Effect of Vanadium (IV and V) on the Hemolysis of Vitamin E-deficient Erythrocytes of Hamsters

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ABSTRACT

Subcutaneous injection of $NaVO_3$ to vitamin E-deficient hamsters contributed to increase the *in vitro* RBC hemolytic index probably because of depletion of anti-oxidants. *In vitro*, although $NaVO_3$ was not hemolytic, $VOSO_4$ at 1 mM was a hemolytic agent for vitamin E-deficient RBC in Hepes-saline buffer.

INTRODUCTION

Vanadium is an essential trace mineral but it causes toxicity in higher animals.[1] In our previous work[2] we determined absorption and tissue accumulation of vanadyl or vanadate compounds using hamsters and chick embryos. In this work we examined the effects of vanadyl(V4) or vanadate(V5) administration on the susceptibility of vitamin E-deficient RBC to the *in vitro* homolysis. We administered $VOSO_4$ and $NaVO_3$ subcutaneously to hamsters fed a vitamin E-deficient or E-sufficient diet and determined the *in vitro* RBC hemolytic index and plasma vitamin E contents one day after the treatment. We also examined whether V4 or V5 compound became an *in vitro* hemolytic agent for vitamin E-deficient RBC when RBC suspension in Hepes-saline buffer was incubated with one of these compounds at 37.5℃.

MATERIALS AND METHODS

Mature male golden hamsters were fed a vitamin E-deficient or E-sufficient purified diet shown in Table 1. We injected subcutaneously 100 μmole of either $NaVO_3$ or $VOSO_4$ into the backs of hamsters of about 120 g body weight with saline. Then 24 h later we collected blood into heparinized tubes. One volume of blood was diluted with 25 volumes of saline and the RBC portion obtained by centrifugation was resuspended with the same volumes of Hepes-saline buffer (pH 7.4) containing 25 mM Hepes (Good's buffer) and 125 mM NaCl. Hemolytic procedure adopted was the same as shown before.[3] Final concen-

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Table 1. Composition (%) of vitamin E-deficient purified diet for hamsters

Casein	19.0
Starch	66.7
Soybean oil	2.5
Linseed oil	2.5
Cellulose	5.5
Vitamin mix.	0.5*
Mineral mix.	3.3**

*One kg of purified diet contained 2.2 mg retinyl palmitate, 25 μg ergocalciferol, 0.5 mg menadione, 1.16 g choline chloride, 1 mg folic acid, 20 mg nicotinamide, 8 mg Ca pantothenate, 3 mg riboflavin, 5 mg thiamine nitrate, 6 mg pyridoxine, and 50 μg cyanocobalamin. (Vitamin E-sufficient diet contained 100 mg DL-alpha-tocopheryl acetate per kg.)

**One kg of diet contained $CaCO_3$ 8.66 g, $CaHPO_4 2H_2O$ 6.58 g, $MgSO_4 7H_2O$ 4.06 g, KH_2PO_4 12.54 g, NaCl 1.27 g, $CuSO_4 5H_2O$ 19.65 mg, $MnSO_4 H_2O$ 153 mg, KI 0.2 mg, $ZnSO_4 7H_2O$ 53 mg, $FeSO_4 7H_2O$ 174 mg, Na_2SeO_3 0.22 mg and NaF 2.2 mg.

trations of Tween 20, ascorbic acid and sodium azide were 0.001%, 1.16 mM and 5.26 mM, respectively and the RBC suspension was incubated 30 min at 37.5℃. Plasma vitamin E was determind by measuring the fluorescence of hexane extract at 295 nm excitation and 340 nm emission as shown before.[4] For the *in vitro* experiment, Tween 20 with ascorbic acid and azide, 4.9 mM H_2O_2, 1 mM $VOSO_4$ with or without 5.26 mM sodium azide, or 1 mM $NaVO_3$ with or without 5.26 mM sodium azide were used to incubate with the RBC suspension in Hepes-saline buffer 1 h at 37.5℃.

RESULTS AND DISCUSSION

In Table 2 vitamin E-deficient animals showed significantly lower plasma vitamin E levels than vitamin E-sufficient ones. Although RBC hemolysis tended to be higher in vitamin E-deficient groups than in vitamin E-sufficient ones, only V5-administered minus-E group showed significantly higher hemolysis. In Fig. 1, five in eight animals of the V5-administered minus-E group showed the highest RBC hemolysis with the lowest plasma vitamin E level. The correlation coefficient between log (plasma vitamin E content) and hemolytic percentage was −0.785 in all animals used. V5 administration may cause more depletion of anti-oxidants such as GSH and vitamin E than V4 administration since *in vivo* vanadate is reduced to vanadyl by intracellular GSH.[5]

Vitamin E-deficient RBC showed much higher hemolysis than vitamin E-sufficient RBC in such hemolytic procedures as Tween 20 with ascorbic acid and azide or H_2O_2 (Fig. 2). Plasma vitamin E content of

Table 2. Effects of subcutaneous injection of $NaVO_3$ (V5) or $VOSO_4$ (V4) on the hemolysis of vitamin E-deficient and E-sufficient RBC (mean ± SD)

Treatment	Diet fed	Plasma vitamin E μg/ml	RBC hemolysis %
None (3)	minus E	1.93 ± 0.64^{a}	31.0 ± 22.9^{ab}
None (3)	plus E	9.20 ± 4.10^{b}	7.2 ± 0.6^{a}
V5 (8)	minus E	2.03 ± 1.18^{a}	55.9 ± 43.2^{b}
V5 (8)	plus E	12.10 ± 4.41^{b}	4.8 ± 3.9^{a}
V4 (5)	minus E	3.10 ± 1.82^{a}	21.9 ± 24.1^{a}
V4 (5)	plus E	9.45 ± 4.60^{b}	8.3 ± 2.9^{a}

The figures in parentheses in Treatment are number of animals used.
There were significant differences ($p < 0.05$) among the values denoted by different superscripts.

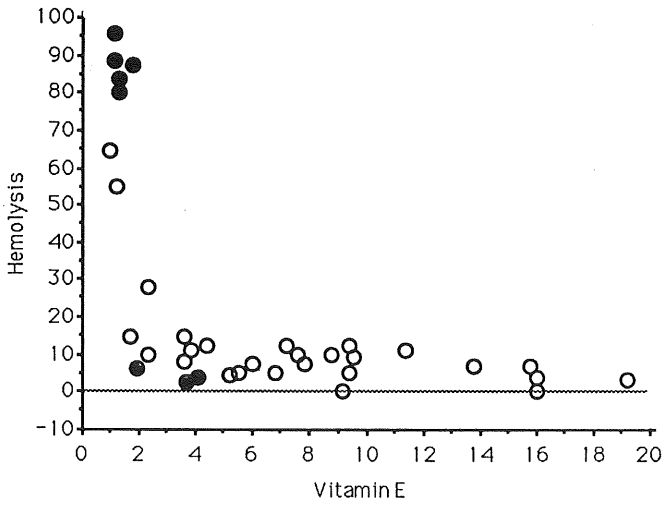

Fig. 1. Scattergram for RBC hemolysis (Y%) and plasma vitamin E content (X μg/ml) in hamsters treated as shown in Table 2
(Black circles show V5 treatment, while open circles the other treatments.)

E-deficient hamsters used here was 1 μg/ml, while that of E-sufficient one was 4 μg/ml. Although V5 at 1 mM could not induce hemolysis, V4 at 1 mM induced extensive hemolysis only in vitamin E-deficient RBC. In contrast to the *in vivo* experimental results, V4 became a hemolytic agent *in vitro* while V5 did not. Addition of azide to V4 or V5 increased hemolysis significantly but only to a small extent. According to Hogan[6], the tetravalent vanadium is the most effective in promoting rupture of isolated erythrocytes and in depressing the erythrocyte count *in vivo*. Vanadyl is the active form of vanadium in initiating conjugated diene formation in micelles from purified fatty acids and hydroxyl radicals are shown to be involved.[7]

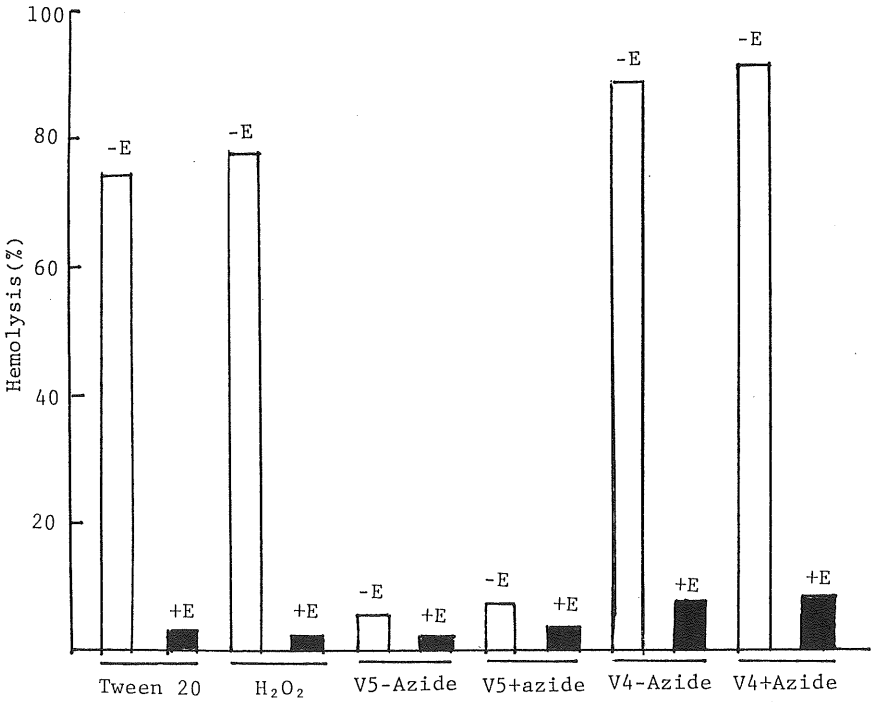

Fig. 2. Comparison of in vitro RBC hemolysis (%) of Tween 20 with ascorbate and azide, H_2O_2, $NaVO_3$ (V5) with and without azide, or $VOSO_4$(V4) with and without azide between vitamin E-sufficient and E-deficient RBC. Each value was the mean of three measurements.

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