Vanadium Distribution in Rats and DNA Cleavage by Vanadyl Complex: Implication for Vanadium Toxicity and Biological Effects

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Vanadium ion is toxic to animals. However, vanadium is also an agent used for chemoprotection against cancers in animals. To understand both the toxic and beneficial effects we studied vanadium distribution in rats. Accumulation of vanadium in the liver nuclei of rats given low doses of compounds in the +4 or +5 oxidation state was greater than in the liver nuclei of rats given high doses of vanadium compounds or the vanadate (+5 oxidation state) compound. Vanadium was incorporated exclusively in the vanadyl (+4 oxidation state) form. We also investigated the reactions of vanadyl ion and found that incubation of DNA with vanadyl ion and hydrogen peroxide (H_2O_2) led to intense DNA cleavage. ESR spin trapping demonstrated that hydroxyl radicals are generated during the reactions of vanadyl ion and H_2O_2. Thus, we propose that the mechanism for vanadium-dependent toxicity and antineoplastic action is due to DNA cleavage by hydroxyl radicals generated in living systems. — Environ Health Perspect 102(Suppl 3):35-36 (1994).

Key words: vanadium, distribution, neutron activation analysis, electron spin resonance (ESR), DNA cleavage, hydroxyl radicals, spin trapping

Vanadium ion is known to be toxic to animals (1). However, vanadium was recently reported to be an agent for chemoprotection against cancers in animals (2). The mechanisms for both toxic and beneficial effects of vanadium are not fully understood. When vanadium compounds in the +4 or +5 oxidation state are given to animals, the vanadium is found exclusively in the vanadyl form (VO_2^+)(3). In living systems, hydrogen peroxide (H_2O_2) is formed by dismutation of superoxide anion radicals (O_2^-), which are generated in several systems such as xanthine oxidase, NADPH oxidase, and NADPH-dependent cytochrome P-450 systems. Thus, hydrogen peroxides are expected to react with vanadyl ion to generate active oxygen species such as hydroxyl radicals (·OH). The generated ·OH may cleave DNA molecules.

To understand the effects of vanadium, we investigated the distribution of vanadium and found that the metal ion in the +4 oxidation state is accumulated in the nuclei of the liver of rats. Further, the ·OH-dependent DNA cleavage was also found. Based on these results, we propose here a new mechanism of a vanadyl-dependent DNA cleavage in the presence of hydrogen peroxide.

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| Subcellular fractions | NaVOS_4 \(0.63\ \text{mg V/kg/day for 3 days}\) | NaVOS_4 \(0.63\ \text{mg V/kg/day for 3 days}\) | NaVOS_4 \(0.63\ \text{mg V/kg/day for 3 days}\) |
|-----------------------|----------------------------------|----------------------------------|----------------------------------|
| Homogenate            | 100%                             | 100%                             | 100%                             |
| Nuclei                | 13.3                             | 17.3                             | 25.1                             |
| Mitochondria          | 41.1                             | 27.3                             | 35.1                             |
| Lysosomes             | –                                | –                                | –                                |
| Microsomes            | 23.9                             | 13.8                             | 10.9                             |
| Cytosol               | 21.6                             | 41.7                             | 28.8                             |

Male Wistar rats weighing 200 to 250 g received ip injections of vanadium in the form of NaVOS_4 or VOSO_4. Vanadium in organs and subcellular fractions of the liver was determined by neutron activation analysis at the Research Reactor Institute of Kyoto University. Electron spin resonance (ESR) spectra were recorded with a JEOL RE-3X ESR spectrometer. ESR spin trapping was performed by using DMPO (5,5-dimethyl-1-pyrroline-N-oxide) as a spin-trapping agent. DNA cleavage activity was estimated according to the reported method (4).

Administration of vanadium in the form of vanadyl or vanadate ion to rats resulted in almost the same distribution of the element in organs, but different subcellular distributions of vanadium were observed in the liver (Table 1). Rats given low doses of vanadium compounds accumulated more vanadium in the nuclei than those of rats given high doses of the compound. However, more vanadium was detected in the mitochondria of rats given high doses of vanadium compounds. Further, when rats were treated with vanadium compounds of the same amount and the vanadyl levels in the blood were monitored with ESR spectra at 77 K, the highest vanadium level was found in rats given vanadate compound, followed by vanadyl.

Figure 1. Changes of vanadium concentrations in the blood of rats treated with VCl_3, VOSO_4, and NaVOS_4 determined by ESR. Each vanadium compound was given to rats at a dose of 5 mg V/kg body weight.

Environmental Health Perspectives 35
and vanadic (+3 oxidation state) compounds (Figure 1). These observations may help explain the higher toxicity provided by vanadate compounds than vanadyl compounds in rats (5).

On the other hand, rats given a vanadyl compound accumulated more vanadium in the nuclei than those given a vanadate compound (Table 1). Vanadium incorporated into the nuclei may prevent chemically induced carcinogenesis. Vanadyl sulfate was recently reported to be effective for protection against experimental carcinogenesis in rats (2). We thus studied DNA cleavage by vanadyl compound.

Addition of \( \text{H}_2\text{O}_2 \) to a solution of vanadyl ion and DMPO at pH 7.8 generated a strong spin adduct ESR spectrum consisting of a 1:2:2:1 quartet with splitting of \( a_m^H=a_m^N=1.49 \text{ mT} \), where \( a_m^H \) and \( a_m^N \) represent the hyperfine splitting of the nitroxylnitrogen and \( \beta \)-hydrogen atom, respectively. This suggests that the formation of DMPO-OH spin adducts is the result of trapping of \( \cdot \text{OH} \) radicals formed during the reaction of vanadyl ion and hydrogen peroxide, by a Fenton-like reaction

\[
\text{VO}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{VO}^2_2 + \cdot \text{OH} + \text{H}^+.
\]

Thus we tested whether DNA cleavage occurs with \( \cdot \text{OH} \) radicals generated by vanadyl ion and hydrogen peroxide at pH 7.8. Reaction time- (Figure 2) and concentration-dependent DNA cleavage were observed, where for maximal DNA cleavage the molar ratio of hydrogen peroxide to vanadyl ion was found to be 1:1. Therefore, hydroxyl radicals may contribute to DNA damage, in a manner consistent with observations on the effects of ionizing radiation and \( \text{Fe}^{2+}\text{H}_2\text{O}_2 \) (Fenton) systems. The DNA cleavage by \( \cdot \text{OH} \) generated in vanadyl ion–\( \text{H}_2\text{O}_2 \) system is an example of the metal ion-induced DNA damage which can be used to understand the mechanism underlying metal ion and hydrogen peroxide-dependent toxicity and antineoplastic activity.

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