# Reactive Oxygen Species Scavenging Effects of Vanadyl Sulfate

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## Abstract

The aim of this study was to investigate the reactive oxygen species(ROS) scavenging effects of vanadyl sulfate. Human Chang liver cells were incubated for 10 passages in media containing deionized distilled water(DDW) and vanadyl sulfate($VOSO_4$, 52 ㎍/L). $VOSO_4$ at 52 ㎍/L significantly showed free radical scavenging effect of superoxide anion and hydroxyl radicals in cell-free system. Furthermore, cells treated with $VOSO_4$ at 52 ㎍/L significantly scavenged intracellular ROS compared to cells treated with DDW, as measured by flow cytometry and confocal microscopy after staining with 2',7'-dichlorodihydrofluorescein diacetate. Our results demonstrated that the antioxidant effects of vanadyl sulfate are mediated by ROS scavenging. (J Med Life Sci 2011;8:50-53)

Key Words : Vanadyl sulfate($VOSO_4$), Human Chang liver cells, Reactive oxygen species

## Introduction

Reactive oxygen species(ROS) is modulated by various physiological functions and represent an essential part of aerobic life and metabolism[1]. Various reports have shown that oxidative stress induced by ROS such as the hydroxyl radical( $\cdot OH$), hydrogen peroxide($H_2O_2$)and superoxide anion($O_2 \cdot^-$). ROS has been implicated as a major cause of cellular damage and cell death[2-4]. An abnormal regulation of ROS has a role in pathological conditions, including inflammation, atherosclerosis, diabetes, aging, and cancer[5-7].

Vanadium is an essential trace element considered to be important for normal cell function and development in mammals. There are several pharmacological applications of vanadium including treatment of diabetes[8, 9], cancertherapy [10], anti-inflammatoryactivity[11]. Recently, we reported that vanadyl sulfate($VOSO_4$) at 8, 13, 26 ㎍/L showed an antioxidant effect via the scavenging of ROS such as superoxide anions and hydroxyl radicals[12]. The objective of this study was to investigate the ROS scavenging effect of $VOSO_4$ at 52 ㎍/L.

## Materials and Methods

### 1. Reagents

Vanadyl sulfate($VOSO_4$), 5,5-dimethyl-1-pyrroline-N-oxide(DMPO) and 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) were purchased from the Sigma Chemical Company(St.Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

### 2. Cell culture

Human Chang liver cells were obtained from the American type culture collection(Rockville, MD, USA), and the cells were maintained at 37 ℃ in an incubator with a humidified atmosphere of 5% $CO_2$ in air, and cultured in DDW or Vanadyl sulfate($VOSO_4$) with RPMI 1640, containing 0.1 mM non-essential amino acids 10% heat-inactivated fetal calf serum, streptomycin(100 mg/ml) and penicillin (100 units/ml).

### 3. Detection of Superoxide Anion

Xanthine/xanthine oxidase was used to generate superoxide anion, which was then reacted with a nitrone spin trap DMPO. The DMPO/$\cdot OOH$ adducts were detected using an electron spin resonance(ESR) spectrometer(JEOL, Tokyo, Japan)[13, 14]. ESR signaling was recorded 5 min after the addition of 20 ml each of xanthine oxidase(0.25 U/ml), xanthine(5 mM), and DMPO(1.5 M), and either DDW or $VOSO_4$. The parameters of the ESR spectrometer were as follows:magnetic field, 336 mT;power, 5.00 mW; frequency, 9.4380 GHz; modulation amplitude, 0.2 mT; gain, 500; scan time, 0.5 min; scan width, 10 mT; time constant, 0.03 sec; and temperature, 25 ℃.

### 4. Detection of Hydroxyl Radical

Hydroxyl radical was generated by the Fenton reaction

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($H_2O_2$+$FeSO_4$) and then reacted with DMPO. The resultant DMPO/•OH adducts was detected using an ESR spectrometer[15, 16]. ESR signaling was recorded 2.5 min after the addition of 20 ml each of 0.3 M DMPO, 10 mM $FeSO_4$, 10 mM $H_2O_2$, and either DDW or $VOSO_4$. The parameters of the ESR spectrometer were as follows: magnetic field, 336 mT; power, 1.00 mW; frequency, 9.4380 GHz; modulation amplitude, 0.2 mT; gain, 200; scan time, 0.5 min; scan width, 10 mT; time constant, 0.03 sec; and temperature, 25 ℃.

### 5. Measurement of Intracellular Reactive Oxygen Species(ROS)

Cells were treated with 25 μM DCF-DA and the fluorescence of 2',7'-dichlorofluorescein was detected using a flow cytometer(Becton Dickinson, Mountain View, CA, USA)[17]. The image analysis for the generation of intracellular ROS was performed by seeding cells on a cover-slip-loaded six-well plate at $2\times10^5$ cells/well. DCF-DA(100 μM) was added to each well followed by incubation for an additional 30 min at 37 ℃. The stained cells were washed with phosphate buffered-saline (PBS) and then mounted onto microscope slide in mounting medium (DAKO, Carpinteria, CA, USA). Microscopic images were collected using the laser scanning microscope 5 PASCAL program (Carl Zeiss, Jena, Germany) of a confocal microscope.

### 6. Statistical Analysis

All measurements were made in triplicate(n=3), and all values are the means ± standard error(SE). Data were analyzed with analysis of variance(ANOVA) using the Tukey test.

## Results and Discussion

### 1. Radical scavenging activity of vanadyl sulfate in cell free system

To investigate whether $VOSO_4$ at 52 μg/L possess the radical scavenging activity, ESR spectrometry assessed to determine DMPO/•OH or DMPO/•OOH spin adducts which were produced by hydroxyl radicals (•OH) or superoxide anion radicals ($O_2^{\bullet-}$), respectively. As shown in Fig. 1, $VOSO_4$ treatment reduced superoxide anion radical generation to average values of 3392 at $VOSO_4$ concentrations of 52 μg/L, respectively, compared to 3756 in DDW. Also, $VOSO_4$ significantly exhibited hydroxyl radical scavenging activity. The quantity of hydroxyl radical (arbitraryunit) in $VOSO_4$ was 3452 respectively compared to 3747 in the DDW group(Fig. 2). These results suggested that $VOSO_4$ has radical scavenging activity in cell-free system.

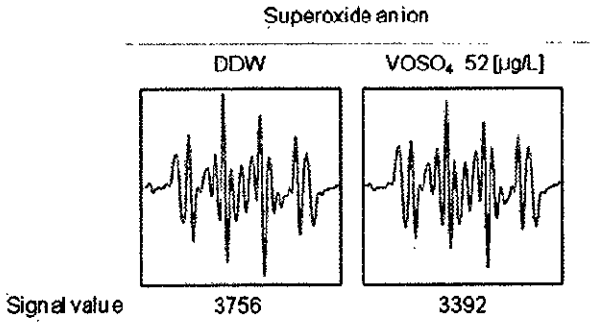


**Figure 1.** Scavenging effect of $VOSO_4$ against superoxide anion. Superoxide anion generated by xanthine and xanthine oxidase was reacted with DMPO, and the resultant DMPO/•OOH adducts were detected by ESR spectrometry.

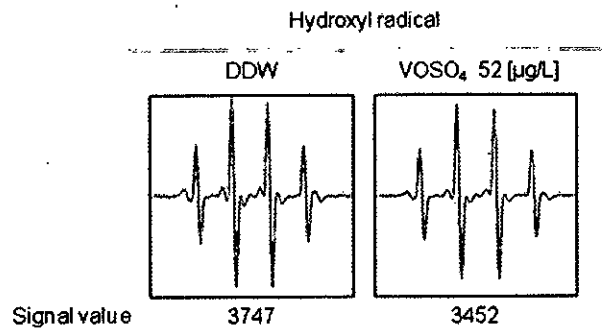


**Figure 2.** Scavenging effect of $VOSO_4$ against hydroxyl radicals. Hydroxyl radicals generated by the Fenton reaction ($H_2O_2$+$FeSO_4$) were reacted with DMPO, and the resultant DMPO/•OH adducts were detected by ESR spectrometry.

### Intracellular ROS scavenging activity of vanadyl sulfate

The DCF-DA method was used to detect the levels of intracellular ROS[17]. In our system, the intracellular ROS scavenging ability of $VOSO_4$ in human Chang liver cells was measured. The level of intracellular ROS detected using a flow cytometer revealed a fluorescence intensity of 234 for ROS stained by DCF-DA fluorescence dye in $VOSO_4$ at concentrations of 52 μg/L and $H_2O_2$ treated cells, respectively, compared to that of 268 in the DDW and $H_2O_2$ treated cells(Fig. 3A). Moreover, confocal microscopy showed that $VOSO_4$ at concentrations of 52 μg/L reduced red

fluorescence intensity with $H_2O_2$ treatment compared to that in the DDW and $H_2O_2$ treated cells(Fig. 3B). Taken together, these results suggest that $VOSO_4$ at 52 $\mu g/L$ was sufficient to inhibit intracellular ROS.

These results suggest that $VOSO_4$ possessed antioxidant effect via ROS scavenging.

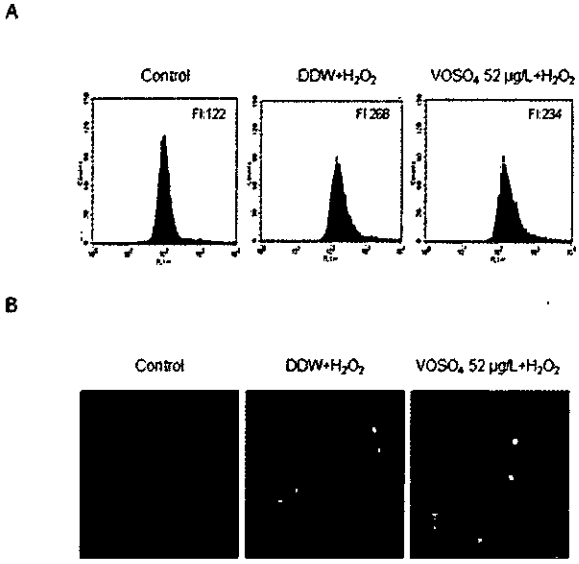


**Figure 3.** Scavenging effect of $VOSO_4$ against intracellular ROS. (A) DDW or $VOSO_4$ cultured cells (10 passages) were treated with $H_2O_2$. After an additional 30 min, the DCF-DA was added and intracellular ROS generated, were detected by flow cytometry. (B) The representative confocal images illustrate increase in the red fluorescence intensity of DCF produced by ROS in DDW and $H_2O_2$ treated cells compared $VOSO_4$ and $H_2O_2$ treated cells.

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