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Lipid Peroxidation and Activities of Antioxygenic Enzymes *In Vitro* in Mercuric Chloride Treated Human Erythrocytes

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Mercury can exist in the environment as metal, as monovalent and divalent salts and as organomercurials, the most important of which is methyl mercury. The average daily intake of mercury from food is 10-12 ug and may be higher on diets comprising of fish from polluted water (Meranger and Smith 1972). Mercury is absent in drinking water while polluted water contain upto 30 ug/L. Intake of metallic mercury vapour amounts to 1 ug/day. Absorption of inorganic mercury compounds from food is about 7-8% and 15% from water, whereas methyl mercury is almost absorbed completely. Absorbed methyl mercury rapidly appears in the blood, where, in man, 80-90 % is bound to the red cells. The greater toxicity of methyl mercury is due to its lipid solubility, which permits it to cross cell membranes. Concentration of mercury below 20 ug/L of whole blood are satisfactory and without any toxic effects (Davidson et al. 1985). There are reports which suggests that heavy metals like mercury, lead and cadmium have prooxidant catalytic activity and can initiate lipid peroxidation by generating free radicals and thereby interferring with the antioxidant system of the cell (De Bruin 1976; Luckey et al. 1982). Higher concentrations of mercury have been used earlier to assess the extent of lipid peroxidation in bovine erythrocytes. However, the interaction between mercury and the red blood cell is not properly understood. Therefore, in the present study the in vitro toxicity by lower concentrations of mercuric chloride on human erthrocytes in relation to their effect on lipid peroxidation and some enzymes which have a protective role in such a condition are reported.

MATERIALS AND METHODS

Healthy male donors (30) were selected for the study. Blood was collected in 2% sodium citrate, centrifuged

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and the plasma and buffy coats removed by gentle aspiration. The red blood cells were harvested and washed three times with 0.1 M phosphate-saline buffer (1:9) pH 7.4 which removed more than 99% of the white cells. Packed cell volume (PCV) was adjusted to 5% with phosphate-saline buffer pH 7.4. The packed cells were pretreated with mercuric chloride at various concentrations (0, 0.01, 0.1, and 1.0 mM) for different time intervals (0,5, 10, 15, 30 and 60 min). The red blood cells were washed three times with phosphate-saline buffer pH 7.4 and adjusted to 5% PCV. As mercury concentration below 20 ug/L of blood has been reported not to have any effect (Davidson et al. 1985), a concentration 10 times higher than this was selected for in vitro studies. The experiments were performed in a 100 fold concentration range to observe the maximum effect due to mercury.

Lipid peroxidation was estimated in 2.5% PCV as malonyldialdehyde (MDA) formed by thiobarbituric acid reaction (Stocks and Dormandy 1971). Superoxide dismutase (SOD) (Nishikimi et al. 1969) and catalase (Aebi 1983) activities were determined in RBC lysate prepared by the method of McCord and Fridovich (1969). Each assay of SOD and catalase was performed in triplicate in two fold concentration range. Glutathione content of blood was estimated by the method of Beutlar et al. (1963) in the control and after the cells were pretreated with mercuric chloride for 30 min. Haemoglobin in blood was estimated by the method of Dacie and Lewis (1984). Protein content in lysate prepared from the erthrocytes was determined by the method of Lowry et al.(1951).

RESULTS AND DISCUSSION

In the red blood cells, protein represents approximately 52% of its mass, lipid 40% and carbohydrate 8% . Distribution of phospholipids is highly asymmetrical, outer layer consists mainly of lecithin, sphingomyelin and glycolipids, the inner layer is mainly of phosphatidyl ethanolamine and phosphatidylserine. Superoxide dismutase is a cytosolic enzyme not attached to the erythrocyte membrane (Fridovich 1974) while catalase is loosely associated with haemogolbin (Nicholls and Schonbaum 1963).

The in vitro effect of mercury on the erythrocytes was assessed by measuring the rate of MDA formation, the activities of SOD and catalase and by measuring the glutathione content of erythrocytes pretreated with mercuric chloride.

Table 1 shows the effect of pretreatment of erythrocytes with mercuric chloride on lipid

Table 1. Lipid peroxidation[a] in human erythrocytes treated with various concentrations of mercuric chloride for different periods

Concentration of $HgCl_2$ (mM)	Incubation Time (min) 0	5	10	15	30	60
0	1.51±0.11	1.54±0.07	1.57+0.09	1.62±0.05	1.67±0.08	1.78±0.08
0.01	1.67±0.05	1.79±0.15	1.80±0.05	1.85±0.12	2.01±0.09	1.96±0.11
0.10	1.78±0.06 NS	1.94±0.08 NS	2.05±0.05 P< 0.05	2.20±0.07 P< 0.01	2.26±0.08 P< 0.03	2.35±0.09 P< 0.05
1.0	2.10±0.05 P< 0.05	2.20±0.14 P< 0.05	2.30±0.09 P< 0.01	2.45±0.11 P< 0.01	2.52±0.08 P< 0.03	2.36±0.07 P< 0.02

Values are mean±SE, NS- non significant

[a] mM of MDA produced/hr/g Hb

Table 2. Activities of superoxide dismutase[a] and catalase[b] and glutathione[c] content in erythrocytes treated with mercuric chloride for different periods

Concentration of $HgCl_2$ (mM)	Incubation Time (min)					
	SOD Activity		Catalase Activity		Glutathione content	
	d	e	d	e	d	e
0	40.2±3.1	61.6±5.4	1.24±0.07	1.20±0.10	27.1±2.50	27.7±2.90
0.01	-	67.8±2.2	-	0.81±0.05	-	18.3±4.21
0.10	-	57.6±3.7	-	0.66±0.77	-	14.2±2.70
				(P < 0.01)		(P < 0.05)

Values are mean±SE, NS- non significant

[a] units/mg protein

[b] mmoles of H_2O_2 decomposed/min/mg protein

[c] umoles/100 ml blood

d and e are time of incubation of erythrocytes with mercuric chloride for 0 and 30 min, respectively.

peroxidation (LPO) as a function of preincubation time with mercuric chloride. The increase in LPO in control cells upon incubation for the specified period was insignificant. Similarly, at low concentration (0.01 mM), preincubation with mercuric chloride upto 60 min did not significantly affect LPO. However, at higher concentration of mercuric chloride (0.1 mM), LPO was significantly increased within 10 min of preincubation. High concentration of mercuric chloride (1.0 mM) caused an immediate increase in LPO. Since, peroxidation of membrane lipids is primarily an outcome of generation of free radicals, it is suggested that mercuric chloride initiates free radical processes in erthyroctyes. The effect of mercuric chloride on antioxygenic enzymes was investigated under such a condition. Data presented in Table 2 show no significant change in SOD activity after pretreatment with mercuric chloride at 0.1 mM for 30 min while catalase activities were significantly reduced under similar treatment. The lowered catalase activity may be due to such toxic effects which may increase production of hydrogen peroxide.

A considerable decline in glutathione content under mercury intoxication was observed (Table 2). The toxic effects of mercury may be due to the binding with sulfhydryl groups and forming free radicals which may further increase lipid peroxidation. The scavenger role of glutathione in removing toxic electrophiles helps the cell to maintain its internal environment to a limited extent at lower concentration of mercury.

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