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Original Research

The Effect of Melatonin Supplementation on Lead, Calcium and Magnesium Distribution in the Tissues of Lead-Exposed Rats

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Abstract

The aim of this study was to determine whether oral supplementation of melatonin (M), a known antioxidant, free radical scavenger and metal chelator, influences lead (Pb), calcium (Ca) and magnesium (Mg) distribution in the blood, bones and teeth of rats exposed chronically to lead. The studies were carried out on male Wistar rats which, from their birth until reaching sexual maturity (for 3 months), drank water containing 1% lead (II) acetate and/or received melatonin in feed (1mg/ kg body wt.) over a 3-month period. Concentrations of Pb, Ca and Mg were analyzed by atomic absorption spectrometry (AAS). Exposure to Pb and Pb plus M resulted in a significant increase in the Pb concentration in the whole blood (but below the threshold level) and bones in both groups.

In rats chronically exposed to lead during their fast development (from birth until reaching sexual maturity), melatonin supplementation did not cause a significant decrease in Pb concentration in whole blood and bones. No changes were observed in blood plasma Ca and Mg concentrations in rats exposed to lead and treated with melatonin. However, their bones were observed to have a lower Ca concentration and higher Mg concentration, and their teeth higher Ca and Mg concentrations.

Keywords*:* lead, calcium, magnesium, bones, teeth, melatonin supplementation

Introduction

The uptake of lead from various sources suggests three compartmental pools for lead metabolism, namely whole blood lead (Pb-B), lead accumulated in soft tissues (collagen and keratin being the target proteins) and the bones, where lead appears to compete with calcium and magnesium for binding sites [1].

Most of the body lead burden in children is contained within the bones (>70%). Because lead is qualitatively a biological analog to calcium, its uptake and release from bones is partly controlled by processes affecting bone growth and turnover [2, 3].

In adults, bone lead (> 90%) is contained within long-lived compartments of cortical (Pb half-life 5-10 years) and trabecular (Pb half-life 1 year) bone, with comparatively small amounts of lead in soft tissues that rapidly exchange lead with extracellular fluid and plasma [2-4].

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The extent of accumulation depends on the blood supply of the tissues and their lead affinity (lead being bound by certain proteins) [5]. In lasting lead exposure of a constant rate, there appears a state of balance among the amounts of lead intake stored and excreted [6].

Due to the fact that the presently used chelating agents that eliminate lead from the system cause side effects (especially in children) [7, 8], it is important to continue studies on new compounds that may enhance the elimination process. It is especially important when the blood lead concentration in children is below 10 μg/dL (still considered harmless) because there are studies reporting neurotoxic lead activity below this threshold [9-11].

Moreover, the results of several cross-sectional studies have indicated that lead toxicity seems to be a result of metabolic disorders, which are connected with a disruption in the balance between pro- and anti-oxidants [12]. At the same time, some studies indicate the protective influence of melatonin in ROS-affected cells [13-16]. Melatonin (5-methoxy-N-acetyltryptamine), a biologically active indol derivative produced by the pineal gland, not only neutralizes ROS, but also regulates the activity of many enzymes responsible for ROS removal. Melatonin also participates in cell detoxification through the regulation of gene expression in some enzymes responsible for ROS excretion, and through the inhibition of enzymes responsible for ROS production [17-19].

Literature on the subject describes many advantages of melatonin supplementation. A wide range of action for this hormone makes it possible to use it in cancer therapy, Alzheimer's disease, Parkinson's disease and diabetes [20]. The prophylactic effect of melatonin in reducing lead-induced toxicity has also been examined by many authors [21-26]. Yet there is little data on the influence of orally administered melatonin on the mineral metabolism of lead-exposed infants (in low concentrations, corresponding to environmental conditions) during their fast development. It seems that because of the ability of melatonin to freely cross all barriers in the human body (cellular membranes, blood-brain barrier), and the wide range of its activity and effectiveness as an anti-oxidant, it could create great new possibilities for negating the effects of lead toxicity.

Therefore, the main purpose of the present work was to determine the effect of oral, low-dose melatonin administration (1mg/ kg body weight.) on lead, calcium and magnesium distribution in the blood, bones and teeth of rats which, from their birth until 3 months old, drank water containing 1% lead acetate.

Materials and Methods

Animals

Pregnant female Wistar rats were purchased from the Nofer Institute of Occupational Medicine, Łódź, Poland. In this study we used newborn males until they reached sexual maturity (3 months old) and a body weight of 250g ± 20 g. For the first two weeks after their birth, the newborns were kept in cages with their mothers and were fed by them. Lactating mothers were kept with the examined and control groups and respectively treated with lead and melatonin. Then, after these 14 days, the young rats were placed in separate cages and ate and drank on their own.

All animals were housed in plastic cages and kept on a 12h light/dark cycle under standard conditions. The animals had free access to rodent chow and water. They were randomly divided into groups for the experiment. The studies were conducted according to the following scheme:

K- control group (n=10) – was given drinking water *ad libitum* from birth for the 3 months of the experiment;

Pb – research group (n=10) – was given *ad libitum* 1% lead acetate drinking water solution from birth for the 3 months of the experiment;

Pb+M – research group (n=10) – were given *ad libitum* 1% lead acetate drinking water and melatonin in feed (1mg/ kg body wt.) over the 3-month period.

M – research group (n=10) was given melatonin (1mg/ kg body wt.) in feed from birth for the 3 months of the experiment.

In all the groups, the animals drank from 30 to 50 ml of water or lead acetate solution per 24 hours. Melatonin was given once per day (in the evening) in the form of powder supplementation in their feed. The amount of water consumed did not differ between the groups. After the 3 months of the experiment, the animals were anaesthetised with pentabarbitone sodium given intraperitoneally in a dose of 60 mg/kg body weight (Biochemie GmbH-Austria), and exsanguinated by cutting the apex cordis. Then the abdominal cavity and chest were opened and bones and teeth were taken. The experimental protocols were approved by the local ethical committee for performing an experimental study on laboratory animals.

Chemicals

For determination of Pb in whole blood, gradient-grade ammonium pyrrolidinedithiocarbamate (APDC), Triton X-100 and isobutyl methyl ketone (IMBK) were obtained from Sigma Chemical Co. For determination of magnesium and calcium nitric acid, lanthanum, strontium was purchased from Merck. Water was filtered thorough a Milli-Q Millipore purification system. All solvents used for HPLC determinations were filtered through 0.22-μm nylon filters (Supelco).

Determination of Lead in Blood and Bones

The blood was collected with single-use plastic Vacutainer-type equipment (Beckton Dickinson). Aliquots of blood serum were transferred to polystyrene tubes using a polyethylene transfer pipet (Eppendorf) for determination of magnesium and calcium. Whole blood (2.5 mL)

was treated with Triton X-100 and extracted into IMBK for lead determination by atomic absorption, following the method of Westerlund–Helmerson [13]. A calibration curve was prepared from known standards (Merck) in concentrations of 0 (background) and background plus 5, 10, 25, and 50 µg Pb/dL. Calibration was performed at the beginning and at the end of the assay. Three standards prepared by the Heavy Metal Toxicology Central Laboratory in Poland were used as the internal control. Quality control was carried out under certified Nycomed standards. A control assay was carried out on every 10 samples.

The bone samples were collected from the thighs of the animals, cleaned of soft tissue, and then frozen at -20^{0}C. In order to completely remove the residues of soft tissues, the bones were then individually boiled in water (Milipoor) (100^{0}C, 1h), with the water changed three times. The samples of bones and teeth were dry ashed at 450^{0}C in a muffle furnace and dissolved in 1M nitric acid (Merck). Concentrations of lead were analyzed by atomic absorption spectrometry AAS-Solar 969, in an air-acetylene flame at 217 nm. The tooth lead concentration was not determined.

Determination of Calcium and Magnesium in Blood Serum, Bones and Teeth

1 ml of the concentrated 65% HNO_3 was added to 10 mg of bone powder samples and 300 uL of serum samples. The samples were then left to solve for 24h at room temperature. From these solutions, 10 µl samples were poured into plastic test tubes, and then 5 ml of H_2O was added. The concentration of calcium and magnesium was determined with an atomic absorption spectrometer calibrated with standard solutions. The calibration curve was determined automatically by the computer connected to the spectrometer. The determination was carried out in an air-acetylene flame with lamps of the following wave lengths: Ca – 422.7 nm (0.5% solution of lanthanum was added as a buffer solution); Mg – 285.2 nm (0.1% solution of lanthanum or strontium was added as a buffer).

Statistical Analysis

Statistical analysis was conducted using v.6.1 of Statistica software. The arithmetical mean and standard deviation (SD) were found for each of the studied parameters. The distribution of results for individual variables was obtained with the Shapiro-Wilk W test. As most of the distributions deviated from the normal Gauss distribution, non-parametric tests were used for further analyses. To assess the differences between the studied groups, the non-parametric Kruskal-Walis ANOVA and the U Mann-Whitney test were used. Correlations between the changes of the parameters were examined with the Spearman's rank correlation coefficient. The level of significance was $p \leq 0.05$.

Results

There were no statistically significant differences between the body weights of 3-month old mature males from the control and experimental groups.

Lead in the Blood and Bones

The exposure of rats to a lead acetate solution in drinking water and to a lead acetate solution in drinking water together with melatonin, resulted in a significant increase in lead concentration in the whole blood and bones of both groups compared with the control group (Table 1). The observed Pb-B concentration in the group that was given melatonin supplementation was about 7% lower that in the group exposed only to lead, but it was not statistically significant. A similar relationship was also observed in bones.

In the study group which drank only a lead acetate solution (Pb), the blood-to-bones ratio was 2.36. In the group with supplementation (Pb+M), it was 2.41 and (M) 2.58. In the control group (K), the ratio was 2.30. The differences were statistically insignificant. The mean lead concentration in the whole blood also correlated with the mean concentration in the bones of the Pb group (rs=+0.62) and Pb+M group (rs=+0.65).

Calcium and Magnesium in the Serum, Bones and Teeth

Serum

No statistically significant change in the Ca concentration in the serum of the lead-exposed animals (Pb) was

Table 1. Concentrations of lead in the whole blood (Pb–B) and bones of rats.

Group	Blood lead (µg/dL)	Bones lead (mg/g dry mass)
Control (K)	1.40 ±0.55	0.60±0.10
Melatonin (M)	1.42 ±0.65	0.55±0.15
Lead (Pb)	9.45±1.35 *	4.00±0.65*
Lead + Melatonin (Pb+M)	9.38±1.23*	3.90±0.53*

The control group K – was given drinking water *ad libitum* for the 3 months of the experiment; M – research group – **melatonin** (1mg/kg b.w./day) was given in feed for the 3 months of the experiment; Pb – research group – was given *ad libitum* 1% (w/v) $(CH_3COO)_2Pb$ drinking water solution for the 3 months of the experiment; Pb+M – research group was given *ad libitum* 1% (w/v) $(CH_3COO)_2Pb$ drinking water and melatonin (1mg/kg b.w./day) was given in feed. Arrows shows intervals with statistically significant differences. Lead concentrations in teeth were not determined. * $p<0.05$ vs. control group.

observed in comparison with the control. For the rats that received only melatonin (M), we observed a significantly higher Ca concentration in the serum compared with the control, but in the group receiving lead and melatonin (PbM), the Ca concentration was similar to the control. (Fig.1)

In addition, no statistically significant change in the Mg concentration in the serum of the lead-exposed rats (Pb) was observed in comparison with the control group. The Mg concentration was statistically significantly higher in the group of rats receiving melatonin compared with control (K), however no significant difference in Mg concentration was observed between the rats which received lead and melatonin, and the control (Fig. 2).

Bones

Calcium concentration in the bones was significantly lower in the group of rats receiving lead (Pb) than in the control (K). Also significantly lower was the Ca concentration in the bones of rats receiving melatonin (M) and in rats receiving lead and melatonin (Pb+M) compared with the control (K). (Fig.1).

No statistically significant difference was observed in Mg concentration in the bones of animals receiving lead (Pb) compared with the control. The Mg concentration in the bones of animals receiving melatonin (M) and treated with lead and melatonin (Pb+M) was also significantly higher than in the control (K) (Fig.2).

Calcium concentration in the bones was strongly negatively correlated with Pb concentration in the bones, both in rats treated with lead (Pb) rs=-0.75 and those treated with lead and melatonin (Pb+M) rs=-0.68. We also observed a strong negative correlation between Pb concentration in the whole blood and Ca concentration in the bones of rats that received lead (Pb) rs= -0.78 and the rats treated with lead and melatonin (Pb+M) rs=-0.65.

Mg concentration in the bones strongly positively correlated with Pb concentration in the bones of rats treated with lead and melatonin (Pb+M) rs=+0.65. A weak posi-

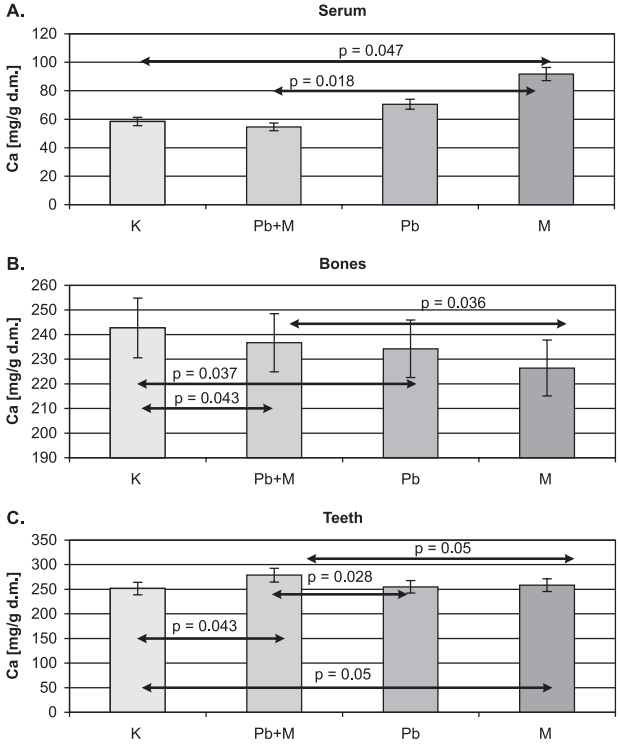

Fig. 1. Mean content of calcium (mg/g d.m) in the rats' serum (A), bone (B) and teeth (C) (depending on the group): control group K – was given drinking water *ad libitum* for the 3 months of the experiment; M – research group – melatonin (1mg/kg b.w./day) was given in feed for the 3 months of the experiment; Pb – research group – was given *ad libitum* 1% (w/v) $(CH_3COO)_2Pb$ drinking water solution for the 3 months of the experiment; Pb+M – research group was given *ad libitum* 1% (w/v) $(CH_3COO)_2Pb$ drinking water and melatonin (1mg/ kg body wt.) was given in feed. Arrows show intervals with statistically significant differences.

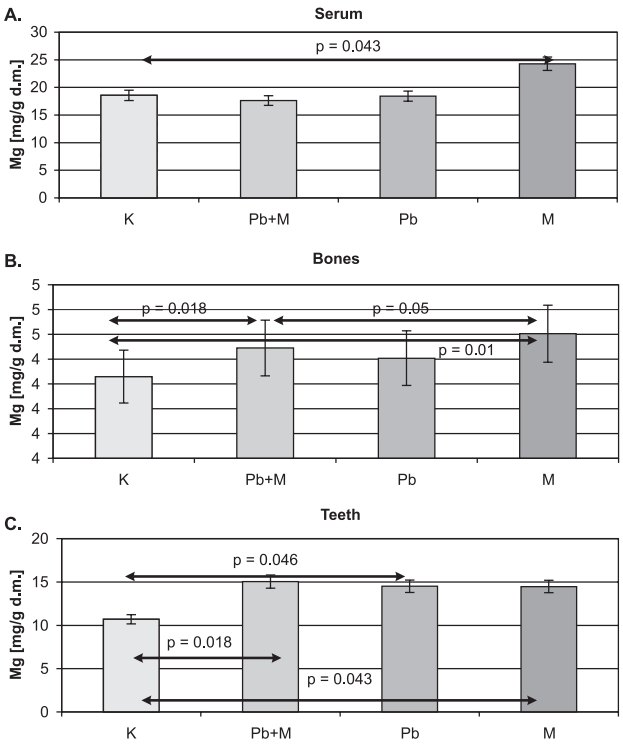

Fig. 2. Mean content of magnesium (mg/g d.m) in the rats' serum (A), bone (B) and teeth (C) (depending on the group): control group K – was given drinking water *ad libitum* for the 3 months of the experiment; M – research group – melatonin (1mg/kg b.w./day) was given in feed for the 3 months of the experiment; Pb – research group – was given *ad libitum* 1% (w/v) $(CH_3COO)_2Pb$ drinking water solution for the 3 months of the experiment; Pb+M – research group was given *ad libitum* 1% (w/v) $(CH_3COO)_2Pb$ drinking water and melatonin (1mg/ kg body wt.) was given in feed. Arrows show intervals with statistically significant differences.

tive correlation was found in rats treated with lead (Pb) rs=+0.41.

We also observed a strong positive correlation between Pb concentration in the whole blood of rats and Mg concentration in the bones of rats treated with lead and melatonin (Pb+M) rs=+0.70 and rats treated with melatonin (PbM) rs=+0.68.

Teeth

No statistically significant difference was observed in Ca concentration in the teeth of animals receiving lead (Pb) compared with the control. In the teeth of rats treated with melatonin (M), Ca concentration was significantly higher than the control (K). Ca concentration in the teeth of animals treated with lead and melatonin (Pb+M) was also significantly higher than in the control (K) (Fig.1).

Mg concentration in the teeth of rats treated with lead (Pb) was significantly higher than in the control group (K). We also observed a significantly higher Mg concentration in the teeth of rats treated with melatonin (M) compared with the control, and in rats receiving lead and melatonin (Pb+M), also compared with the control (Fig.2).

Discussion

Rats treated with a 1% (w/v) solution of lead acetate, and those which were simultaneously given melatonin supplementation, showed a significant increase in lead concentration in the whole blood, but nonetheless still below the threshold level of 9.94 µg lead per dL of blood [28-30], considered 'safe' by the CDC. However, in light of the latest reports, which indicate the neurotoxic activity of lead below this value, the CDC has issued a report in which it is recommended to launch protective measures even against concentrations lower than 5 µg/dL [31, 32].

As it has been mentioned earlier, researchers are still looking for an effective method of eliminating lead from organisms, especially from bones, with their long-term deposits of lead. This deposit may be mobilized quickly and return to the blood during a period of rapid growth and bone development in children, and during lactation, which may result in secondary contamination. Besides, many modern chelating agents used to eliminate lead from organisms cause side effects (especially in children) [7, 8].

Melatonin has been shown so far to protect against the toxicity of various chemicals, including carcinogens and chemotherapeutic agents [33]. The mechanism of melatonin protection has primarily been attributed to an inhibition of oxidative stress [13, 34]. The protective effect of melatonin in combating free radical-induced damage due to lead toxicity in the liver and kidneys of rats was investigated by El-Sokkary et al. [22]. Melatonin co-treatment (10 mg/kg body wt.) to lead administrated rats (100 mg/kg body wt.) for 30 days attenuated the increase of LPO (lipid peroxidation products) and restored the activity of SOD and levels of GSH in the studied tissues. Morphological damage in the liver and kidneys was also reduced.

Flora et al. [24] in their studies on lead-treated rats, examined the therapeutic efficacy of melatonin, the potent thiol-chelating agent meso 2,3-dimercatosuccinic acid (DMSA) and thiol containing antioxidant N-acetylcysteine (NAC), in reducing lead concentration in the blood and soft tissues. The authors observed that combined therapy with melatonin, antioxidant moiety and thiol-chelating agent, provided significant protection to lead disturbed antioxidant defence, decreasing thiobarbituric acid reactive substance (TBARS) levels, and increasing reduced glutatione (GSH) and oxidized glutathione (GSSG) contents in tissues.

In addition, El-Missiry et al. [25] reported that in rats which were injected intramuscularly with lead acetate (10mg/kg body weight for 7 days), daily pre-treatment with melatonin (30 mg/kg body weight) reduced the inhibitory effect of lead on glutathione reductase, glutathione-S-transferase, superoxide dismutase and catalase, as well as nonenzymatic antioxidants such as total sulfhydryl groups and glutathione. This was accompanied by a marked normalization of lipid peroxidation.

The prophylactic effect of melatonin in reducing lead-induced toxicity has been reported by many authors, especially in relation to its neurotoxic activity, as the nervous system is a primary target for low-levels of lead exposure, and the developing brain appears to be especially vulnerable to lead neurotoxicity. Chronic low-level lead exposure causes a significant increase in ROS, neuronal nitric-oxide synthetase, and intracellular calcium levels along with behavioural abnormalities in locomotor activity, learning, and memory that are accompanied by changes in neurotransmitter levels [21, 35-37]. The administration of melatonin to rats (10 mg/kg) exposed to lead (lead acetate given in 100mg/kg doses for 21 days) almost completely attenuated the increase in LPO products and restored GSH levels and SOD activity. In addition, morphological damage was reduced and neuronal density was restored by melatonin [37]. The protective effect of melatonin during exposure to low levels of Pb (0.01 to 10 µM for 48h) in human SH-SY5Y neuroblastoma cell cultures was also reported by Suresh 2006. Pre-treatment with melatonin (10 µM) blocked the effect of Pb on GSH content and caspase-3 activity, and showed significant improvement in reducing levels of prostaglandin E2. The author indicates that some of the neurotoxic effects of Pb may be partly mediated by apoptosis and that pre-treatment with melatonin can prevent these effects.

Some reports suggest another possible mechanism of melatonin influence, namely through decreasing heavy metal concentration in the soft tissues and bones of animals [38, 39]. Chwełatiuk et al. [38] observed that in mice which received drinking water containing 50µg Cd/mL, or 50µg Cd/mL with additional 2,4 or 6 µg/mL melatonin for 8 weeks, melatonin co-treatment caused a dose-dependent decrease in renal, hepatic and intestinal Cd concentration. The Cd and melatonin treatments did not

affect renal lipid peroxidation. The mechanism by which melatonin decreases heavy metal concentrations in the tissue is not known. One possibility is that melatonin, which is capable of forming stable complexes with e.g. Cd [38, 39], inhibits intestinal absorption of this metal, especially its uptake from the intestinal lumen into mucosa. Another possible explanation is that melatonin, which is highly lipid-soluble, can move freely across all cellular barriers, facilitating the removal of metal from soft tissues [40]. In an aforementioned report, El. Missiry et al. [25] observed that apart from influence on the activity of antioxidant enzymes, lead treatment caused a hepatic deficiency in copper and zinc accompanied by a significant elevation of lead concentration in both the plasma and liver. Pretreatment with intraperitoneal melatonin prevented the suppressive effects of lead on heme-synthesizing enzymes, and also prevented iron deficiency and changes in copper and zinc levels in the liver.

In our study, in rats simultaneously administered lead and melatonin, we found no significant differences in lead concentration in the whole blood and bones compared with only lead-treated rats. Moreover, mean lead concentration in the whole blood correlated with mean concentrations in the bones in both of these groups.

As is widely accepted, blood lead concentration reflects the present state of a dynamic equilibrium between the lead which permeates the system, which is absorbed by blood and deposited in soft tissues and bones. So far, no sufficient amount of data has been gathered to determine the exact quantities in these processes, and some data concerning the pharmacokinetics of the transfer of blood lead to other target organs and bones has been discussed by Hu et al. [2], O'Flaherty et al. [3], Silbergeld et al. [44], Rabinowitz et al. [45], Pounds et al. [46].

As is customary with most outcomes of lead intoxication, the effects are dependent upon lead dose, duration of exposure, dietary calcium and other trace elements [45, 46]. Furthermore, the manifestation of lead intoxication in the bone is undoubtedly the result of a complex interplay between systemic endocrine effects, cellular processes in the bone, and chemical processes in the bone matrix. Moreover, skeletal lead toxicity, altered bone mineral metabolism, and bone lead metabolism, must then be identified in the context of a complex regulatory system for bone (systemic regulators are: 1,25 dihydroxyvitamin D_3, parathyroid hormone, glucocorticoids, estrogen; local regulators as: cytokines, growth factors, prostaglandins) and bone minerals [46]. For a more complete review of lead and calciotropic hormones, including interactions of lead with the hormonal regulation of calcium absorption and plasma calcium, see review [44, 46].

In this study, we found that melatonin administered to rats exposed to lead did not significantly change the concentrations of calcium and magnesium in the blood serum, but significantly influenced Ca and Mg concentrations in the bones and teeth. We also observed a negative correlation between Pb concentration in the whole blood of rats and Ca concentration in the bones of both groups. Similar data with reference to calcium in the blood serum were obtained by Koo et al. [47], who studied 105 children, ages 1 to 3 with a detailed history of lead exposure since birth, to determine the effect of chronic low to moderate lead exposure. The average lifetime Pb-B concentration was 4.8-23.6 μg/dL and the current Pb-B concentration 6-44 μg/dL. The children generally had adequate dietary intakes of calcium, vitamin D, and phosphorous. With adequate nutrition, lead exposure at low levels appears to have no demonstrable effect on calcium, magnesium and calciotropic hormones including 1,25 dihydroxyvitamin D3 concentration in the blood serum, and bone mineral content. The significant effect of average lifetime Pb-B concentration was found only for phosphorous.

A decrease in Ca concentration of the bones observed in this study, with an unchanged Ca blood serum concentration, may have been due to the compensatory action of the aforementioned calciotropic hormones and the influence of lead on mineral metabolism, including magnesium in the bones, the increase of which we observed in both examined groups (though in rats treated only with Pb it was statistically insignificant). However, Heard [48] in his study with radioactive lead demonstrated that bone uptake of radioactive calcium was more rapid than lead uptake because, as Heard suggests, red blood cells have a stronger affinity for lead than for calcium. They also exhibit a considerably higher affinity for lead than for magnesium [49]. Simultaneously, blood lead is considered as the most labile compartment with a half-life of over 36 days, and bone lead as the most stable with a half-life of over 10 years.

In the blood serum, there occurs competition between magnesium and calcium concerning albumin bounding site, which

(1) first of all increases magnesium diffusing fraction, and

(2) consequently increases glomerular filtration, and

(3) then increases hypercalcuria – usually associated with a non-typical relative increase of calcium reabsorption in tubules, inhibiting competitive tubular reabsorption of magnesium [49].

It is also possible that magnesium is more quickly built into the bones than calcium or is also more labile [49].

In acute lead poisoning in rats as well as in lead poisoning due to work exposure in people, one may observe magnesuria, causing a decrease in magnesium concentration in the bones, liver and kidneys [30]. Interestingly, increased administration of magnesium decreases lead retention and increases its excretion. This action is less a result of decreased absorption of lead-influenced magnesium than the antagonism between both these elements. Some human and animal studies also show that orally administrated lead can decrease intestinal calcium and magnesium absorption and its concentration in the soft tissues, bones and teeth [41-43].

In our research, rats treated with lead and melatonin had increased Ca and Mg concentrations in the teeth in comparison with the control group. No significant differ-

ence in Ca concentration was observed in the bones and teeth of rats treated with lead and those treated with melatonin, but Mg concentration in the teeth of rats in both examined groups was significantly higher than in the bones. It seems that with chronic exposure at a constant level, we may observe a state of equilibrium between the amount of absorbed, deposited and excreted lead, and it may influence the distribution of other trace elements.

Conclusion

For rats chronically exposed to lead from birth until reaching sexual maturity, supplementation with melatonin did not cause any significant decrease in Pb concentration in the whole blood and bones. No significant changes in Ca and Mg concentrations in the blood serum were observed in rats treated with lead and melatonin. In the bones of these rats we observed a significantly lower Ca concentration and higher Mg concentration, and in the teeth, significantly higher Ca and Mg concentrations.

References

1. GRANDJEAN P., OLSEN N.B. Lead. In: Hazardous Metals in Human Toxicology, Part B.; A. Veromysse (eds.), Elsevier: Amsterdam, **1984.**
2. HU H., RABINOWITZ M., SMITH D. Bone lead as biological marker in epidemiologic studies of chronic toxicity: conceptual paradigms. Environ. Health Perspect. **106**, 1, **1998.**
3. O'FLAHERTY E.J. A physiologically based kinetic model for lead in children and adults. Environ.Health Perspect. **106** (6), 1495, **2000.**
4. GWIAZDA R., CAMPBELL C., SMITH D. A non-invasive isotopic approaches to estimate the bone lead in children: implications for assessing the efficacy of lead abatement. Environ. Health Perspect. **113**, 104, **2005.**
5. FOWLER B.A. Biological roles of high affinity metal-binding proteins in mediating cell injury. Comm. Toxicol. **3**, 27, **1989.**
6. SIMONS T.J.B. Lead transport and binding by human erythrocytes in vitro. Pfugers Arch. **423**, 307, **1993.**
7. KOSTIAL K., BLANUSA M., PIASEK M., RESTEK-SAMARZIJA N., JONEM M.M., SIGM P.K. Combined chelation therapy in reducing lead concentrations in suckling rats. J. App. Toxicol. **19**, 143, **1999.**
8. LISIEWICZ J., MOSZCZYŃSKI P. Industrial Hematology. PZWL: Warsaw, **1999**. [In Polish]
9. CANFIELD R.L., HENDERSON C.R., CORY-SLECHTA D.A., COX C., JUSKO T.A., LANPHEAR B.P. Intellectual impairment in children with blood concentrations below 10 µg per decilitre. N. Eng. J. Med. **348**, 1517, **2003.**
10. DIETRICH K.N., RIS M.D., SUCCOP P.A., BERGER O.G., BORNSCHEIN R.L. Early exposure to lead and juvenile delinquency. Neurotoxicol. Teratol. **32**, 511, **2001.**
11. NEEDLEMAN H.L., FARLAND C., NESS R.B., FIENBERG S.E., TOBIN M.J. Bone lead levels in adjudicated delinquents: a case-control study. Neurotoxicol. Teratol. **24**, 711, **2002.**
12. MARCHLEWICZ M., WISZNIEWSKA B., GONET B., BARANOWSKA-BOSIACKA I., SAFRANOW K., KOLASA A., GŁĄBOWSKI W., KURZAWA R., JAKUBOWSKA K., RAĆ M. Increased lipid peroxidation and ascorbic acid utilization in testis and epididymis of rats chronically exposed to lead. BioMetals **20**, 13, **2006.**
13. PAL S., CHATTERJEE A.K. Possible beneficial effects of melatonin supplementation on arsenic-induced oxidative stress in Wistar rats. Drug. Chem. Toxicol. **29,** 423, **2006.**
14. REITER R.J., TAN D.X., QI W., MANCHESTER L.C., KARBOWNIK M., CALVI J.R. Pharmacology and physiology of melatonin in the reduction of oxidative stress in vivo. Biol. Signals Recept. **9**, 160, **2000.**
15. JOU M.J., PENG T.I., REITER R.J., JOU S.B., WU H.Y.,WEN S.T. Visualization of the antioxidative effects of melatonin at the mitochondrial level during oxidative stress-induced apoptosis of rat brain astrocytes. J. Pineal. Res. **37**, 55, **2004.**
16. KARBOWNIK M., GITTO E., LEWINSKI A., REITER R.J. Introduction of lipid peroxidation in hamster organs by the carcinogen cadmium: amelioration by melatonin. Cell Biol. Toxicol. **17**, 33, **2001.**
17. REITER R.J., TAN D.X., MANCCHESTER L.C, CALVO J.R. Handbook of antioxidants. Marcel Dekker. New York, pp. 565-613, **2002.**
18. SMIRNOV A.N. Nuclear melatonin receptors. Biochemistry (Mosc) **1**, 19, **2001.**
19. ANTOLIN I., RODRIQUES C., SAINZ R.M., MAYO J.C., URIA H., KOTLER M.L., RODRIQUES-COLUNGA M.J., TOLIVIA D., MENENDEZ-PELAEZ A. Neurohormone melatonin prevents cell damage: effect on gene expression for antioxidant enzymes. FASEB J. **10**, 882, **1996.**
20. SRINIVASAN V. Melatonin oxidative stress and neurodegenerative diseases. Indian J. Exp. Biol. **40**, 668, **2002.**
21. SURESH C., DENNIS A.O., HEINZ J., VEMURI M.C., CHETTY C.S. Melatonin protection against lead-induced changes in human neuroblastoma cell cultures. Int J Toxicol. **25**, 459, **2006.**
22. EL-SOKKARY G.H., ABDEL-RAHMAN G.H., KAMEL E.S. Melatonin protects against lead-induced hepatic and renal toxicity in male rats. Toxicology. **213**, 25, **2005.**
23. OTHMAN A.I, AL SHARAWY S, EL-MISSIRY M.A. Role of melatonin in ameliorating lead induced haematotoxicity. Pharmacol Res. **50**, 301, **2004.**
24. FLORA S.J., PANDE M., KANNAN G.M., MEHTA A. Lead induced oxidative stress and its recovery following co-administration of melatonin or N-acetylcysteine during chelation with succimer in male rats. Cell. Mol. Biol. **50**, 543, **2004.**
25. EL-MISSIRY M.A. Prophylactic effect of melatonin on lead-induced inhibition of heme biosynthesis and deterioration of antioxidant systems in male rats. J. Biochem. Mol. Toxicol. **14**, 57, **2000.**
26. KIM Y.O., PYO M.Y., KIM J.H. Influence of melatonin on immunotoxicity of lead. Int. J Immunopharmacol. **22**, 821, **2000.**

27. WESTRLUND-HELMERSON U. Determination of lead and cadmium in blood by modification of the Hassel method. At. Abs. News **9**, 133, **1978.**
28. CDC (Centers for Disease Control). Preventing lead poisoning in young children. United States Department of Health and Human Services: Department of Health and Human Services: Atlanta, **1991.**
29. SEŃCZUK W. Toxicology. PZWL: Warsaw, pp 490-427, **1994**. [In Polish]
30. WHO. Environmental Health Criteria 165. Inorganic lead. Geneva, **1995.**
31. CDC (Centres for Disease Control). United States Department of Health and Human Services: Atlanta, **2004**. http://www.cdc.gov/nceh/lead/ACCLPP/meetingMinutes/lessThan10MtgMAR04.pdf
32. LIDSKY T.I., SCHNEIDER J.S. Lead neurotoxicity in children: basic mechanisms and clinical correlates. Brain **126**, 5, **2003.**
33. KARBOWNIK M., GITTO E., LEWINSKI A., REITER R.J. Introduction of lipid peroxidation in hamster organs by the carcinogen cadmium: amelioration by melatonin. Cell Biol. Toxicol. **17**, 33, **2001.**
34. REITER R.J. TAN D.X., QI W., MANCHESTER LC, KARBOWNIK M., CALVI J.R., Pharmacology and physiology of melatonin in the reduction of oxidative stress in vivo. Biol. Signals Recept. **9**, 160, **2000.**
35. DIETRICH K.N., WARE J.H., SALGANIK M., RADCLIFFE J., ROGAN W.J., RHOADS G.G., FAY M.E., DAVOLI C.T., DENCKLA M.B., BORNSCHEIN R.L., SCHWARZ D., DOCKERY D.W., ADUBATO S., JONES R.L. Effect of chelation therapy on the neuropsychological and behavioral development of lead-exposed children after school entry. Pediatrics **114**,19, **2004.**
36. CANFIELD R.L., GENDLE M.H., CORY-SLECHTA.D.A. Impaired neuropsychological functioning in lead-exposed children. Dev. Neuropsychol. **26**, 513, **2004.**
37. EL-SOKKARY G.H., KAMEL E.S., REITER R. Prophylactic effect of melatonin in reducing lead-induced neurotoxicity in the rat. Cell. Moll. Biol. Lett. **8**, 461, **2003.**
38. CHWEŁATIUK E., WŁOSTOWSKI T., KRASOWSKA A., BONDA E. The effect of orally administred melatonin on tissue accumulation and toxicity of cadmium in mice. J. Trace. Elem. Biol. Med. **19**, 259, **2006.**
39. LIMSON J., NYOKONG T., DAYA S. The interaction of melatonin and its precursors with aluminum, cadmium, copper, iron, lead and zinc: an adsorptive voltammetric study. J. Pineal. Res. **24**, 15, **1998.**
40. REITER R.J., TAN D.X., SAINZ RM, MAYO JC, LOPEZ-BURILLO S. Melatonin: reducing the toxicity and increasing the efficacy of drugs. J. Pharm. Pharmacol. **54**, 1299, **2002.**
41. BODAK E., KOŁACZ R., DOBRZAŃSKI Z. Heavy metals – the conditions of exposure and the defensive mechanisms in animals. Med. Wet. **52**, 619, **1996**. [In Polish]
42. AUNGST B.J., FUNG H.L. Kinetic characterization of in vitro lead transport across the rat small intestine. Toxicol. Appl. Pharmacol. **61**, 39, **1981.**
43. VARNAI V.M., SARIC M., MOKROVIC G., PIASEK M., BLANUSA M., BULJAN C.J., MATEK S.M., KOSTIAL K. The effect of dietary supplementation with calcium salts on skeletal calcium in suckling rats. Arh. Hig. Toksikol. **54**, 119, **2003.**
44. SILDERBERG E.K. Lead in bone: implications for toxicology during pregnancy and lactation. Environ. Health Perspect. **91**, 63, **1991.**
45. RABINOWITZ B.M. Toxicokinetics of bone lead. Environ. Health Perspect. **91**, 33, **1991.**
46. POUNDS J.G., Long G.J. Cellular and molecular toxicity of lead in bone. Environ. Health Perspect. **91**, 17, **1991.**
47. KOO WW., SUCCOP P.A., BORNSCHEIN R.L., KRUG-WISPE SK., STEINCHEN J.J., TSANG R.C., BERGER O.G. Serum vitamin D metabolites and bone mineralization in young children with chronic low to moderate lead exposure. Pediatrics. **87**, 680, **1991.**
48. HEARD M., CHAMBERLAIN A. uptake of lead by human skeleton and comparative metabolism of lead and alkaline earth elements. Health Phys.**47**, 857, **1984.**
49. DURLACH J. Magnesium in clinical practice. PZWL, Warsaw, **1991.**