Effect of Per Os Administration of Mercuric Chloride on Peroxidation Processes in Japanese Quail

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Abstract

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Activities of glucose-6-phosphate dehydrogenase (G-6-PD, E.C. 1.1.1.49), superoxide dismutase (SOD, E.C. 1.15.1.1) in erythrocytes and the amount of malondialdehyde (MDA) precursors in the liver and kidney were examined in Japanese quails exposed to mercuric chloride ($HgCl_2$) (25 $mg.l^{-1}$ drinking water) for 60 days. Lipid peroxidation was determined by the thiobarbituric acid reaction measuring the production of malondialdehyde precursors and the enzymes activities were measured by the spectrophotometric methods. Lipid peroxidation measured by the quantification of MDA precursors was significantly higher in both quail organs treated with $HgCl_2$ than in control groups (liver: 5.3 ± 0.22 in the controls vs. 16.0 ± 0.62 $\mu mol.g^{-1}$, $P < 0.001$; kidney: 7.6 ± 0.21 in the controls vs. 19.0 ± 0.61 $\mu mol.g^{-1}$, $P < 0.001$). The activities of both SOD and G-6-PD in erythrocytes after the administration of $HgCl_2$ were significantly less than in the control group (G-6-PD: 59.98 ± 1.22 in the controls vs. 41.5 ± 3.19 $mU.10^{-9}$ Ec, $P < 0.001$; SOD: 1361 ± 48.37 in the controls vs. 1034 ± 33.11, $U.g^{-1}$ Hb, $P < 0.001$). The results of this study show that the antioxidant protection is not supported by glucose-6-phosphate dehydrogenase and superoxide dismutase.

Antioxidant protection, lipid peroxidation, Japanese quails, mercury chloride

One of the harmful effects of mercury action during its accumulation in a body in a region contaminated by mercury is the excessive release of reactive oxygen species and increased lipid peroxidation in the cells (Lund et al. 1993). Free radicals and intermediate products of peroxidation are capable of damaging the integrity and altering the function of biomembranes, which can lead to the development of many pathological processes (Gutteridge 1993). Various specific enzymes that limit free-radical formation, such as superoxide dismutase (SOD) and glucose-6-phosphate dehydrogenase (G-6-PD), play an important role in the protection of cell membranes against oxidative damage. Protective systems existing in the cells avoid the excessive increment of undesirable oxidizers. Three enzymes form the corner stone of this protection: superoxide dismutase, catalase and glutathione peroxidase. Glucose-6-phosphate dehydrogenase is the key enzyme, catalyzing the first step of pentose phosphate metabolic pathway. The pentose phosphate metabolic pathway is a unique source of NADPH in erythrocyte and synthesis of NADPH decreases in G-6-PD deficiency. One of major roles of NADPH in erythrocyte is detoxication of hydrogen peroxide and oxygen radicals in and on the red blood cells (Deutsch 1983). There is conflicting evidence about the effect of oxidative damage on the activities of these antioxidant-associated enzymes. The aim of this work was to study the effect of administration of mercuric chloride on SOD and G-6-PD activities in erythrocytes as well as on lipid peroxidation in the liver and the kidney of Japanese quails.

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Materials and Methods

Animals

The experiments were carried out on twelve Japanese quails, weighing from 115 to 125 grams, 9 weeks of age. The birds were fed a standard diet for chickens (a complete pelleted feed mixture for poultry of all age groups). The quail were divided into two groups. The experimental group (n=6) was treated with mercuric chloride ($HgCl_2$). Mercuric chloride was added to their drinking water at a concentration of 25 $mg.l^{-1}$ for 60 days before the measurement of enzymes activities. The control group received untreated drinking water for the same period. Water was supplied ad libitum. After 60 days exposure to mercuric chloride, the birds were quickly killed by cervical decapitation and exsanguination. At all times, the quails received care in compliance with international accepted procedures. Blood samples were collected after decapitation into heparinized tubes and the liver and kidney were dissected.

The activities of the superoxide dismutase, glucose-6-phosphate dehydrogenase in the erythrocytes and amount of malondialdehyde precursors in the liver and kidney tissue were measured by spectrophotometric methods on SPEKOL 11 (Carl Zeiss Jena).

Superoxide Dismutase

The activity of superoxide dismutase in blood was measured by the comercial kit (RANDOX Laboratories Ltd., UK). This method uses xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye.

Glucose-6-phosphate Dehydrogenase

The activity of glucose-6-phosphate dehydrogenase in blood was measured by the comercial kit (RANDOX Laboratories Ltd., UK). The enzyme activity is determined by measurement of the rate of absorbance change at 340 nm due to the reduction of $NADP^+$.

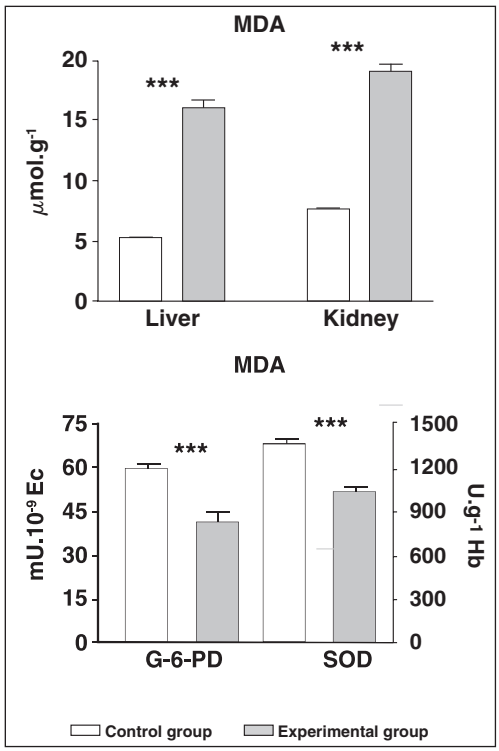

Fig. 1. The levels of malondialdehyde (MDA) in liver and kidney tissue; the activities of glucose-6-phospate dehydrogenase (G-6-PD) and superoxide dismutase (SOD) in the blood of the Japanese quails after 60 days action of the mercuric chloride. Values are means ± S.E.M., n = 6 for each group. Significance: *** = $P < 0.001$.

Lipid Peroxidation

The lipid peroxidation was determined by the thiobarbituric acid reaction measuring the production of malondialdehyde (MDA) precursors in the liver and kidney tissue by the method of Mihara and Uchiyama (1978).

Statistical analysis

The results are given as means ± S.E.M. Statistical analyses of the differences between the control and $HgCl_2$-treated group were performed by the Student's *t*-test according to Snedecor and Cochran (1967).

Results and Discussion

The treatment of Japanese quails with mercuric chloride had effects on the activities of the antioxidative enzymes in the blood and on lipid peroxidation in the liver and kidney tissue (Fig. 1). Our results show that lipid peroxidation was significantly higher in both liver and kidney tissue in the experimental group compared with the control group (liver: 16.0 ± 0.62 to 5.3 ± 0.22 $\mu mol.g^{-1}$, $P < 0.001$; kidney: 19.0 ± 0.61 to 7.6 ± 0.21 $\mu mol.g^{-1}$, $P < 0.001$). The experimental work confirms that Hg ions initiate and stimulate peroxidative reactions both in *vitro* and *in vivo* by a direct effect on the cell membrane with a consequent increase in the release of reactive oxygen species as well as with the inactivation of the body's antioxidants (Andersen and Andersen 1993; Lund et al. 1993).

Huang et al. (1996) have shown that the parenteral administration of mercuric chloride to rats enhances lipid peroxidation in the liver, kidney, lung, testis, and serum, but not in the heart, spleen or muscle. After the subcutaneous injection of mercuric chloride, MDA concentrations in the liver and kidney significantly increased after 9 hours and reached peak values at 24 hours. The intramuscular injection of mercuric chloride enhances lipid peroxidation in the cerebral cortex, cerebellum and sciatic nerves in rats (Anuradha and Varalakshmi 1999).

The activities of G-6-PD and SOD in the blood was significantly lower (G-6-PD: 59.98 ± 1.22 in the controls vs. 41.5 ± 3.19 $mU.10^{-9}$ Ec, $P < 0.001$; SOD: 1361 ± 48.37 in the controls vs. 1034 ± 33.11, $U.g^{-1}$ Hb, $P < 0.001$) after 60 days of mercury chloride administration. These lower activities apparently related to the binding of Hg on sulfhydryl groups of enzymes. Our results extend information about the effect of metal ions on the activities of antioxidant-associated enzymes.

The oxidative load induced by cadmium in rats (Manca et al. 1991), nickel in mice (Dieter et al. 1988) and aluminium in rats (Zaman et al. 1993) also decreases the activities of G-6-PD and SOD. The higher activity of G-6-PD in sheep after an 8-day administration of Hg (Košťová et al. 1995) and during a 3-day application of Pb (Hacker et al. 1990) in rats is probably a compensatory mechanism to counter of excessive peroxidation in the initial phase of the oxidative load.

Our results show that only the long-term administration of mercury decreases the activity of SOD and G-6-PD, which corresponds with the results of Bulat et al. (1998), who measured the lower activities of SOD in the erythrocytes of workers occupationally exposed to mercury. Moreover, Zabinsky et al. (2000) have shown that the long-term exposure of man to mercury vapours significantly decreases the activities of G-6-PD and SOD.

In conclusion, lipid peroxidation, determined as malondialdehyde production is higher in the liver and kidney tissue of Japanese quails after a prolonged exposure to mercury in drinking water. The results of this study show that the antioxidant protection is not supported by glucose-6-phosphate dehydrogenase and superoxide dismutase.

Vplyv orálne podávaného chloridu ortuťnatého na peroxidatívne procesy u japonských prepelíc

Aktivity glukózo-6-fosfát dehydrogenázy (G-6-PD, E.C. 1.1.1.49), superoxid dizmutázy (SOD, E.C. 1.15.1.1) v erytrocytoch a množstvo malondialdehydových (MDA) prekurzorov v pečeni a obličkách boli merané u japonských prepelíc vystavených účinkom chloridu ortuťnatého ($HgCl_2$) v dávke (25 $mg.l^{-1}$ pitnej vody) po dobu 60 dní. Peroxidácia lipidov, ktorá bola meraná reakciou kyseliny tiobarbiturovej na produkciu malondialdehydových prekurzorov a aktivity enzýmov boli merané spektrofotometrickými metódami. Peroxidácia lipidov meraná hladinou MDA prekurzorov bola signifikantne vyššia v oboch orgánoch prepelíc ovplyvnených $HgCl_2$ oproti kontrolnej skupine (pečeň: kontrolná skupina 5.3 ± 0.22, $HgCl_2$ skupina 16.0 ± 0.62 $\mu mol.g^{-1}$, $P < 0.001$; obličky: kontrolná skupina 7.6 ± 0.21, $HgCl_2$ skupina 19.0 ± 0.61 $\mu mol.g^{-1}$, $P < 0.001$). Aktivity SOD a G-6-PD v erytrocytoch po podávaní $HgCl_2$ boli signifikantne nižšie než v kontrolnej skupine (G-6-PD: kontrolná skupina 59.98 ± 1.22, $HgCl_2$ skupina 41.5 ± 3.19 $mU.10^{-9}$ Ec, $P < 0.001$; SOD: kontrolná skupina 1361 ± 48.37, $HgCl_2$ skupina 1034 ± 33.11, $U.g^{-1}$ Hb, $P < 0.001$). Výsledky tejto práce ukazujú, že antioxidačná ochrana japonských prepelíc nie je podporovaná glukózo-6-fosfát dehydrogenázou a superoxid dizmutázou.

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