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Effect of Intoxication with Vanadium Compounds on Copper Metabolism in the Rat

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Vanadium is required in comparatively small quantities for normal growth and differentiation in all organisms. Whether the element is essential and what its precise function is remains difficult to ascertain because the reported evidence is inconclusive (Nielsen 1984; Golden and Golden 1981).

Man's activities in petroleum and metallurgical refining have magnified naturally high concentrations of the metal in certain crude oils and ores (Gough and Severson 1976). The adverse effects of human exposure to the combustion products of vanadium-bearing residual oils and to fumes and dusts in metallurgical refining have stimulated interest in vanadium toxicology. Studies in animals and man have shown that vanadium compounds cause the destructive manifestations in many systems. Signs of toxicity and clinical symptoms associated with vanadium poisoning, include conjunctivitis, rhinitis, pharyngitis, bronchitis, ataxia, tremors, paralysis, depression and cardiovascular disorders (Waters 1977).

The mechanisms by which vanadium exerts its toxic effects are poorly understood, but interference with the normal kinetics and macromolecular binding of the body's other essential metals, such as Zn, Ca, Mg, Fe, Cu may play a significant role. It is well known that nonessential metals, such as Pb, Hg and Cd, exert toxic effects by interacting with essential elements, thereby adversely affecting various metabolic processes (Goyer 1978; Prohaska and Ganther 1977; Chertok et al. 1981).

The purpose of the present study was to determine the influence of intoxication with vanadium compounds ($NaVO_3$ and $VOSO_4$) on the intestinal transport of copper. Serum levels of copper and ceruloplasmin and copper content in the liver were also assayed.

MATERIALS and METHODS

Male Wistar rats 60±10 g used in the experiments were maintained on standard laboratory chow with free access to water.

In the acute exposure study, rats (6 per group) were starved overnight and given orally a single dose of $NaVO_3$-12 mg V/kg (20% LD_{50}) or $VOSO_4$-15 mg V/kg (20% LD_{50}). Control animals received an equal volume of saline. The animals were sacrificed by decapitation 1; 2; 4 and 24 h after treatment with vanadium salts.

In the subchronic study, vanadium was administered to rats orally as $NaVO_3$ in a dose of 3 mg V/kg (5% LD_{50}) or as $VOSO_4$ in a dose of 3.75 mg V/kg (5% LD_{50}) six times a week for 3 months. Control animals received saline. Six rats from each group, experimental and control were sacrificed by decapitation 2; 4; 6; 8; 10 and 12 weeks of intoxication.

The active transport of copper was measured in the proximal duodenal segment of rat intestine using the author's modification of the everted gut-sac technique (Wilson and Waseman 1954; Wróbel et al. 1973). A 7 cm segment of everted intestine, securely ligated at each end was filled with 0.7 ml of buffer solution containing 50 mM imidazol, 20 mM glucose, 100 mM D-mannitol, 70 mM NaCl, 0.4 mM $CuCl_2$ and 8 mM sodium phosphate adjusted to a pH of 7.4. Next, the sac was placed into a 25 ml Erlenmeyer flask containing 5.0 ml of the same buffer. All incubations were conducted for 1 h at 37°C under an atmosphere of O_2 with shaking.

After the incubation period 0.2 ml of mucosal (outer) and serosal (inner) fluids were taken for the total copper measurments. Copper was assayed by atomic absorption spectrophotometry (Jerral Ash, wavelength 324.7 nm, acetylene/air). The ratio of the final Cu concentration in the serosal medium (S) to the final concentration of Cu in the mucosal medium (M) was designated S/M and used as an index of active copper transport.

The levels of copper in serum and liver were determined using atomic absorption spectrophotometry.

Ceruloplasmin in fresh serum samples was measured by the method of Colombo and Richterich (1964) and expressed as mg ceruloplasmin/100 ml.

Statistical evaluation was carried out using the Student's t-test.

RESULTS and DISCUSSION

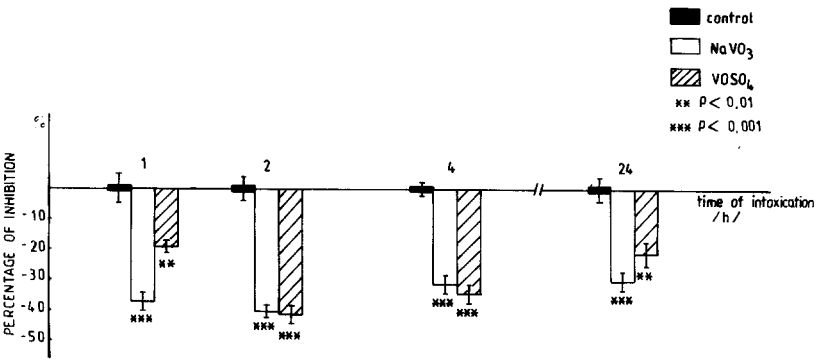

Figure 1. Inhibition of copper transport in the rat duodenum after acute intoxication with vanadium compounds. The transport ratio (S/M) for control was 1.90±0.10.

Table 1. Copper and ceruloplasmin contents in the serum of rats given orally a single dose of sodium metavanadate or vanadyl sulphide.

Time of intoxication (h)	Copper (µg/ml)		
	Control	$NaVO_3$	$VOSO_4$
1	1.33±0.04 (5)	1.31±0.05 (6)	1.31±0.05 (5)
2	1.37±0.03 (6)	1.23±0.06 (6)	1.41±0.04 (5)
4	1.34±0.04 (6)	1.16±0.06[a] (6)	1.17±0.06[a] (6)
24	1.37±0.03 (6)	1.51±0.04[a] (5)	1.51±0.03[a] (6)
	Ceruloplasmin (mg/100 ml)		
	Control	$NaVO_3$	$VOSO_4$
1	43.4±2.7 (5)	43.8±1.6 (6)	45.2±4.9 (6)
2	48.9±2.7 (6)	48.2±5.5 (6)	53.7±4.9 (6)
4	49.1±5.3 (6)	56.3±2.9 (5)	38.9±2.0 (6)
24	38.9±4.4 (5)	32.3±1.6 (5)	33.8±3.6 (6)

a $P < 0.05$ Number of animals in parentheses.

Fig.1 illustrates the effect of acute intoxication with vanadium compounds on the intestinal transport of copper. In both experimental groups copper transfer through the duodenum was significantly decreased.

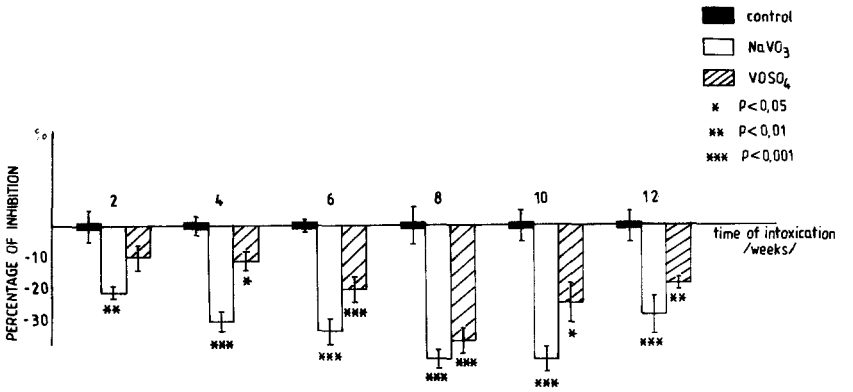

Figure 2. Inhibition of copper transport in the rat duodenum during subchronic intoxication with vanadium compounds.
The control levels expressed as S/M were 1.79 ± 0.08; 1.60 ± 0.05; 1.96 ± 0.04; 2.53 ± 0.16; 2.05 ± 0.09 and 2.03 ± 0.09 after 2; 4; 6; 8; 10 and 12 weeks respectively.

Serum Cu and ceruloplasmin levels for both control and vanadium-treated animals are given in Table 1. In the group of rats poisoned with $NaVO_3$ and those intoxicated with $VOSO_4$ the serum Cu contents were significantly decreased after 4 h of intoxication (by 13% below control value) whereas after 24 h the concentrations of the element in the V-treated animals were higher than in controls.

There were no differences in serum ceruloplasmin levels between control and $NaVO_3$ or $VOSO_4$-exposed rats.

The subchronic investigation confirmed and extended the acute observations. It was shown that the inhibition of copper transfer was more pronounced in the group of rats poisoned with $NaVO_3$ than in those treated with $VOSO_4$ (Fig. 2). In both groups of rats the mean ratio of S/M in the duodenum was significantly suppressed over the entire experimental period.

Generally, the oral administration of vanadium compounds did not cause a significant alterations of serum copper concentrations (Table 2). The significant decrease in this parameter was observed only after 4 ($VOSO_4$) and 6 weeks of intoxication ($VOSO_4$ and $NaVO_3$). The results in Table 2 reveal that continuous oral administration of vanadium compounds produced significant changes in the level of serum ceruloplasmin. In the group of rats intoxicated with $NaVO_3$ the activity of ceruloplasmin was decreased after 4; 6 and 8 weeks of poisoning by 18% ($P < .01$), 26% ($P < .01$) and 24% ($P < .01$) respectivelv

Table 2. Copper and ceruloplasmin contents in the serum and copper concentrations in the liver of rats during subchronic intoxication with $NaVO_3$ or $VOSO_4$.

		Time of intoxication (weeks)					
		2	4	6	8	10	12
Serum Cu (µg/ml)	Control	1.57±0.12 (6)	1.45±0.07 (5)	1.61±0.06 (6)	1.58±0.06 (5)	1.45±0.06 (5)	1.44±0.10 (5)
	$NaVO_3$	1.37±0.10 (6)	1.39±0.07 (5)	1.36±0.07[a] (6)	1.48±0.09 (5)	1.49±0.10 (4)	1.49±0.05 (4)
	$VOSO_4$	1.26±0.13 (5)	1.24±0.08[a] (6)	1.35±0.09[a] (6)	1.49±0.09 (5)	1.44±0.05 (6)	1.42±0.07 (6)
Serum ceruloplasmin (mg/100ml)	Control	48.2±2.7 (6)	48.2±2.0 (6)	35.6±2.2 (6)	43.6±1.2 (5)	37.7±1.9 (4)	39.6±1.5 (5)
	$NaVO_3$	40.2±3.9 (6)	39.5±0.6[b] (6)	26.5±2.1[b] (6)	33.3±1.0[b] (5)	36.6±2.7 (5)	34.5±1.0 (6)
	$VOSO_4$	50.5±3.7 (6)	40.0±1.5[b] (6)	31.6±2.0 (6)	35.8±1.1[b] (5)	37.3±2.5 (6)	34.1±2.8 (6)
Liver Cu (µg/g)	Control	6.37±0.26 (5)	6.42±0.42 (5)	6.28±0.28 (5)	5.96±0.13 (5)	6.19±0.28 (5)	6.48±0.25 (6)
	$NaVO_3$	6.08±0.15 (5)	5.50±0.22 (6)	5.33±0.21[a] (6)	4.54±0.28[a] (5)	4.96±0.28[a] (4)	5.19±0.34[a] (5)
	$VOSO_4$	6.27±0.37 (6)	6.08±0.27 (4)	5.07±0.20[a] (6)	4.44±0.20[b] (5)	5.18±0.13[a] (5)	5.50±0.28[a] (4)

a $P < .05$; b $P < .01$

Number of animals in parentheses

as compared with control. After 4 and 8 weeks serum levels of this parameter in $VOSO_4$-treated animals dropped by about 17% ($P < .01$) below control.

Both vanadium compounds cause a significant decrease in liver copper levels (Table 2). The Cu content decreased significantly in $NaVO_3$ and $VOSO_4$-treated animals after 6; 8; 10 and 12 weeks of intoxication.

The present studies clearly indicate that vanadium compounds ($NaVO_3$ and $VOSO_4$) in the various oxidation states (+5 and +4) may influence copper metabolism in rats. It was shown that intoxication with $NaVO_3$ and $VOSO_4$ significantly reduced the intestinal absorption of Cu. The inhibitory effect was elicited both by acute (single oral dose) and subchronic (12 weeks) administration. Furthermore, it was also found that the levels of ceruloplasmin in serum and the concentrations of copper in liver were decreased in the rats exposed to continuous oral administration of vanadium compounds.

The mechanism of vanadium interaction with copper is unknown but certain conclusions may be drawn.

It is known that active as well as passive transport is involved in the movement of copper from the intestinal lumen to the serosal side of the gut. The intestinal absorption of copper in mammals may also be regulated by at least two copper bindings proteins. Using ligated jejunal segments of rats El-Shobaki and Rummel (1979) have shown that copper is bound to mucosal transferrin and to metallothionein, a small cysteine rich molecule important for intracellular transport of this element.

The inhibitory effect of V-compounds on copper transport through the intestinal wall found in our studies may be associated with their ability to reduce active transport. It was shown that vanadate is a potent inhibitor of Na,K-ATPase and other phosphoryl transfer enzymes in vitro (Ghijsen et al. 1982; Higashino et al. 1983; Cantley et al. 1978; North and Post 1984). This has resulted in the hypothesis that vanadium functions in vivo as a regulator of Na,K-ATPase and thus the sodium pump. Moreover vanadium uncouples mitochondrial oxidative phosphorylation in liver homogenates in vitro (Hatchcock et al. 1966), inhibits succinic dehydrogenase (Aiyar and Sreenivasan 1961) resulting in the depletion of ATP energy stores.

There is also some evidence to suggest that vanadium may directly inhibit synthesis of cystine and cysteine with overall lowering of serum protein sulfhydryl groups. This reduction in cystine content may be related

to excretion of cystine in the urine (cystinuria) because an increase of the neutral sulphur fraction, which is indicative of cystinuria, was observed in the urine of rats fed vanadium (Faulkner-Hudson 1964). Cystinuria is known to be associated with Wilson's disease, in which there is a genetically determined decrease in serum level of the copper protein - ceruloplasmin (ferroxidase I), a plasma globulin implicated in iron metabolism. The decrease in ceruloplasmin level was found in our studies.

A number of elements e.g. Cd, Mn, Mo, Ag, Hg are known to alter the serum levels of ceruloplasmin and Cu content in the liver. For example zinc and molybdenum sulfate in the diet have been shown to diminish uptake of copper by the liver (Gaballah et al. 1965). Manganese on the other hand produces an increase in the hepatic contents of Cu in rats (Murthy et al. 1981). In our studies a significant decrease in the copper concentrations in the liver was demonstrated. This is in agreement with the work of Kennan (1963) who found that in spectrographic analysis of livers of animals exposed to vanadium, the intensity of copper lines diminished as vanadium intensity increased. Though the mechanism by which the liver copper decreases after vanadium compounds administration is not clear, it might be due to the displacement of copper from the liver and/or to the decrease in copper binding protein level. This aspects also require further investigations.

REFERENCES

Aiyar AS, Sreenivasan A (1961) Effect of vanadium administration on CoA metabolism in rats. Proc Soc Exp Biol Med 107:914-916

Cantley LC, Cantley LG, Josephson L (1978) A characterization of vanadate interactions with the (Na,K)-ATPase. J Biol Chem 253:7361-7368

Chertok PJ, Sasser LB, Callaham MF, Jarboe GE (1981) Influence of cadmium on the intestinal uptake and absorption of calcium in the rat. J Nutr 111:631-638

Colombo JP, Richterich R (1964) Zur Bestimmung des Caeruloplasmins im Plasma. Schw Med Wschr 94:715-720

El-Shobaki FA, Rummel W (1979) Binding of copper to mucosal transferrin and inhibition of intestinal iron absorption in rats. Res Exp Med 174:187-195

Faulkner-Hudson TG (1964) Vanadium: Toxicology and Biological Significance. Elsevier, Amsterdam pp.102-110

Gaballah SS, Abood LG, Caleel GT, Kapsalis A (1965) Uptake and biliary excretion of ^{64}Cu in rabbits in relation to blood ceruloplasmin. Proc Soc Exp Biol Med 120:733-735

Ghijsen WE, Jong MD, Van Os CH (1982) ATP-dependent calcium transport and its correlation with Ca^{2+}-ATP ase activity in basolateral plasma membrans of rat duodenum. Biochem Biophys Acta 689:327-336
Golden MHN, Golden BE (1981) Trace Elements. Potential importance in human nutrition with particular reference to zinc and vanadium. Br Med Bull 37:31-36
Gough LP, Severson C (1976) Impact of point source emissions from phosphate processing on the element content of plants and soils. In: Hemphill DD (ed) Trace Substances in Environmental Health. Columbia, University of Missouri p. 225
Goyer RA (1978) Calcium and lead interaction. Some new insights. J Lab Clin Med 91;363-365
Hatchcock JN, Hill ChH, Tove SB (1966) Uncoupling of oxidative phosphorylation by vanadate. Can J Biochem 44:983-986
Higashino H, Bogden JD, Lavenhar MA, Bauman JE (1983) Vanadium, Na,K-ATPase and potassium adaptation in the rat. Am J Physiol 244:F105-F111
Kennan RG (1963) Unpublished data in: Mountain JT Arch Environ Health 6:357-362
Murthy RC, Srivastava RS, Chandra SV (1981) Serum ceruloplasmin in manganese-treated rats. Toxicol Lett 7:217
Nielsen FH (1984) Ultratrace elements in nutrition. Ann Rev Nutr 4:21-41
North P, Post RL (1984) Inhibition of (Na,K)-ATP ase by tetravalent vanadium. J Biol Chem 259:4971-4978
Prohaska JR, Ganther HE (1977) Interaction between selenium and methylmercury in rat brain. Chem-Biol Interact 16:155-167
Waters MD (1977) Toxicology of vanadium. Adv Mod Toxicol 2:147-189
Wilson TH, Wiseman G (1954) The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. J Physiol 123:110-132
Wróbel J, Michalska L (1973) The effect of sodium and some other alkali cations on calcium transport in the rat duodenum. Acta Biochim Pol 20:249-254
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