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Protective Effects of GSH, α-Tocopherol, and Selenium on Lipid-Peroxidation in Liver and Kidney of Copper Fed Rats

S V. S. Rana, S. Verma

Toxicology Laboratory, Department of Zoology, Ch. Charan Singh University, Meerut, 250 004, India

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Copper (Cu) deficiency and copper toxicity, both are known to occur in natural conditions (Underwood, 1977). Liver functions as the central organ in copper metabolism therefore, its damage has been an important feature of all copper storage diseases, As copper induced liver damage has invariably been associated with lipofuscin pigment, Lindiquist (1968) suggested that liver cell damage was caused by Cu-mediated lipidperoxidation. Hochstein *et.al.,* (1980) further suggested that copper might cause lipid peroxidation of erythrocyte membrane by inhibiting glycolytic enzymes and by a concomitant decrease of glutathione concentration in erythrocytes. Although, the ability of copper to catalyse peroxidation of unsaturated fatty acids has been confirmed *in vitro,* the stimulation of lipid peroxidation by copper *in vivo* has been demonstrated only in vitamin E or selenium deficient rats. (Daugherty and Hoekstra, 1982). These findings were corroborated by Dillard and Tappel (1984) who observed increased TBA chromogens in liver, kidney and blood of copper sulphate treated but Vitamin E deficient rats. Moreover, a positive correlation has been observed between copper excretion and glutathione depletion. Therefore, selected nutrients viz. vitamin E, selenium and glutathione seem to be good candidates for further study on their antioxidative activity.

The present report describes the effects of GSH, vitamin E and selenium on lipid peroxidation in the liver and kidney of copper treated rats. Simultaneous effects on copper accumulation and glutathione cycle have been described in this communication.

MATERIALS AND METHODS

Adult (140 ± 10 gms) male Charles Foster rats (*Rattus rattus* albino)

Correspondence to: S. V. S. Rana

selected from the laboratory stock were housed individually in polypropylene cages and maintained under standard laboratory conditions (room temperature 25 ± 5 °C and relative humidity 50-60%). They were offered commercial food pellets (Lipton, India) and tap water *ad libitum.*

After brief acclimatization to these conditions, rats were divided at random into five groups, each containing five rats. Rats of group A were fed on predetermined sublethal doses of copper sulphate (15 mg/100 gm body weight) by gavage on each alternate day for thirty days. Rats of group B were subjected to the same treatment but also injected 30 mg/100 gm body weight of reduced glutathione (Sigma, USA) intraperitoneally on each alternate day for thirty days. Rats of group C were offered copper and α-tocopherol (0.4 international units/100 gm body weight as a suspension in 10% sucrose) both. Similarly rats of group D were fed on copper and sodium selenite (15 mg/100 gm body weight) on each alternate day. Rats of group E were administered normal saline so as to serve as controls.

All the rats were starved overnight on 31st. day and sacrificed by decapitation the next morning. Pieces of liver and kidney were quickly removed and processed for the estimation of malondi-aldehyde by thiobarbituric acid (Placer *et.al.* 1966). Protein was estimated by the method suggested by Lowry *et.al.* (1959).

Other samples of liver and kidney were perfused by ice cold 1.15% potassium chloride, homogenised with four volumes of distilled water and centrifuged. Reduced glutathione was estimated using 5,5, dithiobis-2-nitrobenzoic acid as prescribed by Ellman (1954).

Oxidised glutathione was estimated following the method suggested by Ohmori (1986).

A few pieces of liver and kidney were dried at 100 °C and then ashed at 600 °C. The ash was dissolved in concentrated nitirc acid and perchloric acid (5:1), diluted with double distilled water and analysed quantitatively for copper by inductively coupled plasma emission spectrophotometer, at USIC University of Roorkee (India). Intergroup comparisons were made using student's "t" test Glutathione, α-tocopherol, thiobarbituric acid and 5-5 dithiobis-2 nitrobenzoic acid were procured from Sigma (USA). Sodium selenite and copper sulphate were supplied by E. Merck, Bombay.

RESULTS AND DISCUSSION

Observations on tissue distribution revealed that these nutrients restrict absorption and distribution of copper in the liver and kidney both. Maximum restriction was offered by α-tocopherol followed by selenium and reduced glutathione (Table 1).

Malondialdehyde was significantly high in liver and kidney of copper fed rats, however, α-tocopherol manifested maximum inhibition followed by selenium and reduced glutathione (Table II).

Effects of copper on glutathione cycle were simultaneously studied. More GSSG was available in the liver and kidney both. It further improved on coadministration of antioxidants. However no appreciable improvement in GSH content could be recorded after the treatment with these nutrients. Nevertheless, maximum improvement in total glutathione content was recorded in liver of copper and selenium treated rats.

Table 1. Copper distribution in liver and kidney of rats fed on copper with glutathione, α-tocopherol and selenium.

Group No.	Treatments	Cu (mg/kg)	
		Liver	Kidney
A	Copper	4.19 ± 0.18***	3.27 ± 0.15***
B	Copper + glutathione	2.37 ± 0.12^{NS}	1.78 ± 0.08^{NS}
C	Copper + α-tocopherol	1.77 ± 0.08*	1.85 ± 0.08^{NS}
D	Copper + selenium	1.38 ± 0.09**	1.99 ± 0.08*
E	Control	2.08 ± 0.10	1.83 ± 0.12

Values are mean ± SE of 5 ovbservations in each group.
'P' = *<0.02; **<0.01; ***<0.001 (control versus experimental rats)
NS denotes not significant.

Table 2. Malondialdehyde in liver and kidney of rats fed on copper with glutathione, α-tocopherol and selenium

Group No.	Treatments	Malondialdehyde (n moles/mg protein)	
		Liver	Kidney
A	Copper	0.393 ± 0.45***	0.225 ± 0.25**
B	Copper + glutathione	0.157 ± 0.013^{NS}	0.148 ± 0.042^{NS}
C	Copper + α-tocopherol	0.112 ± 0.11*	0.127 ± 0.010^{NS}
D	Copper + selenium	0.115 ± 0.002*	0.168 ± 0.03*
E	Control	0.140 ± 0.02	0.139 ± 0.015

Values are mean ± SE of 5 ovbservations in each group.
'P' = *<0.02; **<0.01; ***<0.001 (control versus experimental rats)
NS denotes not significant.

Table 3. Reduced and oxidised glutathione (GSH and GSSG) in liver and kidney of rats fed on copper with glutathione, α-tocopherol and selenium.

Group No.	Treatments	GSH (μgm/gm)		GSSG (μgm/gm)	
		Liver	Kidney	Liver	Kidney
A	Copper	1480 ± 6.5***	1250 ± 5.45***	178 ± 7.5***	160 ± 5.4***
B	Copper + glutathione	358 ± 9.4***	248 ± 8.5***	265 ± 5.45***	176 ± 7.25***
C	Copper + α-tocopherol	488 ± 7.6***	265 ± 7.2***	305 ± 9.8***	266 ± 3.5***
D	Copper + selenium	865 ± 3.9***	350 ± 9.5***	242 ± 4.54***	196 ± 6.8***
E	Control	1400 ± 4.68	800 ± 4.95	56 ± 0.25	32 ± 0.20

Values are mean ± SE of 5 ovbservations in each group.
'P' = *<0.02; **<0.01; ***<0.001 (control versus experimental rats)
NS denotes not significant.

Metal ion toxicity has long been related with lipidperoxidation (Sunderman, 1986). However, many workers argue that lipid peroxidation appears to be the consequence of other toxic effects. Inorganic compounds stimulate lipid peroxidation indirectly and not by influencing the oxidative status of cells (Stacey and Kappus, 1982). Copper salts are known to stimulate the decomposition of preformed lipid peroxides to generate alkoxy and peroxy radicals that are the true initiators of lipid peroxidation (Halliwell and Gutteridge, 1984). Present results further indicate that liver is more susceptible to this process than kidney. However, a diet rich in these nutrients inhibit the generation of aldehyde radicals. This observation is supported by the fact that GSH, α-tocopherol and Se decreased tissue Cu load and consequently influenced the process of lipid peroxidation. GSH, Se and α-tocopherol protection seemingly do not follow identical mechanism(s) for this action. We postulate that GSH chelates copper like methionine as suggested by Chaberel and Martell (1959) with an equilibrium constant of 14.75. Copper can complex with glutathione through peptide bonds analogous to the biuret reaction. A low Cu/GSH ratio (after its chelation), allows all the copper to associate with sulphydryl group, possibly as GS-Cu-GS (Albro *et.al.* 1986).

Both Se and α-tocopherol are known to protect biological membranes from oxidative degradation. Chen and co-workers (1993) advanced the hypothesis that vit. E functions as a specific lipid soluble antioxidant in the membrane and that Se functions as the component of cytosolic GSH-peroxidase that reduces peroxides. Although Se-Cu relationships are now known, the mechanism advanced for the detoxifying effect of Se on Hg is the formation of an inactive protein-Hg adduct.

An antagonistic interrelationship between Cd and Se has been reported (Rana and Boora, 1992). Nevertheless, it is suggested that like Se-Hg and Se-Cd, selenium-copper interaction can be brought about by endogenous glutathione that 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 and Rana and Boora (1992). This observation thus explains how Se can alter Cu-distribution in target tissues and thereby alter the progress of peroxidative processes.

Selenium seems to influence glutathione through selenium dependent activity of glutathione peroxidase. Se may function in the metabolism of sulphydryl groups. Liver from Se deficient rats had a higher content of total non-protein sulphydryl groups compared with Se

supplemented rats. Possible biochemical site for the injurious effects of selenium seems to be the removal of sulphydryl groups essential for oxidative processes.

In brief, we can safely conclude that the pathobiological event of copper toxicity can be prevented by supplementing diet with one of these nutrients. A combined effect of all the three nutrients being studied by us might be helpful in elucidating further the biochemical basis of CU-GSH, Cu α-tocopherol and Cu-selenium interactions.

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