Absorption, Tissue Accumulation, and Toxicity of Vanadium in Mature Hamsters and Developing Chick Embryos Administered with Various Vanadium Compounds

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ABSTRACT

Absorption and tissue accumulation of vanadium were determined in mature hamsters fed purified diets containing $NaVO_3$, $VOSO_4$ or vanadyl-cysteine methyl ester at each 10 and 20 ppm as V for a week. The absorption of V from $NaVO_3$ was slightly higher than that from $VOSO_4$ or vanadyl-cysteine methyl ester. Tissue accumulation of V was the highest in the kidneys and second highest in the liver. In order to know the toxicity of vanadate and vanadyl compounds and to find effective agents to cope with the toxicity, a model system using fertile chick eggs was established. Aqueous solution of those V compounds and / or aqueous solution of EDTA-2Na were separately introduced into the air sacs of 14-day-old fertile eggs through holes in eggshells and the treated eggs were further incubated for 2 or 5 days. The accumulation of V in the legs (metatarsus) and toes became an indicator of V absorption and both the embryonic growth and the survival rate were adversely affected by the administration of V compounds. In this experiment vanadyl appeared to be more toxic than vanadate. When $VOSO_4$ and EDTA-2Na were separately introduced into the air sacs, the complex was formed in the air sacs and the toxicity of V was greatly alleviated since such a complex as VO-EDTA was not absorbed from the air sacs to the embryos as efficiently as $VOSO_4$ itself.

INTRODUCTION

Although vanadium (V) is regarded as an essential trace element for the rat and the chick, it becomes a toxic element when excess amounts of V compounds are taken. The tissue accumulation of V from vanadate or vanadyl compounds has been studied by using rats[1-3], chicks[1] and sheep[4]. In the first part of the present work $NaVO_3$ (V-5) as vanadate compound and $VOSO_4$ (V-4) and vanadyl-cysteine methyl ester (Vanadyl)[5] as vanadyl compounds were orally administered to mature hamsters for a week

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and the absorption and tissue accumulation of V were determined. One reason for using hamsters was that hamsters have the forestomach similar to the rumen in herbivores.

In the second part of the present work a new model system of using fertile chick eggs was examined to determine the absorption and toxicity of V from various V compounds. Such V compound was introduced into the air sacs of 14-day-old chick eggs and the treated eggs were further incubated for 2 or 5 days. If some chelating compound such as EDTA is also introduced into the air sacs at the same time, the complex may be formed in the air sacs between a V compound and EDTA and the V absorption might be affected adversely and the toxicity of V will be alleviated.

MATERIALS AND METHODS

Mature male golden hamsters adapted to a purified diet consisting of casein 19.0%, corn starch 45.5%, sucrose 22.7%, soybean oil 2.0%, cellulose 7.0%, and minerals and vitamins 3.8% to afford the NRC requirement of minerals and vitamins for the rat were used for the metabolism and slaughter experiments. Six experimental diets supplemented with V-5, V-4 or Vanadyl at two dietary V levels of about 10 and 20 ppm to the purified diet were prepared and fed for a week and total feces and urine excreted were quantitatively collected in the last 3 days. After a week the animals were slaughtered and the heart, lungs, liver, kidneys and heparinized whole blood were taken for analyses of V. Tissue samples equal to 0.5 g dry matter were immersed into 4 ml H_2SO_4 in Hach Digesdahl (Colorado, USA) and digested at 400℃ by adding H_2O_2. The digested samples were diluted to 100 ml and the V contents were analyzed by using a flameless atomic absorption spectrophotometer (Hitachi Z-8000).

Fertile chick (White Leghorn) eggs were incubated at 37.5℃ and about 80% relative humidity in an incubator with a constant rotating device. Two small holes (ID 1.5 mm) were made in the eggshells just above the air sacs of 14-day-old fertile eggs and a 100 μl solution of the above V compound or VO-EDTA (Dozin Kagaku Co., Kumamoto, Japan) and a 100 μl solution of EDTA-2Na were separately introduced into the air sacs and after the holes were coated with vaseline, the eggs were incubated for a further 2 or 5 days. Then wet embryonic weight was measured and after the joints between the tibia and the metatarsus were cut off, the weight of all legs (metatarsus) and toes was measured. The legs and toes were dried and weighed and subjected to nitric acid digestion with small amounts of H_2SO_4 and H_2O_2. The V contents of the digested samples were analyzed by flameless atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

Data of metabolism and slaughter experiments are shown in Table 1. The absorption rate of V defined as 100 (V intake - V in feces) / V intake showed significant differences between the groups administered V-5 and Vanadyl. Although no significant difference in the V absorption between the groups administered V-5 and V-4, the V absorption (14-15%) in the groups administered a vanadate compound

Table 1. Metabolism experiments of mature hamsters fed purified diets containing $NaVO_3$, $VOSO_4$ or vanadyl-cysteine methyl ester (Vanadyl) and tissue accumulation of vanadium

Feed V level (ppm)	$NaVO_3$		$VOSO_4$		Vanadyl		Pooled SE
	9	16	10	19	10	21	
No. of animals	3	3	3	3	3	3	
(During 3 days collection period)							
Feed intake (g)	15	16	16	17	17	15	1
Water intake (g)	26	40	37	46	31	28	6
V intake (μg)	134	248	164	328	167	327	10
V in feces (μg)	116	216	147	301	153	300	19
V in urine (μg)	15	22	12	23	13	22	2
Absorption rate (%)	14^{a}	15^{a}	11	8	9^{b}	8^{b}	6
(After 1 week of feeding; in fresh weight basis)							
V in heart (ppb)	73	66	60	75	68	65	4
V in lungs (ppb)	61^{c}	84^{bc}	63^{c}	105^{b}	110^{b}	156^{a}	8
V in liver (ppb)	256^{b}	400^{a}	248^{b}	371^{a}	238^{b}	351^{a}	12
V in kidneys (ppb)	519^{cd}	783^{ab}	405^{d}	709^{bc}	581^{bcd}	981^{a}	49
V in blood (ppb)	95^{c}	134^{ab}	106^{bc}	161^{a}	110^{bc}	133^{ab}	7

abcd : There were significant differences ($p < 0.05$) between the values of different letters.

appeared to be slightly higher than those (8-11%) administered vanadyl compounds. However, this absorption rate did not take into account the endogenous fecal loss of V. Apparently feces were a main excretion route for ingested V. According to other works[6,7] the absorbed vanadate is rapidly reduced and vanadyl is a prevalent intracellular form of V compounds.

In Table 1 the highest V concentration was found in the kidneys and second highest in the liver. In these tissues the tissue V concentrations reflected the dietary V levels. This trend is the same as with previous works[2,3]. However, the V concentrations of the heart were the lowest and did not change significantly with the V species or dietary V levels. In the lungs the administration of Vanadyl which is an organic V compound showed significantly higher tissue V concentration than those of other V compounds at both lower and higher dietary V levels. In other tissues there were no significant differences of tissue V concentration between inorganic (V-4) and organic (Vanadyl) vanadyl compounds except in the kidneys of higher dietary V level.

In Fig. 1 the growth curve of normal chick embryos during a 21 days incubation period is shown. Dotted lines show the treatment period. In this period of 14 to 19 days the largest embryonic growth occurred and the treatment such as the V administration might exert a more augmented growth depressing effect compared with other periods of a lesser growth rate. In Table 2 the same V compounds used in hamsters were administered into the air sacs of 14-day-old fertile eggs at dose levels of 0.25, 0.50 and 0.75 μ mole and the incubation was continued for a further 2 days. Embryonic growth rate of Vanadyl was significantly larger than that of V-4 at all dose levels and than that of V-5 at lower and middle

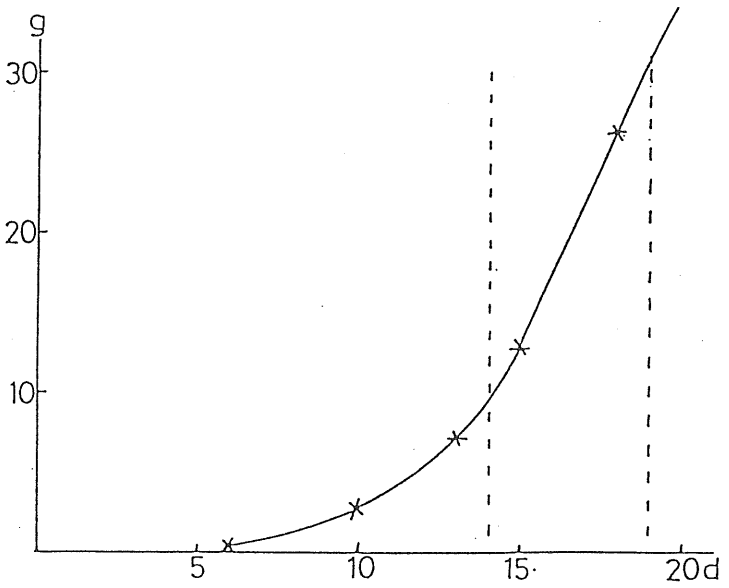

Fig. 1. Growth curve of chick embryos during 21 days incubation.

Table 2. Effects of administration of $NaVO_3$, $VOSO_4$ or vanadyl-cysteine methyl ester (Vanadyl) into the air sacs of 14 days-fertile chick eggs on the growth of embryos and vanadium accumulation in embryonic legs (metatarsus) and toes during 2 days from 14 to 16 days

Treatment (μmole)	Embryos (wet g)	Legs and toes (wet g)	Legs and toes (dry g)	V content of legs and toes (ppm in DM)	Mortality no. in total no.
None	17.1^{ab}	0.80^{ab}	0.122^{ab}	0.11^{e}	0/3
$NaVO_3$-0.25	14.4^{cde}	0.63^{cd}	0.094^{cde}	4.04^{bcd}	0/4
-0.50	14.9^{cd}	0.66^{cd}	0.102^{bcd}	5.70^{bc}	0/6
-0.75	15.4^{bcd}	0.68^{bcd}	0.099^{cde}	8.36^{a}	0/4
$VOSO_4$-0.25	14.0^{de}	0.60^{cde}	0.089^{def}	2.82^{de}	1/4
-0.50	13.1^{e}	0.49^{e}	0.075^{f}	4.70^{bcd}	2/6
-0.75	13.9^{de}	0.58^{de}	0.080^{ef}	4.50^{bcd}	2/4
Vanadyl-0.25	17.3^{a}	0.84^{a}	0.123^{a}	3.74^{cd}	0/5
-0.50	16.9^{ab}	0.79^{ab}	0.116^{abc}	5.50^{bc}	0/4
-0.75	15.5^{bc}	0.70^{bc}	0.104^{bcd}	6.38^{ab}	2/5

abcdef: There were significant differences ($p<0.05$) between the values of different letters.

levels. However, as the experiment of Vandyl was done in a different time period from that of others, the conclusion might be suspended until a further experiment. The administration of V compounds contributed to significant increases in the V concentration of legs and toes. The embryonic death occurred only in the administration of V-4 and Vanadyl, which suggested that for the present experimental system

vanadate which is a known inhibitor of Na, K-ATPase was no more toxic than vanadyl. For the mouse LD_{50} for vanadate compounds is far less than that for vanadyl compounds[8-10], but for the chick both compounds are equally toxic[1]. According to our unpublished data the dose of V-5 causing 50% embryonic death during a 14 to 19 days incubation period was 0.8 μ mole, whereas that of V-4 or Vandyl was 0.55 μ mole. One of the reasons for the species difference may be that the chick and the chick embryo have more sufficient reducing agents in the body than the mouse and that the chick embryo might be more susceptible to a shortage of oxidizing agents.

Table 3 shows the effects of V-4 with or without EDTA and VO-EDTA administered on the embryonic growth, tissue V accumulation and mortality rate during 5 days incubation. The V-4 administration only significantly decreased the embryonic growth rate and significantly increased the V accumulation and the mortality rate. However, the administration into the air sacs of EDTA at the same time contributed to counteract the adverse effects of V-4. The complex between V-4 and EDTA was formed in

Table 3. Effects of administration of $VOSO_4$ (0.78 μ mole), $VOSO_4$ (0.78 μ mole) plus EDTA-2Na (1.0 μ mole) or VO-EDTA complex (0.75 or 1.0 μ mole) into the air sacs of 14 days-fertile chick eggs on the growth of embryos and vanadium accumulation in embryonic legs (metatarsus) and toes during 5 days from 14 to 19 days

Treatment	Embryo (wet g)	Legs and toes (wet g)	Legs and toes (dry g)	V content of legs and toes (ppm in DM)	Mortality no. in total no.
None	26.0^{ac}	1.66^{a}	0.270^{a}	0.20^{a}	$1/20^{a}$
$VOSO_4$	19.2^{b}	1.25^{b}	0.205^{b}	7.49^{b}	$28/46^{b}$
$VOSO_4$+EDTA	23.4^{ad}	1.57^{ac}	0.252^{ac}	2.44^{a}	$2/18^{a}$
VO-EDTA-0.75	27.7^{c}	1.76^{a}	0.286^{a}	1.55^{a}	$0/10^{a}$
-1.0	21.2^{bd}	1.39^{bc}	0.222^{bc}	1.94^{a}	$1/15^{a}$

abcd: There were significant differences ($p < 0.05$) between the values of different letters.

the air sacs and this complex might not pass through the eggshell membrane or the complex might not be absorbed into the embryo through blood vessels of allantoic and york sac membranes as efficiently as V-4 itself. This speculation was examined by administering VO-EDTA itself into the air sacs as shown in Table 3. At approximately the same dose level, VO-EDTA did not cause the adverse effects as those caused by V-4 administration and the V accumulation into the legs and toes from VO-EDTA did not increase significantly. In the rat EDTA completely prevents the toxicity of V apparently by preventing its absorption from the intestinal tract[1]. Judging from these results this experimental method may be suitable for the screening test of many chelating compounds to cope with the toxicity of various V compounds.

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