Bull. Environ. Contam. Toxicol. (1984) 32:405–409

Bulletin of Environmental Contamination and Toxicology

Copper and Zinc in CCl_4 Treated Rats

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The role of two trace metals, copper and zinc, are important in maintaining blood pressure and the effect of carbon tetrachloride (CCl_4) has been found to be a depressor. Copper concentrations increase in liver tissue during exposure to CCl_4 (Lal & Sourkes 1970), and zinc has been found to protect against liver damage due to CCl_4. The influence of trace elements on metabolic pathways is extensive. Copper has been found to inhibit the activity of angiotensin converting enzyme activity, (Bakhle et al. 1971; Dorer et al. 1970). An inter-relationship between copper and zinc metabolism has been reported. (Prasad et al 1978). Both copper and zinc have been found to inhibit the pressor effect of cadmium exposure to rats (Perry et al. 1979).

Experimental renal hypertension has been reduced to normotension after multiple subcutaneous injections of CCl_4, (Loyke et al. 1960), (Loyke 1965). By dose adjustment, the degree of liver damage has been reduced to a level of mild to moderate degree of fatty metamorphosis of the liver (Loyke 1964). Cross perfusion of renal hypertensive rats with CCl_4 treated rats resulted in blood pressure reduction suggesting that a depressor substance was present in the CCl_4 treated animal's blood (Loyke & Hoobler 1982). It is possible that the depressor effect could be mediated by imbalance of copper and/or zinc. In the present study, copper and zinc levels were determined following CCl_4 treatment. Although the studies cited above were completed in renal hypertensive rats, the present work used normotensive rats treated for the same period which, in hypertensive animals, caused the blood pressure to fall.

MATERIALS AND METHODS

Ten male Sprague Dawley rats approximately 8 to 9 weeks old obtained from Beaumanor Farm were housed in individual cages. Purina lab chows and tap water were given ad lib. Blood pressure was measured by the tail microphonic method, (Friedman & Freed 1949), and all animals weighed weekly. Six male Sprague Dawley rats initially weighing 200g were given 0.15ml analytical CCl_4 subcutaneously twice weekly except for the last week for a total of 25 doses. Four animals were untreated and from the same source sampled on the same day as the experimental group. Blood samples were obtained 6 hours after a normal feeding, one day after the last doses of CCl_4, for determination of serum copper and zinc by the atomic absorption graphic furnace method (Peaston 1973). Student's t test was used for computing the significance between CCl_4 and control samples.

RESULTS AND DISCUSSION

Pretreatment weight for the CCl_4 group averaged 200g and the untreated controls weighed 198g. At the end of the experiment, the average weight of the CCl_4 group was 261g $\pm$ 4.01 and of the untreated controls 260g $\pm$ 5.7 (Table 1). The initial systolic blood pressure averaged 120 $\pm$ 2.4mmHg for the CCl_4 group and 125 $\pm$ 3.1mmHg for the controls. The final systolic blood pressures were 136 $\pm$ 4.01mmHg for the CCl_4 and 133 $\pm$ 1.66 for the untreated animals. The mean copper values were 37.6 $\pm$ 2.8ug/dl for the CCl_4 and 80.0 $\pm$ 9.2ug/dl for the control group (Table 1). The mean zinc levels were 1.83 $\pm$ .15ug/dl for the CCl_4 and 2.5 $\pm$ .66ug/dl in the untreated group (Table 1). Using the "t" test for small samples, a significant difference was found ($P > .005$) between the copper values of the CCl_4 and the untreated animals although the zinc levels of the CCl_4 treated were lower, but not to a statistically significant degree ($P > 0.1$), probably due to the small amounts of zinc present.

A statistically significant lower serum value for copper was found after chronic CCl_4 treatment in our male rats compared to untreated controls. Cikrit et al (1975) studying similarly CCl_4 treated female rats found no change in copper values 24 hours after an intravenous load of copper. Their plasma values for copper were greater than our levels probably because of the added injected copper. Since copper has not been found to be pressor (Perry et al. 1979), this metal may be depressor and associated with the formation of the vasodepressor substance demonstrated in

Table 1. Copper and Zinc Levels in Normotensive CCl_4 Treated and Untreated Rats

Animals	CCl_4	Copper (ug/dl)	Zinc (ug/dl)	Systolic blood pressure	Final weight (grams)
n=6	+	6	6	6	6
X=		37.6	1.83	136.6	261.6
SD=		6.91	.37	9.6	9.8
SE=		2.82	.15	4.0	4.0
n=	0	4	4	4	4
X=		80.0	2.5	133.3	260.0
SD=		26.4	1.15	2.8	10
SE=		9.2	.66	1.66	5.7
P=		>.005	>.1	>.5	>.5

our previous report (Loyke & Hoobler 1982). Likewise, using angiotensin I, as a substrate, Huggins et al (1970) found that copper inhibited the activity of angiotensin converting enzyme. Our previous experiments have shown that CCl_4 treatment inhibited angiotensin converting enzyme (Loyke 1965).

After an intravenous zinc load Cikrit et al. (1975) found that plasma zinc levels did not differ between CCl_4 exposed and control rats. Our serum zinc levels were lower in the CCl_4 treated compared to the control animals. This decrease in serum zinc levels in the CCl_4 treated rats was not statistically significant probably due to the low level of mineral in serum. Zinc in low doses has been found by Saldeen (1969) to protect against liver damage due to CCl_4. Since zinc is a constituent of a number of vitally important enzymes (Valley 1955), it is conceivable that a lower level of zinc could alter these enzymes and produce a vasodepressor substance. Furthermore, zinc has been found to be one of the most consistent inhibitors of normal converting enzyme activity with angiotensin I as a substrate (Bakhle et al. 1971) (Dorer et al. 1970).

Cikrit et al. (1975) found that CCl_4 given twice a week (1ml.Kg) for three months to Wistar female rats

did not alter the total excretion of copper or zinc compared to the control rats. The livers impaired by CCl_4 excreted more zinc via bile while the copper excretion did not change (Cikrit et al. 1975). Only a small part of the CCl_4 exposed animals in the Cikrit et al. (1975) experiment exhibited a picture of cirrhosis while the majority of the others merely showed changes which might have led to cirrhosis. Our studies with chronic low dose CCl_4 treatment has shown the rat to develop only a moderate to mild degree of fatty metamorphosis (Loyke 1964).

Copper and zinc were found to be able to inhibit the induction of hypertension by high level cadmium exposure in rats, (Perry et al. 1979). Chronic exposure to CCl_4 by chronic subcutaneous administration did not alter the systolic blood pressure of our normotensive rats. These animals gained weight and did not demonstrate outward signs of toxicity.

Liver damage produced by chronic subcutaneous CCl_4 injections results histologically in only a moderate degree of hepatic fatty metamorphosis (Loyke 1964). No renal tubular changes were found after the CCl_4 injections and adrenal tissue was reported as normal (Loyke 1964).

In conclusion, serum values for copper decreased significantly after chronic CCl_4 treatment. Zinc levels were not significantly lowered in CCl_4 treated rats serum. Because of low levels of copper and especially zinc following the CCl_4 treatment, alterations singly or together may participate in enzymatic alterations and be a factor in the appearance of a vasodepressor substance found in CCl_4 blood.

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Received August 25, 1983; accepted September 22, 1983.