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IMPACT OF MELATONIN ON IMMUNE SYSTEM ACTIVITY IN RATS UNDER HEAVY METAL POISONING

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One of the serious environmental issues the mankind is facing currently is the stable growth of heavy metal compounds found in the soil, water, and air in industrial cities and countries. Accumulation of xenobiotics in the environment is typically manifested through higher rate of general disease incidence with various somatic and immunologic pathologies.

The available literature offers a review of many works describing the effect of a particular metal on biological objects including the human body even though in reality people are subject to the influence of not one yet a set of pollutants, this combination varying among regions. The worst effect is found in anthropogenic factors acting jointly [2,3]. Many researchers have shown, both in vivo and in vitro, that a joint effect of toxicants of different origin may come along with additivity, synergism or antagonistic effects. Despite this there has been nearly no research into the joint effects, and the studies carried out now still have a long way to go.

There has been little attention paid to the chronic action toxicants taken in dosages that are not toxic. Long-term exposure to pollutants, even if taken in small concentrations shall result in the development of a new property in terms of the toxic effect manifestation, which is seen through nonspecific features that obviously being linked to the strain of the general protective homeostatic mechanisms maintaining a constant internal environment. This concerns, above all, the immune system responsiveness.

In view of the urgency there is obvious interest in search for, and study of natural body protection against such chemical aggression. The elimination of the disturbances caused by various xenobiotics including heavy metals can involve not only the liver and the immune system yet other organs and systems of the human body as well.

Among the natural factors protecting humans and animals against any stress including that of chemical origin the pineal gland and its biologically active products are of special interest due to their universal protective role. The stress protective activity of epiphyseal compounds is implemented via various mechanisms including through the immune system modulation [1] by penetrating different pathways of immunogenesis. The antitoxic activity found in the pineal hormone melatonin as well as its obvious immunotropic properties make it possible to consider the need of further study of this hormone viewing it as a potential agent to combat intoxication effects. Melatonin, as having no serious side effects and showing no toxicity of its own, may prove a rather valuable substance for toxicology practice.

Objective: to study the influence that the heavy metal salts mixture has on certain indicators of the immune system activity in experimental animals, as well as their correction with the pineal hormone melatonin.

Material and Methods. The experiment involved 40 white outbred male rats weighing 110–150 g each (March). The animals were divided into four groups: Group 1 (control) was given normal saline (intraperitoneal administration). Chronic intoxication was modeled by injecting metals combination into rats in Group 2, which was done for 10, 30 and 90 days. The metals ratio was around their average ratio in the environmental air and drinking water in Stavropol, namely: Cr : Mn : Ni: Pb : Fe : Cu : Zn=0.05 : 0.1 : 0.1 : 0.03 : 0.3 1: 5.0. The injected dosage contained each of the substances (mg/kg). Group 3 was given melatonin injected intraperitoneally as well, dosage 0.1 mg/kg, in the evening. The animals in Group 4 were injected a combination of heavy metals mixture and melatonin. The immunological examination was done by Level 1 tests. The leukocyte level and the differential blood counting were detected based on the commonly used clinical methods. The T-cell population was studied through the spontaneous erythrocyte rosette assay (by Mendes); the content of T-helpers and T-suppressors was studied employing the theophylline

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loading, while the B-lymphocytes population was investigated using the rosette assay with mouse erythrocytes. The levels of circulating immune complexes (CIC) was detected through photocolorimetry also using 3.5 and 7 % polyethylene glycol (M=6000).

The rats were kept in a vivarium under daylight and maximum standardized temperature and feeding modes with no limit to food and water. The results obtained were then made subject to statistical analysis of the variables using the Student's t-test.

Results and Discussion. The introduction of mixture of heavy metal salts in the concentrations similar to their content in the air and drinking water in Stavropol resulted in a significant reduction of the total leukocyte number, the absolute number of lymphocytes and monocytes (Table 1). There was a significant reduction in eosinophils observed, followed with their total elimination, which came along with an increase in the number of monocytes. The heavy metal salts caused a serious depression of the specific immunity as well, which was manifested, first of all, in a prominent fall in the total number of T-lymphocytes coinciding with a reduction in the number of both T-helpers and T-suppressors. A result of this was a tripled CD4/CD8 coefficient, which is an unfavorable sign indicating the severity of the process (Table 2).

Table 1

Effect of melatonin and heavy metal salts (combined) on the nonspecific blood immunity in rats

		Leukocytes ($x10^9$/l)	Lymphocytes	Monocytes	Neutrophils	Eosinophils
Normal saline	10	18.2±2.19	55.5±3.0 11.5±0.15	2.11±0.3 0.37±0.09	10.5±3.1 1.3+0.08	1.2±0.3 0.25±0.02
	30	13.5±1.25	62.1±2.7 9.7±0.12	2.15±0.2 0.41±0.04	12.2±1.5 1.1±0.2	1.5±0.2 0.22±0.01
	90	10.4±0.57	65.4±1.2 8.4±0.51	2.35±0.1 0.45±0.04	16.1±2.8 1.92±0.22	1.3±0.2 0.20±0.01
Heavy metal salts	10	7.7±0.64**	67±8.33 5.46±0.18**	3.50.±67 0.27±0.09**	26.1±4.9* 6.03+0.05	0.37+0.02** 0.028+0.001**
	30	6.31±4.02*	54.4±11.8 3.16±0.87**	7.4±1.6* 0.21±0.08**	29.1±4.3* 6.7+0.03	none none
	90	8.33±2.56*	51.6±13.7 4.15±0.5**	2.78±0.05 0.24±0.06**	31.3±5* 11.5±0.2**	none none
Melatonin	10	17.7±3.30	69±2.7 11.6±1.93	2.1±0.4 0.35±0.02	26.1±4.9* 4.6±0.08**	5.3±0.25* 0.087±0.009**
	30	18.2±2.03	70.3±1.87 12.8±0.96	2.5±0.27 0.44±0.06**	20±4.9 3.6±0.08**	5.3±0.22* 0.097±0.01**
	90	19±0.20	75±2.5* 13.25±0.8*	3±0.2* 0.49±0.01**	22.5±1.5* 3.37±0.2**	5.3±0.15* 0.093±0.01**
Melatonin + heavy metal salts	10	13.6±1.60##	60.3±3.7 8.14±0.2**##	3.36±0.6 0.48±0.02**#	35.5±4** 4.62±0.06**##	2.21±0.2**# 0.3±0.004**##
	30	15.5±1.25#	62.2±1.9 9.2±0.9*##	3.26±0.4*# 0.49±0.01**##	27.5±3.9* 4.2±0.1**	2.4±0.19** 0.35±0.02**
	90	17.3±2.42#	64.3±1.4 10.2±0.23##**	2.9±0.65 0.56±0.02##	12.1±1.4# 5.2±0.09##***	3.5±0.3*** 0.27±0.04

Note:* $p<0.05$; ** $p<0.001$; *** $p<0.001$ – significance in relation to control group;
– $p<0.001$ – in relation to the group where heavy metal salts were injected.
The upper Figures stand for relative values (%), the lower Figures are absolute values ($x10^9$/L)

Melatonin injection into the animals caused activation of both nonspecific and specific immunity, which revealed itself as an increase in the absolute number of lymphocytes, monocytes and eosinophils. Given the influence of melatonin the number of T-lymphocytes (Table 2) went up, which could be accounted for by an increased synthesis of T-suppressors under a relatively low level of T-helpers. As a result the ratio Th/Ts proved high.

When the heavy metal salts were used combined with melatonin, many immunological parameters went up to, or even exceeded the indices demonstrated in the control group within the same periods of observation (Table 1). The number of leukocytes, for instance, while increasing gradually, was much higher by the end of the experiment (17.3±2.42 vs 10.4±0.5 in the control). The absolute number of lymphocytes demonstrated a similar tendency (10.2±0.23 vs. 8.4±0.51). The absolute numbers of monocytes and neutrophils proved much higher in 10 days already this trend remaining in effect through the entire experiment. The eosinophils concentration remained within the control limits.

However, when comparing the indices in this group with those of the group where the animals were given only mixture of heavy metal salts, then a higher level of activation was observed both in the nonspecific (Table 1) and the specific immunity (Table 2). Thus, in 10 days already after the injections were started there was an increase in the number of leukocytes (13.6±1.6 vs 7.7+0.64), lymphocytes (8.14+0.2 vs 5.46+0.18) and monocytes (0.48+0.02 vs 0.35+0.02).

Table 2

Effect of melatonin and heavy metal salts (combined) on the specific blood immunity in rats

		T-lymphocytes	T-helpers	T-suppressors	CD4/CD8	B-lymphocytes	CIC
Normal saline	10	45.3±4.6 3.29±0.7	33.0±5 2.91±0.66	12.3±1 0.39±0.12	7.6±0.27	5.9±1.09 0.8±0.01	1±0.02
	30	42.3±2.7 2.95±0.2	34.2±2.4 2.54±0.25	10.3±2.1 0.32±0.14	7.9±0.37	6.3±0.92 0.7±0.05	1±0.03
	90	44.4±2.9 3.12±0.4	33.6±3.7 2.72±0.45	10.8±1.5 0.35±0.13	7.7±0.32	6.0±1.01 0.6±0.03	1.1±0.03
Heavy metal salts	10	32±4.7 1.46±0.46*	24.3±4.5 1.38±0.04*	7.5±1.2** 0.08±0.002*	17.2±0.12***	7.63±1.98 0.7±0.03**	2.45±0.41**
	30	30±3.9 1.31±0.12*	23.4±4.2 1.18±0.02*	7.2±1.09* 0.06±0.003*	18.1±2.5***	7.9±2.1 0.52±0.01**	2.45±0.32**
	90	25.9±2.7 1.1±0.09**	20.2±3.1 0.9±0.08**	6.8±0.07** 0.04±0.001**	22.5±5.3*	8.2±1.4 0.45+0.04**	2.8±0.09**
Melatonin	10	34.8±+5.5 8.85±0.11**	28.6±5.8 2.8±0.4	16.2±1.12* 0.7±0.03	3.9±0.6**	10.7±1.75* 1.74±0.42*	1.4±0.05**
	30	39.2±4.8 8.92±0.21**	27.7±7.2 2.4±0.35	16.8±1.43* 0.67±0.01**	3.5±0.82***	9.2±0.7** 1.5±0.35	1.4±0.03**
	90	42.1±3.6 9.3±0.33**	30.1±7.2 2.5±0.46	16.5±1.12* 0.66±0.01**	3.78±0.7**	6.78±0.91 1.3±0.09*	1.2±0.05**
Melatonin + heavy metal salts	10	34.7±4.8 2.45±0.58	22.85±4.3 1.77±0.4	6.69±1.7** 0.68±0.03**##	2.6±0.68***##	4.92±0.91 0.79±0.21	2.75±0.4***
	30	37±3.9 2.74±0.8	27.5±3.3 2.1±0.12##	8.91±2.4 0.72±0.01**##	2.9±0.16***##	4.7±0.62 0.7±0.12**	1.9±0.12**
	90	42+5# 2.91+0.65#	28.5+2.9 2.2+0.09##	10.2+1.2# 0.7+0.06**##	3.1+0.07***#	5.2+0.9 0.79+0.01##	1.2+0.05##

Note:* p<0.05; ** p<0.01; *** p<0.001 – significance in relation to control group; # – p<0,01; ## – p<0,001 – in relation to the group where heavy metal salts were injected.

As for T-, and B-lymphocytes, the upper Figures stand for relative values (%), the lower Figures are absolute values ($x10^9$/L)

A study of the activity in the cell-mediated immunity (Table 1) showed that after 10 days already a combination of melatonin with the heavy metal salts led to a sharp increase in the absolute number of T-suppressors (0.68±0.03) if compared to the control group of animals (0.39±0.12, $p<0.001$). The content of this subpopulation was higher, too, when compared to the Group 2 data. Here, after 10 days of injections the number of T-suppressors grew by 8.5 times while by the end of the experiment it was 17.5 times as high. Note to be made here that an increase in the number of T-helpers was significant when compared to that in the rats injected with heavy metal salts after one month only (Table 2).

A study of the humoral immunity showed that introduction of melatonin could mitigate the effect caused by the heavy metal salts on Day 90 only, and this effect in question was seen through an increase in B-lymphocytes (by 76 %).

Of undisputed interest was the CIC dynamics, which has gained serious importance nowadays as an index of lesion in the hepatobiliary system. In particular, the heavy metal salts, as observed earlier, raised the CIC level significantly. The melatonin injections, too, increased the number of CIC. A combination of the heavy metal salts mixture together with melatonin, in the first days of the experiment, produced no significant shifts in the CIC. The CIC number, when compared to that of the control group remained high: 2.75±0.4 (in the control group 1±0.02, after 10 days) and 1.9±0.12 (1±0.03, after 30 days). Yet, on Day 90 of observation, the number of CIC went down by 57 % if compared to the index found in the animals that were injected with the heavy metal salts mixture alone, while these indices pulled close towards the control Figures.

This all allows noting that chronic administration of melatonin in the dosage as used shall mitigate the toxic effect that the heavy metal salts have on cellular and humoral immunity.

The facts above suggest some modulatory potential that melatonin has on the immune status as a response to the presence of heavy metals. All the immune activities in the body are known to go on with some metals involved so their imbalance might cause certain immunopathologies. For instance, cadmium chloride injected into mice daily in the dosage of 1 mg/kg for five days results in an increased number of B-cells, which produce IgM and IgG at the same time reducing the cell activity in a mixed lymphocyte culture [8]. Similar effects were observed in people – an examination of over 5,000 German children residing in industrial centers showed that heavy metals pollution leads to lymphocytopenia and tonsil expansion [10].

Meanwhile, pineal gland was found to have some immunomodulatory properties, due to which its biologically active compounds amplify the immune reactivity [12,13]. Low dosages of melatonin sharply decrease disturbed production of antibodies, thymus mass reduction, and antiviral resistance, all these associated with stress. At the same time pinealectomy exacerbates immunological defects originating from stress [7]. Tests involving human being yielded similar outcomes. In response to repeated hormone injection to stressed volunteers they revealed a growth in the number of natural killers and null cells [6]. The data offered in the relevant literature well coincide with the outcomes we obtained from our experiments, namely where immune suppression due to heavy metal salts can be reduced with melatonin. Rehabilitation of immune responsiveness by melatonin must be accounted for by several reasons, and can be due to both direct and indirect intervention into the function of immunocompetent cells.

The evidence of the direct effects caused by melatonin can be seen from the experiments involving cultures of human and murine lymphoid cells, whose membranes were shown to contain hormone's specific binding sites. As is seen from the data obtained through mice macrophages the affinity of the mentioned binding sites is rather high to respond to the hormone's low plasma concentration [4].

There is also supposed to be another, secondary, type of immunomodulatory effects in melatonin, in particular pertaining to its antioxidant properties. Incubation of primary culture of rat hepatocytes with chromium has been shown to reduce the cellular DNA content, while melatonin, when added, restricted the cytotoxic effect of the dichromate [11]. In vitro Fe increases significantly the lipid peroxidation in the liver homogenate, while melatonin reduces the disturbances [5]. Melatonin could be possibly employed as a trap for free radicals.

Apart from the potent antioxidant activity a number of authors point at the chelating ability of melatonin. It has been shown that in the culture of pineal gland cells the N-acetylserotonin levels were lower than in the control group after 6 weeks of copper injection. Melatonin prevented N-acetyltransferase inhibition caused by copper and acted as a chelator. Melatonin is capable of binding with such metals as Cu, Fe^{3+} (not Fe^{2+} though), Zn, Pb [9].

There is every reason to believe that when accumulated the metals lead to a changed activity in enzymes responsible for xenobiotics metabolism, destroy the the integrity of the cell membranes, and stimulate lipid peroxidation, while pineal gland melatonin, given its antioxidant and chelating activity, is capable of reducing the toxic effect of heavy metals.

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A set of heavy metal salts cause immunosuppression in rats while the pineal hormone melatonin can mitigate the resulting alterations due to its antioxidant and chelating activity.

Key words: heavy metals, immune system, melatonin

ВЛИЯНИЕ МЕЛАТОНИНА НА АКТИВНОСТЬ ИММУННОЙ СИСТЕМЫ КРЫС В УСЛОВИЯХ ИНТОКСИКАЦИИ ТЯЖЕЛЫМИ МЕТАЛЛАМИ

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Комплексы солей тяжелых металлов вызывают супрессию иммунной системы у крыс, а эпифизарный гормон мелатонин за счет своих антиоксидантных и хелатирующих свойств способен ослаблять возникшие сдвиги.

Ключевые слова: тяжелые металлы, иммунная система, мелатонин