account for multistep carcinogenesis by the selection of pre-existing mutations.

Melatonin has been found to inhibit X-ray induced mutagenesis in human and mouse lymphocytes in vitro [64–66], to reduce Cis-platinum-induced genetic damage in the bone marrow of mice [67], to decrease hepatic DNA adduct formation caused by safrole in rats [68] and to protect rat hepatocytes from chromium(VI)-induced DNA single-strand breaks in vitro [69]. We studied the effect of melatonin on the induction of chromosome aberrations and sperm head anomalies in mice treated with cyclophosphamide, 1,2-dimethylhydrazine and N-nitrosomethylurea and found that melatonin potently inhibited the mutagenicity of these carcinogens [70].

Since melatonin can protect cells directly as an antioxidant [71], and indirectly through receptor-mediated activation of antioxidative enzymes, we applied the Ames test system in vitro [72]. Some of the mutagens used are known to damage DNA through several mechanisms. In order to test these mutagens, we tried to use different strains of *Salmonella typhimurium* that turned out to be able to detect different mechanisms, in a non-toxic range of concentrations, using the preincubation assay. The strains TA 97 and TA 98 are sensitive to different frameshift mutagens. TA 100 detects G-A base-pair substitutions, while TA 102 is sensitive to mutagens damaging A-T pairs, mainly through free radicals formation, and cross-linking agents [72]. We supposed that melatonin, being an antioxidant, could affect mutational events which result from oxidative damage and so could inhibit revertant colony formation in the strain TA 102 specially designed to detect this type of lesions. Thus, melatonin alone turned out neither toxic nor mutagenic in the Ames test.

The Single Cell Gel Electrophoresis assay (SCGE assay or COMET assay) was used for the evaluation of the clastogenic effect of melatonin and it was shown that it had clastogenic activity at the highest concentration tested (100  $\mu\text{M}$ ) in the SCGE assay [72].

As oxidative mutagens we used DMH, bleomycin (with S9-mix) and mitomycin C (without S9-mix) which are believed to generate oxygen radical species [73,74]. Furthermore, mitomycin C acts as a bifunctional alkylating agent [75]. Additionally, we tested eight other intercalating and alkylating agents both direct-acting and those requiring metabolic activation. The 12 mutagens used were 7, 12-dimethylbenz(a)anthracene (DMBA), benzo(a)pyrene (BP), 2-aminofluorene (AF), 1,2-dimethylhydrazine (DMH), bleomycin, cyclophosphamide (CP), 4-nitroquinoline-N-oxide (NQO), 2,4, 7-trinitro-9-fluorenone (TNF), 9-aminoacridine (AA), N-nitrosomethylurea (NMU), mitomycin C and sodium azide tested in the absence or presence of S9 mix. In four *Salmonella typhimurium* tester strains, TA 97, TA 98, TA 100 and TA 102, melatonin significantly reduced the mutagenicity of chemicals which require S9 activation. In the SCGE assay performed on CHO cells, preincubation with melatonin led to a strong inhibition of the clastogenic activities of DMBA and CP, and to a lesser extent with BP and NMU. With

mitomycin C, melatonin exacerbated responses in both tests. It should be noted that melatonin was effective as an antimutagen only at extremely high doses (0.25–2  $\mu\text{mol/plate}$ ) [72]. The modifying activity of melatonin was not related to the mechanism of action of the mutagens, i.e., frameshift mutations or base-pair substitutions. As melatonin displays its protective effect towards promutagens only, we speculate that it can exert its activity on the metabolic activation process, perhaps by inhibiting the cytochrome P 450-dependant mono-oxygenase enzyme system of S9-mix. Another explanation of the S9 mediated properties of melatonin could be that the antimutagenic effect results from metabolites of melatonin, such as 6-hydroxymelatonin. 6-hydroxymelatonin was found to be able to inhibit the thiobarbituric-induced lipoperoxidation in mouse brain homogenates, which was imputed to the production of oxygen free radicals [76].

Generally, the results of the SCGE assay are in accordance with those of the Ames test. Melatonin modulates the clastogenicity of DMBA, BP and cyclophosphamide. Compared to the data obtained in the Ames test, melatonin inhibited the clastogenicity of the chemicals at lower concentrations (0.1–1 nM). In combination with mitomycin C a significant, dose-related, exacerbating effect of melatonin has been observed in both tests [72]. The available data shows that melatonin may play an important role in defending cells from DNA damage induced, not only by oxidative mutagens, but also by different alkylating agents.

## 5. Effect of melatonin on apoptosis

In a series of studies it was shown that melatonin inhibits apoptosis in cells of the brain, induced by reactive oxygen species (ROS) [28], kainate [77], amyloid  $\beta$ -peptide, related to Alzheimer disease [78], neuromediators [79] and neurotoxins [80], but not by N-methyl-D-aspartate [77], staurosporine or neurotoxin ethylcholinaziridine [81]. It was suggested that the protective effect of melatonin on neurocyte apoptosis depends on the model used and is not mediated by caspase-dependent programmed cell death, but involves prevention of glycolysation derivative-induced necrosis [81]. In contrast, exaggeration of neuronal damage by melatonin was observed in native cultures as well as after induction of apoptosis [81]. Melatonin supplementation suppresses NO-induced apoptosis via induction of Bcl-2 expression in immortalized pineal PGT- $\beta$  cells [82]. A similar pathway of inhibitory effects of melatonin on apoptosis induced by ischemic neuronal injury has been determined [83].

Low doses of melatonin ( $10^{-7}$ – $10^{-9}$  M) inhibit apoptosis in both dexamethasone-treated, cultured thymocytes and the intact thymus [84]. This effect of melatonin was mediated by its inhibitory influence on proliferation of thymocytes [85]. Long-term (over 8 months) administration of melatonin at a daily dose of 40–50  $\mu\text{g}$  per mouse prevents thymic involution in very old animals, an effect brought about by the inhibitory action of melatonin on dexamethasone-induced apoptosis in thymocytes and splenocytes [86]. The administration of melatonin to mice in the drinking water (15 mg/l) for 40 days also attenuated the apoptotic thymocyte death caused by free radicals and



stimulated thymocyte proliferation in thymus [87]. Thus, both *in vitro* and *in vivo* studies demonstrated an inhibitory effect of melatonin on apoptosis in the thymus.

The administration of melatonin in the drinking water (20 mg/l) during the dark phase to rats exposed to 1,2-dimethylhydrazine failed influence an apoptotic index in normal colon mucosa but significantly (by 1.8 times) inhibited it in colon tumors [88]. Treatment with the potent hepatocarcinogen aflatoxin B1 leads to direct or indirect caspase-3 activation and consequently to apoptosis in the rat liver. Melatonin treatment enhances the rat hepatic antioxidant/detoxification system, which consequently reduces the apoptotic rate and necrobiotic changes in the liver [89].

## 6. Effect of melatonin on life span and longevity in rodents

Effects of melatonin on life span in mice and rats have been discussed recently [15,16]. In Table 2, we summarized available

data on survival and tumor incidence in mice exposed to long-term treatment with melatonin.

Melatonin did not induce malignancies in male C57BL/6 mice when administered at 10 mg/l (1.5–2.0 mg/kg) in the night drinking water from 19 months [10,94]. Lipman et al. [95] observed lymphomas in 77.9% of male C57BL/6 mice that received melatonin with food (11 ppm or 68 µg/kg) from the age of 18 months and survived to a 50% mortality (26.5 months). In controls only 28.6% of mice developed lymphomas. Leukemia was detected in 70–98% of C57BL/6 mice and 78% of CC57Br mice (both males and females) treated subcutaneously with melatonin at a dose of 2.5 mg/mouse (~ 80 mg/kg) twice a week for of 2.5–5 months [92,93]. Thus, being administered in significantly high dose (about 80 mg/kg) melatonin induced lymphomas and leukemias in C57BL/6 mice. At low dose (from 68 µg/kg to 1.5–2.0 mg/kg) it induced them only in one small group (7 mice). In a previous our study with CBA mice, melatonin given in night drinking water in an interrupted (course)

Table 2  
Summary of experiments on the effect of melatonin on life span and spontaneous tumor incidence in mice

Strain	Sex	Nos. of mice C/M	Age at the start of treatment, months	Treatment with melatonin	Age at the end of observation	Effects of melatonin on		References
						Mean life span	Tumor incidence	
Balb/c	Female	26/12	15	10 mg/l in night drinking water	ND	+18%	No data	[90]
Balb/c	Male	50/50	18	10 mg/l in night drinking water	ND	Shift to right of the survival curve	No data	[91]
C57BL/6	Male & female	25/45	1.5	2.5 mg/mouse s.c. twice a weeks×5 months	22 months	-13%	Increases	[92]
C57BL/6	Male & female	29/57	1.5	2.5 mg/mouse s.c. twice a weeks×2.5 months	22 months	-20.6%	Increases	[93]
CC57Br	Male & female	26/57	1.5	2.5 mg/mouse s.c. twice a weeks×2.5 months	22 months	-12%	Increases	[93]
C57BL/6J	Male	10/10	19	10 mg/l in night drinking water	ND	+20%	No data	[94]
C57BL/6	Male	20/15	19	10 mg/l in night drinking water	ND	+17%	No data	[10]
C57BL/6	Male	1:20/20	18	11 ppm (68 µg/kg) with lab chow ad libitum	1: 24 months	No effect	1: No effect;	[95]
		2:7/13			2: 50% survival		2: Increases;	
		3:38/30			3: died <2 years		3: No effect	
CBA	Female	50/50	6	20 mg/l in night drinking water	ND	+5%	Increases	[96]
C3H	Male	20/20	1	2.5 mg/kg/day in night drinking water	23 months	+20%	No data	[97]
	Female	20/20	8		27 months	No effect		
C3H/He	Female	14/15	12	10 mg/l in night drinking water	ND	No effect	Increases	[10]
C3H/Jax	Female	16/39	3 weeks	25–50 mg/mouse/day with drinking water	12 months	No data	Decreases	[98]
HER-2/neu	Female	30/27/22	2	2.5 mg/kg/day in night drinking water 5 days/monthly or constantly	ND	M1: No effect; M2: -13%	M1: No effect; M2: Decreases	[99]
NOD	Female	25/30	1	4 mg/kg s.c. at 4:30 PM, 5 times a weeks, 4–38 weeks	50 weeks	C:32% survivors M:90% survivors	No data	[100]
NOD	Female	29/17	1	10 mg/l in night drinking water, 5 times a week, 4–38 weeks	50 weeks	+17%	No data	[100]
NZB	Female	10/10	4	10 mg/l in night drinking water	20 months	Survivors: C:0; M: 40%	No data	[10]
NZB/W	Female	15/15/15	8	2–3.5 mg/kg s.c. daily at 8–10 h (M1) or at 17–19 h (M2)×9 month	34 weeks	Survivors: C: 20%; M1: 60%; M2: 60%	No data	[101]
SAMP-1	Female	20/20	3	20 mg/l in night drinking water	ND	No effect	No effect	[102]
SAMR-1	Female	10/12	3	20 mg/l in night drinking water	ND	-11%	No effect	[102]
SHR	Female	50/50/50	3	2 or 20 mg/l in night drinking water	ND	No effect;	M1: ↓ 1.9-fold	[103]
						M1, M2: +3 months. MLS	M2: No effect	

Note. C=control group; M=melatonin-treated group; MLS=maximum life span; ND=animals were survived until natural death; NOD=non-obese diabetic; SAMP-1=senescence accelerated mouse-prone; SAMR-1=senescence accelerated mouse-resistant.

regimen at a relatively low dose (3–3.5 mg/kg) was carcinogenic. Lymphomas and lung adenocarcinomas developed in CBA mice treated with melatonin whereas no such malignancies developed in controls [96]. In female SHR mice, melatonin given approximately in the same dose (20 mg/l; 2.7–3.3 mg/kg) failed significantly increase the total incidence of tumors or tumors of any localization [103]. Strain differences in susceptibility to chemical carcinogens are well known.

There is one strong critical comment on results of long-term experiments with melatonin in mice. It was claimed that murine strains used in some studies do not synthesize melatonin as a result of a genetic defect (BALB/c, NZB, and C57BL/6) [104]. Later it was shown that the pineal gland did produce melatonin in the above-mentioned mouse strains with genetic defects, but the production night peak was very short, so its presence was difficult to detect [100]. It is worthy to note that the major signal transduction cascades in the pineal gland did not differ between melatonin-proficient C3H mice and melatonin-deficient C57BL mice [105].

Thus, with exception of the results of Romanenko's studies, which used a very big dose of the compound, being administered into mice of various strains regardless the age of mice at start of the treatment, and sex, melatonin increased life span in 12 from 20 experiments (60%) and has no effect in 8 experiments (40%) ( $P > 0.05$ ). In males, 4 from 5 experiments resulted in the life extension with melatonin, while in females, 8 experiments were positive and 7 were negative. If the treatment was started early, 8 of 14 cases observed the increase in longevity and 6 had no effect.

We believe that these discrepancies related mainly to the variations and defects in experimental design and strain susceptibility to melatonin. In the majority of these studies the number of mice per group was rather small (20 and less) that is not sufficient for correct conclusion in long-term studies devoted to the evaluation of effect on both longevity and carcinogenicity [15,16]. It is worth to note that among 4 experiments, which were conducted according to the standard protocol (50 animals per group) 3 resulted in the increase of the life span under the influence of melatonin.

In an experiment with male CD rats melatonin was given with drinking water (4 mg/l) during the whole day starting at 11–13 months age during 16 months [106]. Additional groups of rats received with drinking water melatonin antagonist M-(1,4-dinitrophenyl)-5-methoxytryptamin (ML-23) at the dose of 0.4 mg/l or combination of melatonin and ML-23 in the same doses. Observation was stopped when the age of rats was 26–29 months. In the control group to this age survived 7 from 16 rats (44%), whereas in the group exposed to melatonin alone—13 from 15 rats (87%). Surprisingly, in the group exposed to the melatonin antagonist ML-23 survived 6 from 10 mice; and in the group treated with combination of melatonin and ML-23—survived 8 from 10 rats. Body weight was similar in all groups. Five of seven rats from the control group revealed a pneumonia at autopsy whereas in the group treated with melatonin there were no cases of pneumonia. The serum level of testosterone was 2.8 times higher in rats treated with melatonin as compared to the controls. Authors believe that melatonin antagonist ML-

23 induces chronic deprivation of melatonin receptors followed by their hypersensitivity to the melatonin. It is worthy of note that in this experiment was small number of animals per group and the experiment was finished before natural death of all animals.

Vinogradova and Shevchenko [107] administered melatonin in the night drinking water (10 mg/l) to 50 female and 50 male outbred LIO rats since the age of 3 months until natural death. Survival of melatonin-treated animals was significantly higher as compared with intact controls at the age of 2 years (80% and 57%, respectively,  $p < 0.05$ ). At the same time melatonin significantly inhibits the development of spontaneous tumors both in males and females as compared with the intact controls. The treatment with melatonin slowed down of the age-related increase in the incidence of irregular estrous cycles. There was observed a normalization of age-related changes in activity of antioxidative enzymes and antioxidant status of rats under the treatment with melatonin [108].

## 7. Effect of melatonin on longevity in fruit flies

We have studied the effects of melatonin on longevity in the *D. melanogaster* strain HEM [109]. Melatonin was added to the nutrition medium (100 µg/ml) during the 2nd to 3rd age of larvae. Exposure to melatonin was followed by a decrease in the level of conjugated hydroperoxides and ketodienes in females. Furthermore, melatonin failed to influence the activity of catalase in males, whilst it was increased in females by 24% ( $p < 0.02$ ) and Cu, Zn-superoxide dismutase (SOD) activity was unaltered in both males and females. Melatonin did not influence the life span of this strain of fruit fly.

The life span of *D. melanogaster* wild strain Canton-S was studied under treatment with melatonin at a concentration of 800 mg/l [110]. The hormone was introduced into the nutrient medium at the larvae stages. Five experiments with melatonin have shown variable effects of melatonin on life span: the mean life span in males varied from –10.0% to +18.5%, whereas in females the range was +2.3% to 12.1%. An inverse correlation was observed between the change in life span after melatonin supplementation and the life span in the corresponding control group. For a relatively low life span in the population from which the control and experimental group were formed, the geroprotector effect of melatonin was the most distinct; for a relatively high life span, the effect of melatonin was either not detected or appeared as a reduction in life span due to a toxic effect.

The effect of melatonin on life span was studied also in *D. melanogaster* Oregon wild strain [111]. Melatonin, added daily to the nutrition medium at a concentration of 100 µg/ml throughout the experiment, significantly increased the life span of the flies. The maximum life span was 61.2 days in controls and 81.5 days in the melatonin-fed group (+33.2%). Relative to controls, the percentage of the melatonin-fed flies was 19.3% in the onset of 90% mortality and 13.5% in median life span. The authors have also shown that melatonin treatment increased the resistance of fruit flies to superoxide mediated toxicity of paraquat and to thermal

stress. Thus, if melatonin is added to food for the entire life span, it statistically significantly increases the longevity of fruit flies.

## 8. Effect of melatonin on carcinogenesis

The role of the pineal gland in tumor development has been under intensive study during the last years [112–115]. In cancer patients the morphological signs of pineal function decrease and disturbances in the circadian secretion pattern of the main hormone of the pineal gland, melatonin, were observed [112–115]. For a variety of tumors their growth is accelerated in pinealectomized animals. The inhibitory effect of melatonin is well established in relation to mammary tumors [113,116–118] and colon cancer [119,120]. There are few data on the effect of melatonin on tumors of other localizations. In this section we will review available data on anticarcinogenic effects of melatonin.

### 8.1. Melatonin and mammary carcinogenesis

The evidence of oncostatic action of melatonin on mammary tumor growth was obtained both in *in vitro* and *in vivo* experiments. MCF-7 human breast cancer cell line mostly used as a model for study of melatonin effect *in vitro* [116–118]. This cell lines originated from the pleural effusion of women with metastatic breast carcinoma, and contains both estrogen and progesterone receptors. It was shown that a growth of MCF-7 cells is estrogen dependent and estrogens regulate the levels of some RNAs and proteins in them [116]. The inhibitory effect of melatonin on MCF-7 cells was firstly observed by Blask and Hill [121]. A lot of experiments on effect of melatonin on MCF-7 cell growth was reviewed recently by Cos and Sanchez-Barcelo [116,117]. It was shown that melatonin, at physiological concentration in a culture medium, inhibits cell proliferation and invasiveness of the cells, suppress mitotic activity of estradiol-17 $\beta$  and EGF, thus blocks proliferation at stage G<sub>0</sub>/G<sub>1</sub> of mitotic cycle. Only those lines of MCF-7 and other breasts carcinoma cell lines mammary which have estrogen receptors (T47D, ZR75-1) have been shown susceptible to inhibitory effect of melatonin [116–118,122,123]. Lemus-Wilson et al. [124] observed inhibitory effect of melatonin on proliferative effect of prolactin and human growth hormone on MCF-7 cells. There is suggestion that melatonin suppress autocrine and paracrine secretion of some growth factors (EGF, TGF- $\alpha$ , insulin, IGF-I and II [125].

Anisimov et al. [126] have shown inhibitory effect of melatonin on transplantable mammary carcinoma RSM in female  $\tilde{N}3\tilde{A}$  mice. Melatonin was given subcutaneously in a daily dose of 50  $\mu$ g/mouse and 51% decrease of tumor size has been observed.

According Hamilton and Sneddon [127], morning administration of melatonin to rats was followed by the increase in the incidence of mammary carcinomas induced by DMBA in female rats. However it was shown in several experiments that the evening treatment with melatonin inhibits DMBA-induced mammary carcinogenesis [128–134]. Melatonin also attenuated the stimulating mammary carcinogenesis effect of pinealectomy

[135]. Another mammary carcinogen, N-nitrosomethylurea (NMU) was also used in experiments with melatonin. This model has some advantages in comparison to DMBA-induced carcinogenesis. In contrast to DMBA-induced mammary adenocarcinoma susceptible mostly to prolactin, the growth of NMU-induced mammary carcinoma is more depend on the levels of estrogens, that is more relevant to human breast carcinoma [136]. In several studies have been observed inhibitory effect of melatonin on NMU-induced carcinogenesis in mammary gland of rats [129,137,138]. It is interesting that melatonin failed reveal the dose-dependent inhibitory effect in this model. The effect of melatonin was similar to those of estrogen antagonist tamoxifen [67].

Subramanian and Kothari [139] reported a suppressive effect of melatonin on the development of spontaneous mammary carcinomas in female C3H/Jax mice treated from the age of 3 weeks until the age of 12 months. in female CBA mice, administration of melatonin with night drinking water from the age of 6 months failed change the incidence of spontaneous mammary adenocarcinomas [140].

The oncostatic effects of melatonin on the mammary gland have been studied in transgenic mice carrying the *N-ras* proto-oncogene under the control of the MMTV-LTR [141]. Female (4-week-old) virgin mice with positive transgenic pedigrees were injected with melatonin (200  $\mu$ g/mouse/ day, 5 times a week) or vehicle late in the evening. After 5 months of treatment, animals were sacrificed and the mammary glands were dissected for whole mounts, histology, and immunohistochemical analysis with a mouse monoclonal antibody specific for *N-ras* protein. Mammary glands of control transgenic mice showed different densities of hyperplastic alveolar nodules (HANs) consisting primarily of dysplastic epithelial cells with nuclear atypia and prominent nucleoli. The epithelial cells of HANs showed a high expression of *N-ras* while no immunostaining was detected in the unaffected mammary parenchyma. Only one (10%) of the control transgenic mice presented an infiltrating ductal carcinoma with the neoplastic cells over-expressing *N-ras* protein. The mammary glands of melatonin treated mice had a lower density of HANs, absence of epithelial dysplastic cells, and weak immunostaining of *N-ras* protein in comparison to the vehicle-treated group. None of the melatonin treated animals developed mammary carcinomas during the observation period. The lymph nodes of the inguinal mammary glands of all the vehicle-treated transgenic mice presented hyperplasia and two animals even had lymphomas, whereas in melatonin-treated animals there was less hyperplasia (two cases were atrophic) and a lack of lymphomas. Authors conclude that in the mammary glands of MMTV-LTR/*N-ras* transgenic female virgin mice, melatonin reduces the incidence of HANs and the expression of *N-ras* protein in focal hyperplastic lesions, completely prevents the development of epithelial cell atypia and mammary adenocarcinomas, and also reduces the hyperplasia of the mammary lymphoid tissue and prevents the development of lymphomas.

Four-week-old TG.NK female mice with MMTV/*c-neu* oncogene were gavaged with melatonin at 50, 100 or 200  $\mu$ g/kg dissolved in corn oil [142]. Melatonin delayed the appearance

of palpable mammary tumors and the growth of tumors with a dose-related statistically significant negative trend for the incidence of tumors.

The administration of melatonin during the night time significantly decreased the incidence of mammary adenocarcinomas in FVB/N female mice transgenic for oncogene HER-2/neu [143]. Melatonin administration decreased the size of mammary adenocarcinomas ( $p < 0.05$ ) and the incidence of lung metastases ( $p < 0.07$ ) as compared with controls. In order to evaluate whether the decreased incidence of mammary tumors observed in mice was due to an effect of melatonin on mammary gland, we performed RT-PCR analysis for HER-2/neu gene expression in the mammary tumors from mice. mRNA for HER-2/neu gene was greatly expressed in saline treated mice whereas it was significantly (2.5-fold) decreased in animals chronically treated with melatonin [143].

### 8.2. Melatonin and colon carcinogenesis

We have studied the effect of melatonin on colon carcinogenesis. Intestinal tumors induced in rats by 1,2-dimethylhydrazine (DMH) and its carcinogenic metabolites azoxymethane (AOM) and methylazoxymethanol at present are the most popular models used in experimental oncology to study various aspects of the morphology, pathogenesis, prevention and treatment of colorectal cancer [144]. It was shown that colon carcinomas induced by DMH are morphologically similar to human colon tumors and, like other malignancies, a colon carcinoma needs at least several stages (events) for development in bowel mucosa.

Three-month-old male LIO rats were exposed to 5 or 15 weekly s.c. injections of 21 mg/kg DMH [145,146]. Part of DMH-treated rats was given melatonin in tap water (20  $\mu\text{g}/\text{ml}$ ) at night time during the period of the carcinogen treatment. Rats were sacrificed in 6 months after first DMH injection. The exposure to melatonin was followed by the decrease of intestinal tumor incidence in jejunum and ascending colon as compared to controls. Melatonin inhibits multiplicity and size of colon tumors as well as increases tumor differentiation. The level of immunohistochemically detected melatonin 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 + melatonin it was in control ranges [147]. 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 + melatonin a normalization of free radical processes in serum and colon has been observed [146]. MLT also inhibits the mutagenic effect of DMH in vivo (chromosome aberration and sperm head anomalies tests) and in vitro (Ames' test). Melatonin 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 [148].

In experiments of Weisburger et al. [149], male F344 rats were injected subcutaneously with an aqueous solution of 15 mg/kg azoxymethane on day 50 and day of 57, and a group was also given the 0.5 mg melatonin solution 5 times per week at 4:30 pm. Controls were handled identically without melatonin. The rats given azoxymethane were examined for foci of aberrant crypts in the colon after 8 weeks of test. In rats given azoxymethane and the melatonin injections, there were fewer foci of aberrant crypts in the colon and the average number of crypts was fewer as well.

DLD-1 human colon carcinoma cells were insensitive to high concentrations of melatonin ( $10^{-4}$  M) [112]. Using the CaCo-2 human colon carcinoma cell line Pentney [150] failed to observe an antiproliferative effect of melatonin in physiological concentrations. The only effect occurred at suprapharmacological concentrations ( $10^{-3}$  M). Thus, these data have shown that melatonin affect initiating, promoting and, in less degree, progression stages of colon carcinogenesis.

### 8.3. Melatonin and cervico-vaginal carcinogenesis

In our experiments, forty female CBA mice aged 3–4 months were exposed twice a week during 2 months to intravaginal applications of polyurethane sponges impregnated with 0.1% solution of DMBA in triethyleneglycol [151]. Three hours after each application the sponges were taken out. Starting from the day of the 1st DMBA application a part of mice was exposed five times a week during 4 months with melatonin in tap water (20  $\mu\text{g}/\text{ml}$ ) given at night time. All mice were sacrificed in 6 months after start of the experiment. Seven of 20 mice exposed to DMBA alone developed malignancies in the vagina and cervix uteri and two mice developed benign cervical tumors. No malignancies in vagina and uterine cervix and three vaginal papillomas were observed in mice exposed to DMBA + melatonin. Thus, these data indicate that melatonin inhibits cervical and vaginal carcinogenesis induced by DMBA in mice.

### 8.4. Melatonin and endometrial carcinogenesis

Experiments with spontaneous endometrial carcinomas in BDII/Han rats have shown that melatonin prolonged survival of rats only if chronic night-time administration in drinking water was initiated by day 30 of life [152], i.e., shortly before beginning of puberty, whereas life-long treatment was ineffective if treatment was started on day 50, when animals had attained puberty [153]. Since the pineal hormone delays pubertal development in both male and female rats it can be anticipated that the action of melatonin on endometrial cancer is exerted by delaying reproductive maturation [115].

### 8.5. Melatonin and hepatocarcinogenesis

Six-week-old male F344 rats were given a single dose of N-diethylnitrosamine (DENa, 200 mg/kg b.w., i.p.). Starting 2 weeks later, there were treated with 0, 1, 5, 10 and 20 ppm melatonin in their night drinking water for 6 weeks [154]. At week 8, the experiment was terminated and the animals were



sacrificed. Although clear dose dependence was not apparent, both numbers and areas of glutathione S-transferase (GST-P)-positive liver foci, liver preneoplastic lesions, in the liver were decreased in the melatonin treated groups, this being significant for the number in the 10 ppm melatonin group. Administration of melatonin reverted the DENA induced alteration in GSH and GSH related enzyme activities in the liver [155] and inhibited DENA induced hepatocarcinogenesis in male Wistar rats [156]. Sugie et al. [157] reported that melatonin inhibited development of hepatocellular adenomas and adenocarcinomas induced by DENA and phenobarbital in both initiation and promotion phases of carcinogenesis.

Blask et al. [158] have shown that both physiological and pharmacological levels of melatonin exhibit substantial anticancer activity in tissue isolated rat hepatoma 7288 CTC via melatonin receptor-mediated blockade of tumor uptake of linoleic acid and its metabolism to the mitogenic signaling molecule 13-hydroxyoctadecadienoic acid. Melatonin receptor antagonist S20928 completely blocked the effects and prevented the intratumoral accumulation of melatonin.

#### 8.6. Melatonin and lung carcinogenesis

Female A/J were injected subcutaneously with a single dose of urethane at 49 days of age and injected with 0.1 mg melatonin solution in aqueous ethanol 5 times per week [149]. The mice given urethane and melatonin displayed insignificantly fewer pulmonary tumors, than the controls on urethane alone, after an 18 week period.

In our experiments, SHR/u mice at the age of 3 months were given a single i.p. injection of 1 mg/kg urethane and a part of mice was exposed to melatonin (2 or 20  $\mu\text{g/ml}$ ) in night drinking water [159]. At the 28 week after the injection of the carcinogen the experiment was terminated. The number of lung adenomas was  $23 \pm 2.63$  per mouse in the groups treated with urethane alone,  $11 \pm 0.72$  in the group exposed to lower dose of urethane and  $14 \pm 1.2$  in the group treated with 20  $\mu\text{g/ml}$  melatonin. Serum malonic dialdehyde level was increased by 62% in mice exposed to urethane alone as compared to intact mice, whereas treatment with both doses of melatonin decreased serum MDA level in urethane treated animals.

#### 8.7. Melatonin and skin carcinogenesis

The effect of melatonin on benzo(a)pyrene (BP)-induced two stage skin carcinogenesis in mice was evaluated [160]. It was observed that it cannot only decrease the number of animals bearing papillomas but also the number of papillomas per animal both in the initiation and promotion stages of skin carcinogenesis. It was also found that melatonin treated animals have low levels of lipid peroxides and that it can also prevent the binding of BP or its metabolites to DNA.

#### 8.8. Melatonin and carcinogenesis in subcutaneous tissues

Daily melatonin injections (50  $\mu\text{g/day}$ ) following the induction of fibrosarcomas in DBF1 mice with 3-methylcholanthrene

results in a suppression of tumorigenesis during the early stages of carcinogenesis [161].

Three-month-old Swiss-derived SHR/u mice were subcutaneously injected with 2 mg of BP dissolved in 0.1 ml of olive oil. After the injections of the carcinogen two groups of mice were given melatonin with night drinking water at the doses of 2 mg/l or 20 mg/l and one group of mice was not treated with melatonin and served as a control [162]. At the 28th week after the carcinogen administration the experiment was stopped and animals were sacrificed. The results show that melatonin treatment inhibits BP-induced carcinogenesis, decreases the incidence of subcutaneous sarcomas, increases their latency and survival of mice. The level of MDA in the serum of BP-induced tumor-bearing mice was increased 2.6 times ( $p < 0.01$ ) and in the tumors was increased by 11.1% ( $p < 0.01$ ) as compared to intact control mice. Treatment with melatonin significantly decreased the level MDA both in the serum and in the tumor tissue. The activity of catalase in the serum of BP-induced tumor-bearing mice was increased by 12.1% as compared to the intact control mice ( $p < 0.01$ ) and was unchanged in the tumor tissue. Treatment with melatonin at the dose 2 mg/l significantly decreased activity of catalase in the serum (by 31.7%,  $p < 0.01$ ) and in the tumor tissue (by 2.6 times,  $p < 0.01$ ) as compared to the animals treated with BP alone. Thus it was shown inhibitory effect of melatonin on malignancies of mesenchymal origin induced by BP, and have shown that the lower dose of melatonin appeared to be more effective in the inhibition of lipid peroxidation and tumorigenesis induced by chemical carcinogen than higher one.

In Table 3, we summarized available data on effect of melatonin on carcinogenesis.

#### 8.9. Effect of melatonin on transplantable tumors in vivo and tumor growth in vitro

There are a number reports on inhibitory effect of melatonin on the growth of tumors in vitro (Table 4) and growth transplantable tumors of different histogenesis in vivo (for review see [113,115,163]).

#### 8.10. Effect of melatonin in cancer patients

The results of clinical application of melatonin in cancer patients comprehensively reviewed in several recent publications [113–115,164–166,183–185]. Mills et al. [17] conducted a systematic review of 10 randomized controlled trials of melatonin in solid cancer patients and its effect on survival at 1 year. The reports included totally 643 patients. It was shown that melatonin reduced the risk of death at 1 year (relative risk: 0.66, 95% CI=0.59–0.73). Effects were consistent across melatonin dose, and type of cancer. It is worthy to note that no severe adverse events were reported.

### 9. Effect of melatonin effects on glucose and lipid metabolism

The insulin/insulin-like growth factor-1 (IGF-1) signaling pathway plays a fundamental role in animal physiology,

Table 3  
Summary on anticarcinogenic effect of melatonin in rodents

Target tissue	Tumor type	Strain	Carcinogen	References
<i>Mice</i>				
Mammary gland	Adenocarcinoma	SHR	Spontaneous	[103]
		C3H/Jax	MMTV	[98]
			ras-MMTV-LTR <sup>a</sup>	[141]
		TG.NK	MMTV/c-neu <sup>a</sup>	[142]
Uterine cervix and vagina	Squamous-cell papilloma and carcinoma	FVB/N	HER-2/neu <sup>a</sup>	[143]
		CBA	DMBA	[151]
Skin	Papilloma	Swiss	Benzo[a]pyrene + croton oil	[160]
Subcutaneous tissue	Sarcoma	DBF1	3-Methylcholanthrene	[161]
		SHR/u	Benzo[a]pyrene	[117]
Lung	Adenoma	A/J	Urethane	[149]
		SHR/u	Urethane	[162]
<i>Rats</i>				
Mammary gland	Mastopathy Adenocarcinoma	Outbred	Spontaneous	[107,186]
		Sprague–Dawley; Holzman	DMBA	[128,129,134,135]
		Sprague–Dawley; LIO	NMU	[130,131,137,187]
Liver	Preneoplastic liver foci (GST-positive) Hepatocarcinoma	Wistar:Han	$\gamma$ -rays + DMBA	[188]
		F344	DENA	[154]
Endometrium	Adenocarcinoma	Wistar	DENA	[155–157]
Colon	Adenocarcinoma Foci of aberrant crypts	BDII/Han	Spontaneous	[153]
		LIO	DMH	[145]
		F344	Azoxymethane	[149]

<sup>a</sup> Transgenic mice.

influencing longevity, reproduction, and diapause in many species [189,190]. Despite a large number of studies, the role of melatonin on glucose metabolism is rather controversial. There are reports that a lack of melatonin has effects on the action of insulin in rats [191], on the stimulation of blood glucose and insulin levels in rats [192], and causes a marked decrease of insulin secretion by rat pancreatic islets in response to glucose in vitro [193]. In postmenopausal women, the oral administration of 1 mg of melatonin reduced glucose tolerance and insulin sensitivity [194]. Melatonin administration at middle age decreased visceral fat, plasma insulin and IGF-1 levels in rats [195]. It also enhanced the utilization of liver carbohydrates but suppressed hepatic lipolysis [196]. Long-term treatment with melatonin reduced hyperinsulinemia, hypertriglyceridemia, hyperleptinemia and restored hepatic delta-5 desaturase activity in type 2 diabetic rats [197]. The administration of melatonin to normal rats resulted in significant decreases in serum leptin levels compared to those of control animals, whereas a pinealectomy significantly increased it level [198]. Melatonin significantly augmented the level of total, free, and esterified cholesterol, as well as high-density lipoprotein cholesterol in rat blood [192]. Supplementation of the diet with melatonin led to an increase in the surface area of atherosclerotic lesions in the proximal aorta of hypercholesterinemic mice and increased the susceptibility to ex vivo oxidation of atherogenic lipoproteins isolated from the plasma during the fasting period [28]. However, there are reports on the inhibitory effect of high doses of melatonin on the oxidation of low density lipids in rodents and humans [199,200]. Thus, these observations suggest that caution should be taken with high melatonin dose supplementation.

Further investigations should be performed on animals and humans to clarify the effect of melatonin on carbohydrate and lipid metabolism.

## 10. Effect of melatonin on immune system

Immunopharmacological activity of melatonin has been demonstrated in various experimental models [201–203]. In 1986, Maestroni et al. [204] first showed that inhibition of melatonin synthesis causes inhibition of cellular and humoral responses in mice. Melatonin receptors are detectable in monocyte/macrophage lineage and melatonin binding to these receptors stimulates the production of GM-CFU cells [203]. Melatonin stimulates the production of progenitor cells for granulocytes and macrophages (GM-CFU) and has a general stimulatory action on hemopoiesis [201–203]. The action of melatonin on monocyte production can be partly due to its direct action on melatonin receptors or may be due to an increase of monocyte sensitivity to stimulants like IL-3, IL-4, IL-6 or GM-colony stimulating factor (GM-CSF).

Treatment with melatonin increases production of antibodies to sheep erythrocytes and immune response to primary immunization with T-dependent antigen [205]. There is evidence of an involvement of melatonin in complex relationships between nervous and endocrine system [201,206,207]. There are melatonin membrane receptors on  $\hat{O}$ -helpers (Th). Activation of melatonin receptors leads to the increase of release of some type Th1 cytokines, including  $\gamma$ -interferon, interleukin-1 and opioid cytokines related to interleukin-4 and dinorphine [201,207]. Melatonin stimulates production of interleukines-1, -6 and -12 in

Table 4  
Effect of melatonin on tumor growth in vitro

Tumor strain	Effective dose of melatonin	Effect of melatonin	References
Human breast carcinoma MCF-7	1 nM	↓	[121,164]
Murine melanoma M2R	0.1–10 nM	↓	[165]
Murine melanoma B16	10 <sup>-3</sup> M	↓	[166]
Hamster melanoma B7	μM	↓	[167]
Human melanoma	μM	↓	[168]
Primary human melanoma cell line	0.001 pm–10 μM	↓	[169]
Ovarian carcinomas SK-OV-3 and JA-1	mM	↓	[170]
Human serose cystadenocarcinoma KF	200 μM	↑	[171]
Ovarian carcinomaEFO-27	0.1 mM–0.1 pM	↓	[172]
Human endometrial carcinoma RL95-2	mM	↓	[173]
Human cervical carcinoma HeLa	1 nM	=	[174,175]
	<1 nM	=	
Human cervical carcinoma ME-180	100 μM	↓	[176]
	2 μM	=	[176]
	2 mM	↓	
Trophoblastic tumor JAR	4–500 μM	↓	[177]
Human prostate cell line LNCaP	mM	↓	[178]
Hamster transplantable hepatoma	mM	=	[175]
Human neuroblastoma SK-N-SH	10 pM–1 nM	↓	[164]
Rat pituitary prolactinoma	0.1 μM–10 pM	↓	[179]
Rat DES-induced prolactinoma	10 <sup>-7</sup> –10 <sup>-11</sup> M	↓	[112]
Human epidermoid carcinoma	<1 mM	=	[174]
Human colon carcinoma DLD-1	10 <sup>-4</sup> M	=	[112]
Human colon carcinoma CaCo-2	10 <sup>-3</sup> M	↓	[150]
Human transition-cell bladder carcinoma RT112	0.5 mM	↓	[180]
Rat pheochromocytoma PC12	1–10 nM	↓	[181]
	10 μM	↑	
Erhythroleukemia K562	MM	↓	[172]
Sarcoma-180	MM	Weak effect	[177]
Murine lymphoid tumor	MM	Weak effect	[177]
Human myeloma	200 μM	↑	[182]
Human lymphoblastoid TK6 cells	0.1 μM–0.1 nM	=	[175]
Human osteogenic sarcoma MG-63	0.1 μM–0.1 nM	=	[175]

Note: ↓ — inhibition; ↑ — stimulation; = — no effect.

human monocytes. These mediators can prevent stress-induced immunodepression defending mice from virus- and bacteria-induced lethal disease [207]. Important chain in mechanism of influence of melatonin on hemopoiesis includes the effect of melatonin-induced opioids on κ-opioid receptors at stromal macrophages of bone marrow [207]. It is worthy to note, that γ-interferon and colony-stimulating factors (CSFs) can modulate a production of melatonin in the pineal gland [207]. The loss of thymocytes with age is the main cause of structural thymic

atrophy and thymic weight loss. Treatment with melatonin inhibits apoptosis in thymocytes and increased their total number in old mice [208,209]. The reversal of age-associated thymic involution by melatonin added further support to the concept that melatonin can be a potential therapeutic agent for correcting immunodeficiency state associated with aging and possibly other immunocompromised states like severe stress [203]. Thus, melatonin it was shown has rather immunostimulatory potential.

## 11. Conclusion

The analysis of available data on the effect of melatonin on longevity support the hypothesis of a geroprotective effect of melatonin. At the same time, a critical review of real results has shown that the majority of studies are invalid when considering the current guidelines for long-term testing of chemicals for carcinogenic safety [15,16] and, to some extent, from the point of view of the correctness of the gerontological experiment. In some experiments, melatonin was given to a small number of animals (from 10 to 20); the treatments started when the animals were old; the observations were stopped when the animals reached 50% mortality or at some other arbitrary time, but not at the natural death of the last survivor; an autopsy and correct pathomorphological examination was not always performed; the body weight and food consumption of the animals were not monitored; and so on. We believe that the study of long-term effects of melatonin at a variety of doses in different strains and species (e.g., in rats) will be useful for making a conclusion on its safety. In adequately designed experiments (50 animals per group), melatonin given during the night time in relatively small doses (2.5–3 mg/kg) delayed the onset of age-related disturbances in estrous function and increased survival of animals.

There is evidence on suppressive effect of melatonin on the development of spontaneous and chemically induced carcinogenesis at various sites in mice and rats as well as on the tumor growth in vitro. There are also observations of positive effect of melatonin in the treatment of advanced cancer patients [17,163,212]. Thus, melatonin is a potent geroprotector, anticarcinogen and antitumor compound. The results of the clinical administration of melatonin are promising [17,210–212].

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