Melatonin and Metformin Inhibit Skin Carcinogenesis and Lipid Peroxidation Induced by Benz(a)pyrene in Female Mice

T. A. Man'cheva, D. V. Demidov, N. A. Plotnikova, T. V. Kharitonova, I. V. Pashkevich, and V. N. Anisimov*

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Outbred female SHR mice (n=200) were divided at random into 4 groups, 50 per group. Benz(a)pyrene solution in acetone was applied onto a skin site on the back during 26 weeks. In parallel, the animals received melatonin, metformin, or both. Melatonin, metformin, and their combination promoted significant reduction of the number and size of skin tumors. In mice receiving no therapy, benz(a)pyrene applications increased the concentrations of malonic dialdehyde in the serum and skin tumor tissue in comparison with the concentrations in the sera and skin of intact mice. Melatonin, metformin, and their combination normalized LPO level.

Key Words: *metformin; melatonin; skin carcinogenesis; benz(a)pyrene; malonic dialdehyde; catalase*

In view of growing incidence of skin tumors during the last decades, the search for new means for prevention of skin tumors remains a pressing problem [10]. The formation of reactive oxygen species (ROS) is assumed to play an important role in the mechanisms of carcinogenesis and aging, while antioxidants can prevent these processes [2]. Antidiabetic biguanides (metformin, buformin, phenformin), apart from their hypoglycemic effects, can eliminate the signs of metabolic immunosuppression, which suggests their use in practical oncology for normalization of some metabolic disorders characteristic of cancer patients [8]. Melatonin is a hormone produced in the pineal gland mainly during the night hours; it is characterized by biorhythmological, antioxidant, immunomodulating, and oncostatic effects [6]. In addition, melatonin is one of the most potent endogenous absorbents of free radicals [6].

N. P. Ogarev Mordovian State University, Saransk; *N. N. Petrov Institute of Oncology, Federal Agency for Health Care and Social Development, St. Petersburg, Russia. ***Address for correspondence:*** aging@mail.ru. V. N. Anisimov

We studied combined effects of melatonin and metformin on carcinogenesis induced by benz(a)pyrene (BP) in the skin and the levels of free radical processes in mice.

MATERIALS AND METHODS

The study was carried out on outbred SHR female mice (n=220; 2 months, 20-22 g) from Laboratory Animal Breeding Center (Ufa). The animals were kept under standard vivarium conditions at 12:12 light:darkness regimen, with free access to water and granulated fodder. At the age of 3 months, 200 animals were divided at random into 4 groups, 50 per group. The remaining 20 mice served as intact control. Experimental animals received applications of 0.2 ml 0.05% BP solution (Fluka) in acetone. The agent was applied onto a shaven skin site 1.5-2.0 cm in diameter 2 times a week.

Group 1 animals (controls) received drinking water without drugs. Group 2 animals starting from the next day after the start of carcinogen application re-

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ceived 2 mg/liter melatonin (Sigma) with drinking water during the night hours (from 19.00 till 07.00). Melatonin was dissolved in several droplets of 96% ethanol and brought to the needed concentration with water. Group 3 animals received metformin (200 mg/liter; Siofor 500, Berlin-Chemie AG/Menarini Group) with drinking water throughout 24 h. Group 4 animals received metformin (200 mg/liter) during 24 h and melatonin (2 mg/liter) during night hours. Melatonin and metformin solutions were prepared daily *ex tempore*.

The animals were regularly examined and palpated 2 times weekly in order to detect tumors. The time of tumor detection, its location, and the size and degree of tumor nodule ulceration were recorded. The latent period of tumor development and animals' life-span after tumor appearance were determined. The duration of the experiment was 26 weeks. The mice surviving until this term were sacrificed by ether overdosage. All animals were autopsied and a thorough pathomorphological study was carried out. The skin and viscera were studied, skin tumors were counted and their sizes measured. All tumor nodes and tissues and organs with suspected tumors were resected and fixed in 10% neutral formalin. After routine histological processing, the tissues were embedded in paraffin. Histological sections were stained with hematoxylin and eosin and examined under a microscope. Tumors were classified according to IARC recommendations [12].

At the end of the experiment, the blood and skin tumor specimens were collected from 5-8 animals of each experimental group for biochemical studies. The intensity of LPO processes in the serum and tumor tissue homogenate was evaluated by MDA level, the function of antioxidant system was evaluated by catalase activity. In intact mice these parameters were studied in the serum and normal skin tissue homogenate.

The results were statistically processed using Student's t test, exact Fisher's test, and χ^2 test.

RESULTS

Tumor-like tuberous formations appeared at the site of carcinogen applications in experimental mice starting from week 12 of the experiment. The tumors, initially mobile, fixed to the underlying tissues as they increased in size, and were often ulcerated. Metformin treatment significantly reduced the development of multiple tumor nodes, while melatonin, metformin, and their combination reduced significantly the mean size of the tumors (Table 1). Both drugs alone and their combination were inessential for the mean latent period of tumor development and the mean life-span of animals with tumors.

Generation of ROS and LPO are the mechanisms of realization of the carcinogenic effect of BP. The time course of LPO activity in experimental animals was evaluated by MDA level determining the severity of pathological changes and by catalase activity reflecting protective characteristics of the organism. The levels of MDA increased significantly ($p<0.05$) in group 1 animals in comparison with intact mice: 2.1 times in the serum and 2.3 times in skin tumor tissue homogenate.

TABLE 1. Melatonin and Metformin Effects on the Development of Skin Tumors Induced by BP Applications in Mice ($M\pm m$)

Parameter	Group			
	1	2	3	4
Total number of animals	50	50	50	50
Effective number of animals*	46	47	49	45
Total number of animals with tumors(%**)	31 (67.4%)	25 (53.2%)	30 (61.2%)	20 (44.4%)$^{++}$
Number of animals with malignant tumors, squamous cell hornifying skin carcinomas (%**)	23 (50.0%)	16 (34.0%)	18 (36.7%)	11 (24.4%)$^{++}$
Number of animals with papillomas (%)	16 (34.8%)	13 (27.7%)	14 (28.6%)	12 (26.7%)
Mean number of tumors per mouse with tumors	3.4±0.4	2.4±0.4	2.1±0.3^{+}	2.3±0.4
Mean diameter of tumors, cm	3.4±0.2	1.6±0.2^{+++}	2.1±0.2^{++}	2.0±0.3^{++}
Mean latent period of tumors, days	126.0±3.9	137.0±5.4	132.0±5.3	135.0±6.6
Mean life span of mice after tumor detection	96.0±2.5	100.0±2.5	103.0±2.5	103.0±3.5

Note. *Number of animals surviving till detection of the first tumor in experiment. **Incidence (from effective number of animals). Here and in Table 2: $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ compared group 1.

TABLE 2. Effect of Applications of BP Alone and in Combination with Melatonin and Metformin MDA and Catalase Levels in the Serum and Skin Tumor Tissue in Mice ($M \pm m$)

Group	MDA level, mmol/liter		Catalase activity, μcat/liter	
	serum	tumor tissue	serum	tumor tissue
Intact control	4.05±1.35	4.17±0.19*	1.97±0.11	2.73±0.03*
1	8.42±1.05^{o}	9.62±0.94^{oo}	3.03±1.13	2.52±0.11
2	1.53±0.14^{+++}	6.56±1.14	1.83±0.15	2.06±0.08^{ooo+}
3	1.72±0.15^{+++}	5.40±0.78^{++}	1.94±0.08	2.17±0.15^{o}
4	2.40±0.44^{+++x}	5.14±0.90^{++}	1.70±0.10	2.14±0.11^{o}

Note. *Level in normal skin homogenate. $^{o}p<0.05$, $^{oo}p<0.01$, $^{ooo}p<0.001$ compared to intact control; $^{x}p<0.05$ compared to group 2.

Serum levels of MDA in the group receiving melatonin were significantly lower in comparison with intact animals (2.6 times; $p<0.05$) and controls (5.5 times; $p<0.001$; Table 2). Metformin alone and in combination with melatonin led to a reduction of serum MDA level: 4.9 times in comparison with intact ($p<0.001$) and 3.5 times ($p<0.001$) compared to controls. A similar effect was observed in skin tumor tissue. Catalase activity decreased negligibly in the serum and significantly (though not much) in skin tumor tissue.

Hence, melatonin and metformin inhibited tumor growth induced by BP in mice, which manifested in lower incidence of development of all and malignant tumors and by lesser multiplicity of tumors and their smaller size. The greatest inhibitory effect was observed after combined melatonin and metformin treatment, resulting in a 2-fold lesser incidence of malignant tumors of the skin.

Analysis of the results and comparison of the parameters with the control group (induced carcinogenesis) suggest more marked corrective effect of combined therapy with endogenous hormone melatonin and antidiabetic biguanide metformin on LPO reactions in tumor tissue in comparison with monotherapies with these drugs. Previous study demonstrated an inhibitory effect of melatonin on skin tumor development on a two-staged carcinogenesis model, with applications of BP and croton oil in Swiss mice [9]. Daily melatonin treatment in a dose of 10 μg resulted in a 2-fold lesser incidence of skin papillomas and their lower multiplicity. In addition, that report described the development of skin papillomas in mice after applications of BP and croton oil. In our study, squamous cell carcinomas of the skin were diagnosed in the majority of cases. The differences were presumably explained by differences in carcinogenesis models.

The results of our experiments are in line with the data on melatonin and metformin inhibition of the development of tumors induced by various carcinogens and of spontaneous tumors in laboratory rodents [1,3,4,6,7,11] and indicate good prospects of their combined use [5].

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