Acta Veterinaria Hungarica 51 (3), pp. 297–304 (2003)

EFFECT OF INGESTED HEAVY METALS (Cd, Pb AND Hg) ON HAEMATOLOGY AND SERUM BIOCHEMISTRY IN RABBITS

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(Received November 4, 2002; accepted April 1, 2003)

In order to investigate the effects of exposure to possible environmental pollutants such as Cd, Pb and Hg on haematological and serum biochemistry values, New Zealand White female rabbits were treated orally with distilled water solutions of $CdSO_4 \cdot H_2O$, $Pb(NO_3)_2$ and $HgCl_2$ (n = 4/treatment) in concentrations of 2.3, 4.1, and 30 mg/kg dry matter, respectively, for 28 days. The initial concentrations of Cd, Pb, and Hg in serum were significantly increased by the treatment. Exposure to Pb significantly decreased the red blood cell (RBC) count, haemoglobin (Hgb) concentration and the haematocrit (Hct) value. The Zn-protoporphyrin concentration did not change as a result of Pb exposure. Pb and Hg loading significantly increased the aspartate aminotransferase (AST) activity. Alanine aminotransferase (ALT) activity was also increased by both Hg and Cd exposure. Comparing the treated and the control rabbits, all the trace elements studied significantly reduced the activity of enzymes in the pancreatic tissues. The haematological results indicate that hyperchromic macrocytic anaemia developed in rabbits treated with Pb. The increased activities of both AST and ALT indicate pathophysiological changes of the liver parenchyma, which was verified by focal fatty infiltration seen histopathologically. Cd exposure could exert a toxic effect on the kidneys, although the slight tubulonephrosis developed would not possibly affect the renal function. The reduced activities of amylase, trypsin, protease and lipase induced by Cd, Pb and Hg suggest toxicity to the pancreas.

Key words: Heavy metals, rabbit, haematological values, Zn-protoporphyrin

Biomonitoring of the heavy metal exposure of humans has commanded increasing interest not only in the case of occupational exposures but also in normal populations (Drasch et al., 1997). The purpose of this study was to obtain information about the changes occurring in the haematological values of rabbits after oral ingestion of relatively small amounts of toxic metals (cadmium, lead and mercury). The rabbit was considered to be as a model, testing the possible

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consequences of intake of some human foods loaded with toxic elements. The applied doses have been selected on the basis of the authors' previous experience (Bersényi et al., 1999).

Materials and methods

Animals and housing

Twelve New Zealand White (NZW) female rabbits, obtained from a state-registered, conventional outbred rabbitry (LAB-NYÚL Bt., Gödöllő, Hungary), were used. The rabbits were housed in individual metabolic cages, fed commercial rabbit pellets and were provided with tap water *ad libitum*. During the experiment, the rabbits were considered clinically healthy on the basis of the veterinary examination.

Treatments

Heavy metals including cadmium, lead and mercury were administered in the form of inorganic salts such as $CdSO_4 \cdot H_2O$, $Pb(NO_3)_2$ and $HgCl_2$ in concentrations of 2.3, 4.1 and 30 ppm (in dry matter of the daily ration), respectively. Four NZW rabbits per each microelement were treated orally with a daily dose of 0.2 ml solution of the described salts for 28 days. The dissolved trace elements were administered through a metal catheter**.**

Sample collection

Blood was taken from the marginal ear veins of twelve rabbits prior to the treatments (Day 0) and after the withdrawal of trace elements (on Day 28). For the determination of haematological values [white blood cells (WBC), red blood cells (RBC), blood haemoglobin (Hgb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT) and Zn-protoporphyrin (ZPP)], K-EDTA was added to the tubes as anticoagulant. Serum was used for the determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and creatinine (CREA) concentration.

In order to determine the influence of heavy metals on the selected organs, the rabbits were euthanised at the end of the trial by an overdose of intraperitoneal pentobarbital injection (Nembutal inj. A.U.V., Sanofi-Phylaxia, Budapest, Hungary). For histopathological investigation, both liver and kidneys were completely removed, fixed in phosphate-buffered 10% paraformaldehyde, stained with haematoxylin and eosin, and examined by light microscopy.

Changes in the activities of amylase, trypsin, protease and lipase in both the pancreatic tissues and the intestinal content were also determined.

Procedures

Heavy metals were determined by atomic absorption spectrometry (Perkin-Elmer Model 5000 AAS, HGA-500, MHS-10) using flame and graphite furnace atomisation for Cd and Pb, and cold vapour technique for Hg.

ZPP concentrations were measured by haematofluorometry of plasma. The instrument used (Model AVIV) measures the ratio of ZPP fluorescence to haem absorption, and the results are reported as μmol ZPP/mol haem.

For enzyme analysis, the pancreas and small intestinal content samples were homogenised in distilled water using a Potter-Elvehjem instrument, then centrifuged (5000/min) for 5 min, and the supernatant was decanted for the determination of enzyme activities. Alpha-amylase activity was determined by the method of Ceska et al. (1969) using Phadebas Test (Pharmacia Diagnostics AB, Sweden). Trypsin activity was measured by calorimetry (Kakade et al., 1969), and lipase activity as proposed by Schön et al. (1961).

Protein content of the samples was assayed by the method of Lowry-Folin (Herd, 1971), with bovine albumin used as reference standard.

Unit enzyme activity (mE) was defined as the quantity of enzyme required to split 10^{-6} M substrate in 1 min under the given test conditions. The specific activity values are related to 1 mg tissue protein.

Statistical methods

Results were presented as mean and standard deviation (SD) of four rabbits in each group. An unpaired two-tailed Student's *t*-test was calculated with the SPSS version 3.0. Statistical significance was accepted if $P < 0.05$.

Ethical allowance

The experiment was approved (No. 15/2001) by the Animal Use and Care Administrative Advisory Committee of the Municipal Veterinary Service for Animal Protection and it is in agreement with the Ethical Codex of the Hungarian Association of Laboratory Animal Science.

Results

Data on the concentrations of heavy metals in the serum of rabbits treated with cadmium, lead or mercury are summarised in Table 1. Initial concentrations of *cadmium,* before ingestion, in the serum of the 12 rabbits proved to be lower than 0.1 μg/L. After 4 weeks of cadmium ingestion, the Cd concentration significantly ($P < 0.001$) increased to 0.13 μg/L (range 0.11–0.16 μg/L).

Table 1

Concentration of heavy metals in the serum of rabbits treated with cadmium, lead or mercury (n = 4/treatment; mean ± SD and range)

Heavy metal	Concentration (µg/L)		P
	d 0	d 28	
Cd	< 0.1	0.13 ± 0.02 (0.11–0.16)	***
Pb	22.23 ± 3.31 (17–28)	46.50 ± 4.80 (40–51)	***
Hg	< 1.0	97.58 ± 21.12 (72–123)	***

P: level of significance; ***P < 0.001

Initial concentrations of *lead,* before ingestion, in the serum of the 12 rabbits ranged from 17–28 µg/L with a mean value of 22 µg/L. After 4 weeks of lead ingestion, the Pb concentration significantly ($P < 0.001$) increased to 40–51 µg/L with an average of 46 µg/L.

Initial concentrations of *mercury,* before ingestion, in the serum of the 12 rabbits was lower than 1.0 µg/L. After 4 weeks of mercury ingestion, the Hg concentration significantly ($P < 0.001$) increased to an average value of 98 µg/L (range 72–123 µg/L).

The haematological values found in the serum of rabbits treated with cadmium, lead or mercury are summarised in Table 2. The initial means of RBC, Hgb, and Hct ($6.56 \pm 0.82 \times 10^{12}$/L, 120.95 ± 11.39 g/L, and 37.41 ± 4.51%, respectively) were significantly ($P < 0.05$) decreased ($5.04 \pm 2.74 \times 10^{12}$/L, 96.75 ± 49.30 g/L, and 29.55 ± 16.00%, respectively) as a result of Pb exposure. However, the initial mean of MCV, MCH, and MCHC (57.08 ± 1.63 fl, 18.53 ± 0.91 pg, and 324.32 ± 11.63 g/L, respectively) were significantly ($P < 0.05$) increased (59.10 ± 1.12 fl, 20.03 ± 2.17 pg, and 341.50 ± 35.33 g/L) after the 4-week ingestion of Pb.

The initial mean of WBC was significantly ($P < 0.001$) decreased by both Pb and Hg exposure ($8.69 \pm 2.34 \times 10^9$/L vs. 4.03 ± 3.58 and $3.80 \pm 2.03 \times 10^9$/L, respectively).

Cd ingestion did not cause any changes in the haematological values.

The initial ZPP concentration changed non-significantly as a consequence of Pb ingestion (106.00 ± 19.78 µmol/mol haem *vs.* 114.57 ± 37.80 µmol/mol haem).

The plasma biochemistry values of rabbits treated with cadmium, lead or mercury are summarised in Table 3. Pb and Hg loading significantly ($P < 0.01$) increased AST activities as compared to the baseline value (41.25 ± 15.27 and 43.23 ± 12.75 U/L vs. 20.23 ± 6.92 U/L, respectively). ALT activities were also significantly increased ($P < 0.01$) by both Hg and Cd exposure (64.15 ± 17.48 and 61.13 ± 12.93 U/L vs. 49.53 ± 8.08 U/L, respectively).

Table 2

Haematological values of rabbits before and after the 28-day heavy metal exposure (n = 4/treatment; mean ± SD)

	WBC (10^9/L)	RBC (10^{12}/L)	Hgb (g/L)	MCH (pg)	Hct (%)	MCV (fl)	MCHC (g/L)	PLT (10^9/L)
Initial value	8.69[a]	6.56[a]	120.95[a]	18.53[a]	37.41[a]	57.08[a]	324.32[a]	382.00[a]
± SD	± 2.34	± 0.82	± 11.39	± 0.91	± 4.51	± 1.63	± 11.63	± 121.31
Cd exposure	7.20[a]	7.23[a]	135.50[c]	18.80[a]	41.45[a]	57.40[a]	327.50[a]	405.00[a]
± SD	± 2.77	± 0.58	± 5.26	± 0.82	± 2.67	± 1.21	± 9.54	± 8.44
Pb exposure	4.03[d]	5.04[b]	96.75[b]	20.03[b]	29.55[b]	59.10[b]	341.50[b]	268.67[a]
± SD	± 3.58	± 2.74	± 49.30	± 2.17	± 16.00	± 1.12	± 35.33	± 144.35
Hg exposure	3.80[d]	6.10[a]	116.25[a]	19.10[a]	34.98[a]	57.53[a]	332.50[a]	272.75[a]
± SD	± 2.03	± 1.07	± 17.35	± 1.02	± 5.33	± 2.18	± 5.80	± 122.68

a-a: NS; a-b: $P < 0.05$; a-c: $P < 0.01$; a-d: $P < 0.001$; WBC: white blood cells; RBC: red blood cells; Hgb: haemoglobin; MCH: mean corpuscular haemoglobin; Hct: haematocrit; MCV: mean corpuscular volume; MCHC: mean corpuscular haemoglobin concentration; PLT: platelets

Table 3

Serum biochemistry values of the rabbits before and after the 28-day heavy metal exposure (n = 4/treatment; mean ± SD)

	AST (U/L)	ALT (U/L)	CREA (μmol/L)	UREA (mmol/L)	UREA/ CREA
Initial value	20.23[a]	49.53[a]	95.27[a]	5.11[a]	0.05[a]
± SD	± 6.92	± 8.08	± 17.74	± 0.80	± 0.01
Cd exposure	26.73[a]	61.13[c]	115.72[b]	4.93[a]	0.04[a]
± SD	± 11.03	± 12.93	± 6.96	± 0.65	± 0.01
Pb exposure	41.25[c]	62.23[a]	99.09[a]	5.13[a]	0.05[a]
± SD	± 15.27	± 25.33	± 9.84	± 0.75	± 0.01
Hg exposure	43.23[d]	64.15[c]	106.00[a]	5.01[a]	0.05[a]
± SD	± 12.75	± 17.48	± 16.24	± 0.76	± 0.01

a-a: NS; a-b: $P < 0.05$; a-c: $P < 0.01$; a-d: $P < 0.001$; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CREA: creatinine

Results for the enzyme activities, including the activities of amylase, trypsin, protease and lipase measured in the pancreatic tissue and in the intestinal contents of rabbits treated with cadmium, lead or mercury, are summarised in Tables 4 and 5. Amylase activity in pancreatic tissue was significantly ($P < 0.001$) reduced by Cd, Pb and Hg as compared to the controls (9.52 ± 0.62, 5.87 ± 1.16 and 4.51 ± 0.55 vs. 12.37 ± 0.97, respectively). Trypsin activity in pancreas

was also significantly reduced by Cd ($P < 0.05$) and Hg ($P < 0.001$) as compared to the controls (5.01 ± 0.36 and 3.58 ± 0.72 vs. 6.33 ± 0.57, respectively). Protease and lipase activities in pancreas were significantly reduced by Pb ($P < 0.05$ and $P < 0.01$, respectively) and Hg ($P < 0.01$ in both cases) as compared to the controls (41.31 ± 4.79, 175.38 ± 10.72 and 36.74 ± 6.74, 160.17 ± 14.46 vs. 51.68 ± 5.71, 217.07 ± 19.35, respectively). The activities of trypsin and lipase in the small intestinal content were significantly reduced by Cd, Pb, and Hg, while the activities of amylase and protease were lowered only by Pb and Hg.

Table 4

Activity of amylase (U/mg protein), trypsin, protease and lipase (mU/mg protein) in the pancreatic tissue of rabbits treated with heavy metals (n = 4/treatment; mean ± SD)

	Amylase	Trypsin	Protease	Lipase
Control	12.37[a]	6.33[a]	51.68[a]	217.07[a]
± SD	± 0.97	± 0.57	± 5.71	± 19.35
Cd exposure	9.52[d]	5.01[c]	44.39[a]	192.19[a]
± SD	± 0.62	± 0.36	± 6.21	± 17.65
Pb exposure	5.87[d]	5.53[a]	41.31[b]	175.38[c]
± SD	± 1.16	± 0.98	± 4.79	± 10.72
Hg exposure	4.51[d]	3.58[d]	36.74[c]	160.17[c]
± SD	± 0.55	± 0.72	± 6.74	± 14.46

a-a: NS; a-b: $P < 0.05$; a-c: $P < 0.01$; a-d: $P < 0.001$

Table 5

Activity of amylase (U/mg protein), trypsin, protease and lipase (mU/mg protein) in the small intestinal content of rabbits treated with heavy metals (n = 4/treatment; mean ± SD)

	Amylase	Trypsin	Protease	Lipase
Control	0.62[a]	1.96[a]	21.76[a]	1.05[a]
± SD	± 0.09	± 0.16	± 1.57	± 0.14
Cd exposure	0.50[a]	1.61[b]	19.30[a]	0.78[b]
± SD	± 0.09	± 0.14	± 2.04	± 0.11
Pb exposure	0.44[b]	1.42[d]	18.14[c]	0.55[d]
± SD	± 0.05	± 0.10	± 0.77	± 0.10
Hg exposure	0.31[d]	1.20[d]	15.81[d]	0.43[d]
± SD	± 0.05	± 0.14	± 1.04	± 0.07

a-a: NS; a-b: $P < 0.05$; a-c: $P < 0.01$; a-d: $P < 0.001$

Histopathological changes

After ingestion of both Pb and Hg damage of the liver parenchyma manifested in focal fatty infiltration was seen, while Cd ingestion induced slight tubulonephrosis.

Discussion

Serum lead concentration of the treated rabbits increased approximately 2-fold (from 22 μg/L to 46 μg/L) after 4 weeks as a consequence of the mild Pb burden. Mean blood lead concentrations in clinically healthy laboratory rabbits have been reported to be between 20 μg/L and 270 μg/L (Gerken and Swartout, 1986). Although our results were within the reference range, the increase of lead concentration may be related to the lead intake.

While lead concentration in the serum doubled, the ZPP concentration remained unchanged during the same period, which is in agreement with previously reported tendencies (Peter and Strunc, 1983). In that study, elevated lead concentrations associated with normal ZPP values were found. This situation corresponds to acute lead poisoning in humans, in which high lead concentration appears almost immediately after lead loading, while the ZPP value is still well within the normal range. The same phenomenon occurs in lead-poisoned rabbits. Such findings can be explained by assuming a dual effect in acute toxicity, namely interference with iron utilisation, combined with inhibition of ferrochelatase enzyme activity. These findings clearly prove the limitation of the ZPP test. From the data presented it can be suggested that both blood lead and ZPP analysis should be performed when lead intoxication is suspected and that the increase of ZPP concentration can occur as a consequence of chronic Pb exposure (Labbe and Rettmer, 1989; Leung et al., 1993).

RBC, Hgb and Hct decreased while MCH and MCV increased in this study. These haematological data of the present study indicate that macrocytic hyperchromic anaemia developed in rabbits treated with Pb, although a mild to moderate normocytic normochromic anaemia is usually seen only in chronic lead toxicity (Kaneko, 1989).

Both the significantly increased AST activity induced by Pb and Hg loading and the significantly elevated ALT activity caused by Hg exposure are suggestive of a damage of the liver parenchyma. These results have been confirmed by the histopathological finding of focal fatty infiltration. Cd exposure increased the concentration of CREA. This can be explained by toxicity to the kidneys, which was reflected in the development of slight tubulonephrosis. The activity reductions of amylase, trypsin, protease and lipase induced by trace elements (especially by Pb and Hg) suggested the toxicity of these elements to the pancreas, resulting in a potential decrease in exocrine and endocrine pancreatic functions (Banerjee and Bhattacharya, 1997; Chowdhury et al., 1993).

Based on our results, haematology and blood biochemistry can be considered as a good but unspecific indicator of heavy metal (Cd, Hg, Pb) exposure in mammals including humans and companion animals.

Acknowledgements

We thank Dr. Róbert Glávits (Central Veterinary Institute, Budapest) for the histopathological examinations, Aranka Hudák ('J. Fodor' Public Health Center) for ZPP and Dr. Emma Kósa for enzyme activity determinations. This work was supported by a grant from the Hungarian Scientific Research Fund (OTKA F 02539).

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