EXPERIMENTAL STUDY

Changes in ascorbic acid and malondialdehyde in rats after exposure to mercury

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Abstract

Background: **Mercury is one of the ubiquitous pollutants participating in generation of reactive oxygen species leading to the formation of oxidative stress.**
Methods: **Changes in plasma concentrations of ascorbic acid and malondialdehyde were measured in rats after per os exposure to different doses of mercury (as mercuric chloride) for 30 d.**
Results: **The production of ascorbic acid and malondialdehyde was related to the dosage of mercuric chloride. Within the group of rats receiving mercuric chloride in the concentration of 10 μmol/L (corresponding to the LD_{50} of $HgCl_2$), the concentrations of ascorbic acid and malondialdehyde were increased by 22.19 % and by 6.88 % respectively when compared with the control group. The doses of mercuric chloride lower than LD_{50} reduced the production of ascorbic acid and malondialdehyde statistically.**
Conclusions: **Mercury is one of the inducers of oxidative stress in plasma of rats. The oxidation damage caused by mercuric chloride is proven by increased malondialdehyde formation and subsequently compensated by an overproduction of ascorbic acid *(Tab. 3, Ref. 22)*.**
Key words: mercuric chloride, rats, oxidative stress, ascorbic acid, malondialdehyde.

Mercury is a serious pollutant (environmental and occupational) with toxic effects in all living organisms (Berlin, 1987). Primary exposure occurs through environmental contamination as the result of mining, smelting, extensive industrial and agricultural usage including inhalation and ingestion via the food chain. Mercury enters an organism in a variety of chemical forms (elemental, inorganic and organic), exhibiting its toxicologic characteristics including neurotoxicity, nephrotoxicity and gastrointestinal toxicity with ulceration and hemorrhage (Clausen, 1993; Hua et al, 1996; Langauer-Lewowicka and Zajac-Nedza, 1997; Deleu et al, 1998; Gassó et al, 2000).

Most of inhaled (Hg^0) vapor generated from metallic mercury is highly diffusible and lipid soluble and rapidly oxidizing to bivalent ionic mercury by complex catalase-hydrogen peroxide in the blood, and is distributed through blood to various organs. The content of catalase in tissues and production of hydrogen peroxide are the limiting factors of oxidizing Hg^0 to Hg^{2+} and subsequently their diffusion through membrane of cells (Livardjani et al, 1991). The oxidation is inhibited by ethanol. Oxidized mercury reacts with sulfhydryl groups of proteins, namely enzymes causing their inhibition. Mercury causes induction of metallothionein (MT) synthesis in kidneys and hepar. MT is a cysteine-rich, low-molecular intracellular protein with high affinity for metals and plays an important role in the homeostasis of essential metals such as zinc and copper, as well as in metabolism and detoxification of potentially toxic heavy metals including mercury (Yoshida et al, 1999).

The toxicity of mercury and its ability to react with and deplete free sulfhydryl groups are well known (Goyer, 1991). Depletion of protein-bound sulfhydryl groups results in the production of reactive oxygen species such as superoxide anion, hydrogen peroxide, and hydroxyl radical. The formation of oxidative stress results in tissue-damaging effects. The metal plays the key role in the initiation and propagation of free radical-induced peroxidative damage (Stohs and Bagchi, 1995).

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Tab. 1. Characteristics of experimental groups and intake of mercuric chloride.

Group	Concentration of $HgCl_2$	N	Age (days)	Weight (g)	Intake of $HgCl_2$ (mg/kg) for 30 d	1 d
Control	-	10	91	304±23 (270-355)	-	-
1	10 µmol/L	10	91±1	300±22 (260-330)	37.00	1.23 (1.07-1.35)
2	5 µmol/L	10	90±1	293±22 (230-340)	18.50	0.62 (0.49-0.68)
3	2.5 µmol/L	10	95	291±34 (240-360)	9.25	0.31 (0.26-0.36)
4	1.25 µmol/L	10	88±3	276±41 (220-340)	4.63	0.15 (0.12-0.17)

Data are expressed as mean±SD (min-max), N — number of cases

Tab. 2. Effect of mercuric chloride treatment on the concentration of ascorbic acid and malondialdehyde in rats.

Group	Concentration of $HgCl_2$	Ascorbic acid (µmol/L)	95% CI	Malondialdehyde (µmol/L)	95% CI
Control	-	37.26±7.69	30.82-43.70	3.63±0.45	3.25-4.00
1	10 µmol/L	45.53±12.89	36.30-54.75	3.88±0.79	3.31-4.45
2	5 µmol/L	44.96±9.67	38.03-51.87	3.41±0.88	2.72-4.09
3	2.5 µmol/L	31.16±7.78**	24.65-37.65	2.55±0.29***	2.30-2.79
4	1.25 µmol/L	34.68±9.44	26.78-42.58	2.95±0.51**	2.52-3.37

Data are expressed as mean±SD, statistical significance in comparison to the first group: ** $p<0.01$, *** $p<0.001$

The present work reports evidence of the effects of mercuric chloride on the concentration of thiobarbituric acid-reactive substance (TBARs, malondialdehyde) as stress indicator and the concentration of ascorbic acid (vitamin C) as antioxidant and radical scavenger in the plasma of rats.

Material and methods

Adult male Wistar albino rats aged 91±2 (88–95 d) with an average body weight of 293±29 (220–360 g), were housed in an environmentally controlled animal facility, operating on a 12 h dark/light cycle at 22–24 °C.

The experimental animals were divided into 5 groups (n=10 per group) according to Table 1. The LD_{50} of $HgCl_2$ received per os by rats is 37 mg/kg b. w. (Levis and Tatken, 1979). The animals received standard Larsen diet with drinking water containing mercury as mercuric chloride ($HgCl_2$) in a concentration of 10 µmol/L (what corresponded to dose LD_{50}), 5 µmol/L (1/2 LD_{50}), 2.5 µmol/L (1/4 LD_{50}), 1.25 µmol/L (1/8 LD_{50}) for 30 days. The rats in the control group received drinking water without $HgCl_2$. The intake of drinking water and food was controlled and rats were weighed daily. The real mercury exposure was calculated according to the dilution of $HgCl_2$ and the consumption of drinking water for different experimental groups of animals.

After 30d of administration of mercuric chloride to rats blood samples were taken from heart (by puncture) under total anaesthesia with Natrium pentobarbital (50 mg/kg b.w.; i.p.; Pentobarbital Spofa). The blood samples were immediately collected using heparin (Heparinum natricum 5.000 IU/ml inj. solution) as anticoagulant. The collected samples of blood were centrifugated at 1500 G for 15 min for plasma separation.

Plasma specimens were used for determination of ascorbic acid concentrations using a spectrophotometric method based on the development of colour product as a result of the reaction of dehydroascorbic acid with dinitrophenylhydrazine (Roe and Kuether, 1943). The degree of oxidative activity was evaluated from the plasma concentration of lipid peroxidation products, mainly malondialdehyde (MDA) which were determined by the spectrofluorometric method of Yagi to be thiobarbituric acid-rective substances (TBARs). Malondialdehyde reacts with thiobarbituric acid while creating a fluorescent product measured using a fluorescent spectrophotometer at 553 nm Perkin-Elmer emission wavelength.

The data are presented as mean±SD. Statistical analysis used Student's t-test and ANOVA to determine significance. Statistical significance was accepted at $p<0.05$.

Results

The mean concentrations of ascorbic acid and malonialdehyde are summarized in Table 2. In the control group the mean concentration of ascorbic acid was 37.26±7.69 µmol/L and the malondialdehyde 3.63±0.45 µmol/L.

Within the group of rats receiving mercuric chloride in a concentration of 10 µmol/L (LD_{50}), the concentrations of ascorbic acid and malondialdehyde were increased by 22.19 % (45.53±12.89 µmol/L) and by 6.88 % (3.88±0.79 µmol/L), respectively, compared with those in the control group. These results indicate oxidative damage caused by treatment of mercuric chloride, the fact of which is proven by increased malondialdehyde formation (3.88 vs 3.63) and subsequently compensated by an overproduction of ascorbic acid.

Tab. 3. Body weight changes in rats during the experiment.

Group	Body weight (g)		
	before experiment	after experiment	p
Control	304±23	324±27	0.001
1	300±22	349±30	0.001
2	293±22	337±37	0.01
3	291±34	322±31	0.05
4	276±41	313±56	0.01

Data are expressed as mean±SD.

Mercuric chloride in a concentration of 5 µmol/L (1/2 LD_{50}) decreased the production of malondialdehyde in comparison with the control group, although the concentration of ascorbic acid was further increased. In the groups receiving $HgCl_2$ in concentrations of 2.5 µmol/L and 1.25 µmol/L there was a similar deficit of ascorbic acid (-16.4 % and -7 %), and deficit of malondialdehyde (-29.8 % and -18.8 %) compared with control rats. Statistically significant was the comparison of group LD_{50} of $HgCl_2$ (10 µmol/L) with the group receiving 1/4 LD_{50} of $HgCl_2$ (2.5 µmol/L) for ascorbic acid ($p<0.01$), for malondialdehyde ($p<0.001$), and with the group receiving 1/8 LD_{50} of $HgCl_2$ (1.25 µmol/L) for ascorbic acid ($p<0.06$) and for malondialdehyde ($p<0.01$).

In all experimental groups, body weights of rats were significantly increased and results are summarized in Table 3.

Discussion

One notable factor of environmental and occupational pollution is an accumulation of such elements resistant to biological degradation including heavy metals (cadmium, mercury, lead). Both occupational and environmental exposures to heavy metals remain a serious problem (Lumb, 1995; Evans, 1998; Korenekova et al, 2002). Organic and inorganic mercury compounds have been reported to be potent toxic and/or carcinogenic agents in humans and animals, although the exact mode of action is still not certain. Numerous studies indicated that mercuric ions can interact with glutathione (GSH) in the presence of hydrogen peroxide, leading to the generation of reactive oxygen species. However, the mechanism of the generation of radical species including the redox cycle of the metal and the formation of oxidative DNA modification by mercury compounds has not been studied. The reactive oxygen species subsequently induce lipid peroxidation measured by the thiobarbituric acid reaction for malondialdehyde in liver, kidney, lung, testes and serum. Increased MDA was reduced in these target tissues after pre-treatment with antioxidants and chelators in $HgCl_2$-treated rats. The results of this experimental work indicated that the lipid peroxidation is one of the molecular mechanisms for cell injury in acute $HgCl_2$ poisoning and is associated with a decrease of cellular antioxidants such as glutathione, superoxide dismutase (SOD) and catalase (CAT) (Huang et al, 1996). Inorganic mercury decreased total SOD, CuZn-SOD and Mn-SOD activities significantly in the cerebellum while glutathione peroxidase activity was affected in cerebellum and brain stem. Oxidative stress may contribute to the development of neurodegenerative disorders caused by mercury intoxication (Husain et al, 1997).

Lund et al (1993) have also demonstrated that the administration of mercury as Hg^{2+} (1.5 or 2.25 mg $HgCl_2$/kg) to rats results in increased hydrogen peroxide formation, glutathione depletion and lipid peroxidation in kidney mitochondria. The exposure of mice to mercury, chromium or silver results in enhanced production of MDA in liver and kidneys (Rungby and Ernst, 1992).

The effects of antioxidants clearly demonstrate the generation of reactive oxygen species by metals. It is likely that the protective effects of antioxidants on metal-induced damage are dependent on many factors such as the animal species, tissues, and cell types, as well as the level of antioxidants and metals inside cells. High levels of dietary-α-tocopherol protect against methyl mercury chloride-induced hepatic lipid peroxidation and enhanced the activity of selenium-dependent glutathione peroxidase activity. Excess dietary beta-carotene did not provide protection and has no effect on glutathione peroxidase activity (Andersen and Andersen, 1993).

One important antioxidant in blood plasma and tissues with a very wide spectrum of biological effects is ascorbic acid (vitamin C). Ascorbic acid is produced from the ultimate hexose precursor D-glucose. After the pathway of ascorbic acid biosynthesis had been established, it was soon revealed that in tissues of humans, monkeys and guinea pigs, there is no activity of the terminal enzyme of the pathway, L-gulono-gamma-lactone oxidase (GLO), (Nishikimi and Yagi, 1996). Mice, rats and rabbits synthesize vitamin C in their livers. Our experiment showed that the exposure of male Wistar albino rats to mercuric chloride results in enhanced production of MDA in the plasma of rats. Increased malondialdehyde was compensated by ascorbic acid overproduction. The lower doses of chronic administration of mercuric chloride in concentrations of 2.5 µmol/L (1/4 LD_{50}) and 1.25 µmol/L (1/8 LD_{50}) reduced the production of ascorbic acid and malondialdehyde. From this point of view ascorbic acid had a prophylactic effect on mercuric chloride toxicity and may contribute to the reduction of the negative affects of intoxication.

Our experimental data have consequently evoked the questions as to what saturation with vitamin C would have been sufficient in human organisms being unable to produce it and what exposures to mercury from different sources (industrial waste, dental amalgam) are required to eliminate mercury negative impacts on human health.

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