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Influence of Intoxication with Vanadium Compounds on the Intestinal Absorption of Calcium in the Rat

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Calcium is transferred to the plasma after absorption from the gastrointestinal tract and by resorption from the bone.

It is recognized that many environmental poisons, e.g. heavy metals, pesticides etc. cause alterations in calcium homeosthasis in human beings and experimental animals. For example it is generally agreed that cadmium (Chertok et al. 1981), lead (Goyer 1978) and tin (Yamaguchi et al. 1982) affect the intestinal absorption of calcium. Although vanadium is not considered to be as important a health hazard to man as lead or cadmium it must be nevertheless regarded as a dangerous pollutant. There exists an obvious risk of pollution by and poisoning due to the high vanadium content of crude oil and the industrial use of vanadium as a steel additive (Waters 1977).

The toxic effects of this element and its compounds in many biological systems have been reviewed in detail (Waters 1977; Nielsen 1982) but little is known about vanadium influence on calcium metabolism.

The present study was undertaken to determine the effect of various treatments with vanadium compounds, containing vanadium as VO^{2+} ($VOSO_4$) and VO_3^- ($NaVO_3$) ions, exert on calcium transport through the rat duodenum.

MATERIALS and METHODS

Male Wistar rats 5 weeks old, with body weights ranging from 50 to 60 g were used in all experiments. They were fed a standard laboratory diet and received water ad libitum.

In the acute experiment the rats were randomly assigned to three groups. The rats were starved overnight and given orally a single dose of $NaVO_3$ (12 mg V/kg or 20% DL_{50}) or $VOSO_4$ (15 mg V/kg or 20% DL_{50}). Control

animals received an equal volume of saline. Rats were sacrificed by decapitation 1; 2; 4; 6 and 24 h after treatment with vanadium compounds.

In the subchronic study 54 rats were used. Vanadium was administered to rats orally as $NaVO_3$ in a dose of 3 mg V/kg (5% DL_{50}) or as $VOSO_4$ in a dose of 3.75 mg V/kg (5% DL_{50}) six times a week for 42 days. Animals which received an equal volume of saline were served as control. Six rats from each experimental and control group were sacrificed after 2,4 and 6 weeks of intoxication. In this experiment the body weight gain and weights of the liver, the right kidney and the right femur carefully separated from adjacent tissue were recorded regularly for the control and experimental animals throughout the study.

Transport of calcium in the rat duodenum was studied in vitro by the method of everted gut sacs described by Wilson and Wiseman (1954) and modified by Wróbel and Michalska (1973).

The animals were sacrificed and a 10 to 15 cm segment of the intestine proximal to the pyloric sphincter was freed from the mesenteric attachments, isolated, everted on a stainless steel rod and throughly washed in cold 0.9% NaCl. The duodenum sacs (5.0 cm long) were filled with 0.5 ml of a buffer solution containing 50 mM imidazol, 20 mM glucose, 100 mM D-mannit, 70 mM NaCl, 0.4 mM $CaCl_2$ and 8 mM sodium phosphate adjusted to a pH of 7.4. Next the sacs were placed in an Erlenmayer flasks containing 5.0 ml of the same buffer. The media were saturated with O_2 and the flasks were shaken at 100 strokes/min in metabolic shaker at 37°C for 30 min.

After an incubation period 0.2 ml of mucosal (outer) and 0.2 ml of serosal (inner) fluids were taken for the total calcium measurments. Calcium was assayed by AAS (Jerral Ash).

The transport of calcium was expressed as the ratio (S/M) of the total Ca concentration (μg/0.2 ml) in the serosal and the mucosal medium.

In the calcium transport study levels of Ca in serum were determined using AAS method.

Additionally 18 rats were randomly assigned to three groups of six rats each and used for study of ^{45}Ca tissue distribution. Fourty-two days after the pretreatment with $NaVO_3$, $VOSO_4$ or saline (control) as described in the subchronic study, each rat was given

orally 10 µCi $^{45}CaCl_2$ in saline solution. Next the animals were placed in metabolic cages equipped for the collection of urine and feces and received food and water ad libitum.

The rats were sacrificed 24 h after $^{45}CaCl_2$ administration and the tissues (liver, duodenum, kidney, white muscle, brain, heart and femur) were removed. The slices of each organ (weight about 150-200 mg) and feces (200 mg) were then solubilized in 1.0 ml of the tissue solubiliser (NCS) overnight at 50°C. After dissolution 0.1 ml of glacial acetic acid and 10.0 ml of toluene scintillation fluid were added. The samples containing the serum and urine (0.2 ml) were filled with scintillation fluid only. The radioactivity of samples were measured with an ISOCAP 300 liquid scintillation spectrometer.

The results for tissues were expressed as dpm/g wet wt, for serum as dpm/0.2 ml and urine and feces as dpm/total value daily excreted.

Statistical evaluations were carried out using the Student's t-test.

RESULTS and DISCUSSION

The results of calcium transport through the rat duodenum after the acute vanadium compounds intoxication are shown in Table 1. In rats which received 12 mg V/kg as $NaVO_3$ calcium transfer was significantly decreased 2; 4; 6 and 24 h after treatment (respectively 31%, 24%, 25% and 10% below control values) whereas after 1 h the transport ratio (S/M) did not differ statistically from control animals.

In group of rats treated with $VOSO_4$ (15 mg V/kg) the greatest inhibition of calcium transfer was observed 2; 4 and 6 h after intoxication (by 30% as compared with control). After 1 and 24 h no significant changes in the S/M ratio were found.

Both vanadium compounds did not cause the significant alterations of serum calcium levels.

Continuous oral administration of vanadium compounds confirmed and extended observations from the acute exposure. The mean ratio of S/M in the duodenum of rats treated with $NaVO_3$ was suppressed throughout the experimental period (Table 2). After 4 and 6 weeks of intoxication the calcium transport was decreased respectively by 24% ($P<.001$) and 37% ($P<.001$) as compared with control. In the same experimental periods the values of S/M in the animals which received $VOSO_4$

Table 1. The mean ratio (S/M) of calcium in the serosal, S , and in the mucosal fluid, M , and calcium levels in serum after acute intoxication with $NaVO_3$ and $VOSO_4$.

Time of intoxication (hours)	Ca (S/M ± SD)			Calcium level in serum (mg/100 ml)		
	Control	$NaVO_3$	$VOSO_4$	Control	$NaVO_3$	$VOSO_4$
1	2.00±0.02 (6)	1.92±0.04 (6)	1.89±0.10 (6)	10.24±0.14 (6)	9.96±0.21 (6)	10.10±0.11 (6)
2	2.41±0.03 (6)	1.67±0.02^c (6)	1.77±0.03^b (6)	9.87±0.15 (6)	9.84±0.18 (6)	10.28±0.35 (6)
4	1.94±0.03 (6)	1.46±0.11^b (6)	1.30±0.01^c (6)	8.93±0.18 (6)	8.61±0.18 (6)	8.75±0.05 (6)
6	3.33±0.24 (6)	2.50±0.18^a (6)	2.28±0.29^a (6)	9.52±0.16 (5)	9.46±0.15 (5)	9.53±0.12 (6)
24	2.38±0.04 (6)	2.23±0.04^a (6)	2.59±0.10 (6)	9.13±0.16 (5)	9.21±0.12 (6)	9.40±0.08 (6)

Table 2. The mean ratio (S/M) of calcium in the serosal, S , and in the mucosal fluid, M , and calcium levels in serum after subchronic intoxication with $NaVO_3$ and $VOSO_4$.

Time of intoxication (weeks)	Ca (S/M ± SD)			Calcium level in serum (mg/100 ml)		
	Control	$NaVO_3$	$VOSO_4$	Control	$NaVO_3$	$VOSO_4$
2	2.42±0.08 (6)	2.17±0.10 (6)	2.37±0.09 (6)	10.02±0.30 (6)	10.19±0.16 (6)	10.19±0.05 (6)
4	2.35±0.08 (6)	1.78±0.05^c (6)	1.98±0.06^b (6)	10.17±0.16 (6)	10.16±0.33 (6)	10.14±0.14 (6)
6	2.78±0.14 (6)	1.74±0.06^c (6)	2.12±0.07^b (6)	10.86±0.53 (6)	11.19±0.38 (6)	11,50±0.42 (6)

a $P < .05$; b $P < .01$; c $P < .001$

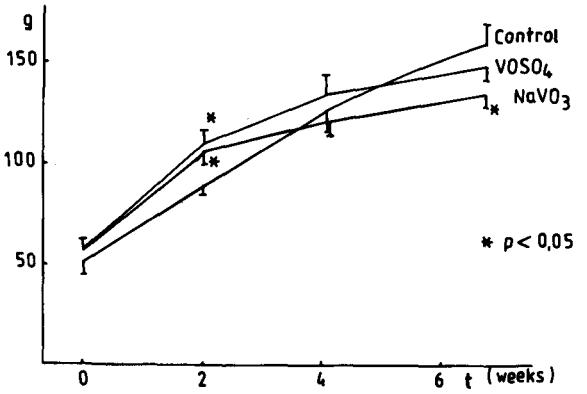

Figure 1. The body weight of rats during subchronic intoxication with vanadium compounds.

Each value is the mean ± SD of six rats.

dropped by about 15% (P<.01) and 25% (P<.01) below control. In both groups there were no significant changes in this parameter after 2 weeks of intoxication.

There were no differences in calcium concentrations in serum between control and vanadium-treated animals.

In the subchronic exposure the body weight of the control animals increased from 50±5 g to 160±10 g. After 2 weeks the $NaVO_3$ and $VOSO_4$-treated animals showed an increase in their body weight. Nevertheless at the end of experiment (6 weeks) those rats weighed less than the control. Their weight amounted to 84% ($NaVO_3$) and 94% ($VOSO_4$) of the body weight of the control group (Fig. 1).

The wet weights of livers, kidneys and femurs are shown in Table 3. The significant changes in these parameters were observed only after 6 weeks of intoxication. The animals which received $NaVO_3$ had significantly heavier livers (P<.05), kidneys (P<.05) and femurs (P<.05) while in the group of rats which were treated with $VOSO_4$ the livers were lighter (P<.05) and the femurs heavier (P<.05).

The results of ^{45}Ca distribution in the rat tissues are given in Fig. 2. In the $NaVO_3$-exposed rats the radioactivity of ^{45}Ca was significantly decreased in the liver, brain, heart, femur, kidney and serum and increased in the urine and feces. In the $VOSO_4$-treated animals the content of ^{45}Ca in the duodenum, brain, femur and serum fell down. However the excretion of ^{45}Ca into the feces and urine was greater as compared

Table 3. Liver, kidney and femur wet weight (g/100 g body weight).

Tissue	Group of animals	Time of intoxication (weeks)		
		2	4	6
Liver	Control	3.87±0.14 (6)	3.63±0.07 (6)	3.50±0.20 (6)
	$NaVO_3$	3.77±0.07 (4)	3.74±0.07 (5)	4.26±0.20[a](4)
	$VOSO_4$	3.91±0.17 (4)	3.72±0.05 (5)	3.79±0.07 (6)
Kidney	Control	0.52±0.02 (6)	0.41±0.02 (6)	0.38±0.01 (6)
	$NaVO_3$	0.51±0.02 (6)	0.41±0.01 (6)	0.42±0.01[a](4)
	$VOSO_4$	0.50±0.02 (6)	0.40±0.01 (6)	0.35±0.01[a](6)
Femur	Control	0.39±0.01 (6)	0.39±0.01 (6)	0.29±0.01 (6)
	$NaVO_3$	0.41±0.01 (6)	0.36±0.01 (6)	0.34±0.02[a](4)
	$VOSO_4$	0.39±0.01 (6)	0.37±0.01 (6)	0.33±0.01[a](6)

a $P < .05$

to control.

No difference could be detected in the radioactivity in the liver, kidney, muscle and heart between the control and the $VOSO_4$-exposed rats.

To summarize it was shown that the acute as well as the subchronic intoxication with vanadium compounds ($NaVO_3$, $VOSO_4$) in the various oxidation states (+5 and +4) caused inhibition of calcium transfer through the duodenal wall. Also the isotopic studies demonstrated that the residual effects of continuous $NaVO_3$ and $VOSO_4$ administration changed the distribution of ^{45}Ca in the rat tissues and body fluids.

The mechanism of vanadium compounds action on calcium metabolism is unknown but it is possible that they may influence calcium homeosthasis by any of three ways: (1) vanadium may affect the absorption of calcium in the gastrointestinal tract either directly or indirectly by influencing vitamin D activity, (2) vanadium may alter parathyroid glands activity or (3) it may affect the renal regulation of the calcium-phosphorus balance.

The biological action of vanadium has been subject of

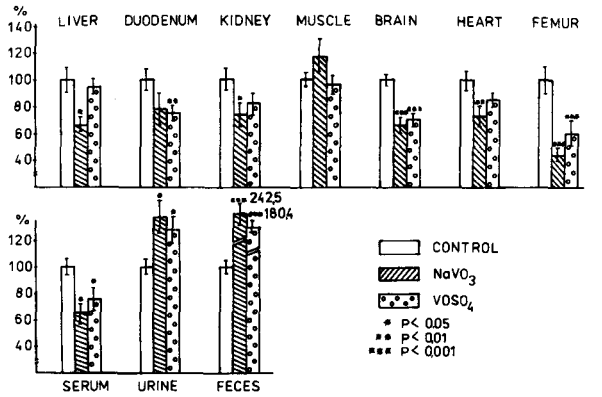

Figure 2. Concentrations of ^{45}Ca in the rat tissues, serum, urine and feces after six weeks intoxication with $NaVO_3$ or $VOSO_4$ expressed as a percent of control. For details see "Material and Methods". Each value is the mean ± SD of six rats.

intensive research ever since it was found that it is an efficient inhibitor of Na,K-ATP ase (Cantley et al.1978; Hansen 1983). Next O'Neal et al. (1979) and Bond and Hudgins (1980) have reported that vanadium compounds are potent inhibitors of calcium stimulated ATP ase and alkaline phosphatase (Lopez et al. 1976; Ghijsen et al.1982). Norman et al. (1970) have demonstrated that an increase in alkaline phosphatase activity exactly parallels a similar increase in calcium transport activity. These studies suggest that inhibition of the ATP ase and alkaline phosphatase activities by vanadium may be responsible for the decreased active calcium transport across the intestine found in our studies. Moreover it is widely known that vanadium compounds uncouple mitochondrial oxidative phosphorylation in the liver homogenates in vitro (Hatchcock et al.1966) and inhibit succinic dehydrogenase (Aiyar and Sreenivasan 1961) resulting in the depletion of ATP energy stores.

The kidneys as well as bone and liver are the principal targets of vanadium (Hansard et al. 1982) so this element may inhibit the activity of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol. Finally, vanadium may influence some endocrine functions e.g. the parathyroid glands (Peabody et al. 1976).

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