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Antiperoxidative Mechanisms Offered by Selenium against Liver Injury Caused by Cadmium and Mercury in Rat

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Hepato-toxic manifestations of cadmium and mercury, two most hazardous metals, have been paid considerable attention during last few years. Now it has been established that they conjugate with glutathione and lead to the formation of mercapturic acids (Cherian and Vostal, 1977; Refsvik, 1978). Further, glutathione is known to be a carrier for their efflux across the canalicular membrane. Although several reports confirm that selenium offers protection against their toxicity by altering their tissue distribution and protein binding capacity (Chen et al., 1974; Whanger, 1976) and also by enhancing their biliary excretion (Stowe, 1976), its concommitant effects on lipid peroxidation are poorly known. Hence, a study on lipid peroxidation was proposed in the liver of rats fed simultaneously on selenium and cadmium and selenium and mercury. Since glutathione cycle constitutes an important part of antioxidant defense mechanisms, glutathione and glutathione peroxidase were also studied.

MATERIAL AND METHODS

Male Charles Foster rats (150±25 gm) were selected from the laboratory stock and maintained in wire-woven cages on pelleted food and tap water ad libitum. After acclimating them to laboratory conditions (room temperature 25°±5°C and relative humidity 60±10%) for two weeks, they were divided into five groups, each containing five rats. Rats of group A were fed on cadmium (as cadmium chloride (95% pure.) obtained from E. Merck, Bombay (India) whereas rats of group B were fed on cadmium and sodium selenite (E.Merck. Bombay,India) by gavage on each alternate day for 30 days. Similarly rats of group C were fed on mercury (as mercuric chloride (95% pure.) obtained from E.Merck, Bombay, (India) and rats of group D were fed on mercury and sodium selenite by gavage for thirty days. Rats of group E that were administered only saline served as controls.

After schedule treatments, the rats were starved overnight and sacrificed by decapitation. For the determination of malondialdehyde, microsomes were purified from liver homogenates by differential centrifugation at 1000,000 xg for 60 minutes using a

Hitachi 55 PA, automatic preparative ultracentrifuge. They were washed twice with 0.25 M sucrose and used for the estimation of malondialdehyde by thiobarbituric acid (Jordan and Schenknan, 1982). A standard was prepared by dissolving 24.6 mg of 1,1,3 tetramethoxypropane in 100 ml of deionized distilled water. Working standards from the stock solution were prepared by diluting the stock solution 1:50, 1:75, 1:100 and 1:150 with 0.01 N HC1. A 1:100 dilution of stock solution contained 15 n-moles of malondialdehyde/mg protein. Protein was determined using bovine serum albumin (Sigma, USA) as the standard (Lowry et al., 1951).

Liver samples were perfused with 1.15% potassium chloride, homogenized in four volumes of distilled water and centrifuged. GSH was determined using dithionitrobenzoic acid (DTNB) as prescribed by Ellman (1954).

The activity of glutathione peroxidase in liver homogenate was assayed with a coupled enzyme system (Wendel,1980) where GSSG reduction was coupled to NADPH oxidation by glutathione reductase. The oxidation of NADPH was followed spectrophotometrically at 340 nm.

Results were expressed as mean values ± standard error. A value of $p < 0.05$ was considered to be significant (Fisher,1950).

RESULTS AND DISCUSSION

As shown in table 1, cadmium and mercury both induced lipid peroxidation in rat liver. However, a concommitant treatment with selenium inhibited (36 and 32%) the rate of peroxidative decomposition in both the groups.

Formation of reduced glutathione was stimulated in the liver of cadmium fed rats. Further, higher values were obtained in rats treated with cadmium and selenium both. Similar results were obtained in the liver of mercury and selenium fed rats also.

Results on glutathione peroxidase, however, did not exhibit a strict relationship with reduced glutathione or malondialdehyde. Nevertheless, the enzyme activity declined after selenium treatment (Table 1).

Causative role of lipid peroxidation in metal toxicity has been studied by number of workers (Sugawara, 1984; Rana and Kumar, 1984; Stacey and Kappus, 1982; Sato et al., 1983). It was Ganther (1978) who pointed out that selenium could be a part of various systems defending against lipid peroxidation. Present observations support that cadmium and mercury when fed with selenium fail to raise TBA chromogens and also confirm their interaction with GSH. Selenium like other anti-oxidants may protect tissue damage directly through conjugation reactions or by enhancing the formation of reduced

Table 1. Malondialdehyde, GSH and glutathione peroxidase in the liver of rats fed on cadmium and mercury with selenium.

Group No.	Treatment	Malondialydehyde (n moles/mg protein)	GSH (ug/g fresh liver)	GSH peroxidase (n moles NADPH used/ mg protein/minute
A	Cadmium	39.00 ± 4.08***	1700 ± 2.93***	59.32 ± 2.90**
B	Cadmium + Sodium selenite	33.90 ± 4.00**	3800 ± 3.07***	26.40 ± 1.69***
C	Mercury	32.80 ± 3.01**	1400 ± 2.68 N.S.	20.57 ± 0.97***
D	Mercury + sodium selenite	28.25 ± 3.62*	1600 ± 1.94***	17.18 ± 0.83***
E	Control	14.20 ± 2.64	1400 ± 2.68	43.40 ± 1.81

Values are mean ± S.E. of five observations in each group.

'p' = * < 0.02; ** < 0.01; *** < 0.001 (Control vs experimental rats).

N.S.- Not significant.

glutathione. Present results agree with this statement. According to some authors Se-Hg and Se-Cd interaction is brought about by endogenous glutathione which reduces selenite to a selenide compound (Iwata et al 1981). The high lipo-affinity of this compound may alter their distribution and toxicity in critical tissues as suggested by Masukawa et al (1982).

Treatments of cadmium and mercury with selenium were found to raise GSH level in the liver. A number of xenobiotics can raise hepatic glutathione (Kaplowtiz, 1981). GSH is also known to be involved in metabolism and detoxication of endogenous and exogenous substances (Ketterer et al 1983). Moreover, glutathione dependent enzymes could have an important function in Se-Hg and Se-Cd antagonism. Since glutathione peroxidase is a Se-dependent enzyme, the subject needs further research. Flohe (1979) studied the kinetic behaviour of GSH peroxidase and concluded that selenocysteine residues of GSH peroxidase shuttle between different redox states during catalysis. At physiological levels, however, the enzyme is largely reduced. Secondly, GSH peroxidase may prevent lipid peroxidation by scavanging hydrogen peroxide thereby slowing down H_2O_2 dependent free radical attack on lipids. However, overlapping mechanisms of other enzymes like glutathione-S-transferase and catalase can not be overlooked. A recent report from this laboratory (Rana et al., 1990) suspects a functional competition between catalase and glutathione peroxidase. The data presented in this communication convincingly support that one of the avenues through which selenium can antagonize with cadmium or mercury involves glutathione and glutathione peroxidase.

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REFERENCES

Chen RW, Wanger PA, Hockstra WG and Ganther HE (1974) Affinity labelling studies with Cadmium 109 and Cadmium induced testicular injury in rats. J Reprod Fertil 38:293

Cherian GM and Vostal JJ (1977) Biliary excretion of Cadmium in rat. Dose dependent biliary exretion and the form of cadmium in the bile. Toxicol Environ Health 2:945-954

Fisher RA (1950) Statistical methods for research workers 11th ed. London: Oliver and Boyd

Flohe L (1979) In "Oxygen free radicals and tissue damage", Ciba Foundation Symposium. 65-95

Ganther HE (1978) Modification of methyl mercury toxicity and metabolism by selenium and vitamin E: possible mechanism. Environ Hlth Perspect 25:71-76

Iwata H, Masukawa T, Kitto H, Hayashi M (1981) Involvement of tissue sulfhydryls in the formation of a complex of methyl mercury with selenium. Biochem Pharmacol 30:3159-3163.

Jordan RA and Schenkman (1982) Relationship between malondialdehyde production and archidonate consumption during NADPH supported microsomal lipid per- oxidation. Biochem Pharmacol 31: 1393-1400

Kaplowitz N (1980) The physiologic significance of the glutathione-s-transferases. Am J Physiol 239:439-444

Kaplowitz N (1981) The importance and regulation of hepatic glutathione. The Yale J Biol Med 54: 497-502

Ketterer B, Coles B, Meyer DJ (1983) The role of glutathione in detoxication. Environ Hlth Perspect 49:56-69

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193:265-275

Masukawa T, Kitto H, Hayashi M, Iwata H (1982) Formation and possible role of bis (methyl mercuric) Selenide in rats treated with methyl-mercury and selenite. Biochem Pharmcol 31:75-78

Rana SVS, Rastogi S, Boora PR, Agarwal S (1990) Pathophysiological significance of catalase in liver injury. Life Sci.(In Press)

Refsvik T (1978) Excretion of methyl mercury in rat bile, the effect of diethylmaleate, cyclohexene oxide and acrylamide. Acta Pharmacol Toxicol 135:141-42

Sato M, Yamanobe K, and Nagai Y (1983) Sex related differences in cadmium induced lipid-peroxidation in rat.Life Sci 33:903-908

Stacey NH and Kappus H (1982) Cellular toxicity and lipid peroxidation in response to mercury. Toxicol Appl Pharmacol 33:903-908.

Stowe HD (1976) Biliary excretion of cadmium by rats: effects of zinc, cadmium and selenium pretreatments. J.Toxicol Environ Health 2:45-53

Suguwara N and Suguwara C (1984) Selenium protection against testicular lipid-peroxidation from cadmium. J Appl Biochem 6:199-204

Wendel A (1980) Glutathione-peroxidase. In : Jakoby WB (ed.) Enzymatic basis of detoxication. Academic Press, New York, pp.333-353

Whanger PD (1976) Selenium versus metal toxicity in animals. In: Proceedings Symposium selenium telluruim in the environment, Industrial Health Foundation,Pittsburgh PA,pp.234

Wisniewska-Knypl JM and Kolakowski J (1984) Stimulation of lipid peroxidation and heme oxygenase activity with inhibition of cytochrome p-450 monoxygenase in the liver of rats repeatedly exposed to cadmium. Toxicology 32:267-276

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