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Iron diminishes the *in vitro* biological effect of vanadium

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ABSTRACT

Mechanistic pathways underlying inflammatory injury following exposures to vanadium-containing compounds are not defined. We tested the postulate that the *in vitro* biological effect of vanadium results from its impact on iron homeostasis. Human bronchial epithelial (HBE) cells exposed to vanadyl sulfate ($VOSO_4$) showed a time- and dose-dependent increase in vanadium relative to PBS. HBE cells exposed to $VOSO_4$ and then exposed to ferric ammonium citrate (FAC) significantly increased intracellular iron import supporting an interaction between the two metals. Following exposure to $VOSO_4$, there was an increase (336 ± 73%) in RNA for divalent metal transporter 1 (DMT1), a major iron importer. With inclusion of $VOSO_4$ in the incubation, vanadium could be measured in the nuclear and mitochondrial fractions and the supernatant. Non-heme iron in the nuclear and mitochondrial fractions were decreased immediately following $VOSO_4$ exposure while there was an increased concentration of non-heme iron in the supernatant. Provision of excess iron inhibited changes in the concentration of this metal provoked by $VOSO_4$ exposures. Using Amplex Red, $VOSO_4$ was shown to significantly increase oxidant generation by HBE cells in a time- and dose-dependent manner. HBE cells pre-treated with FAC and then exposed to $VOSO_4$ demonstrated a decreased generation of oxidants. Similarly, activation of the transcription factor NF- B promoter and release of interleukin-6 and -8 were increased following $VOSO_4$ exposure and these effects were diminished by pre-treatment with FAC. We conclude that an initiating event in biological effect after exposure to vanadyl sulfate is a loss of requisite cell iron.

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1. Introduction

Vanadium appears to be a required element for some lower organisms but does not have a defined role in higher forms of life [1]. In mammalian tissues, vanadium is in low concentrations (0.014 to 7.2 μM) with the total mass of the metal approximating 100 to 200 μg in humans [2]. While it is unclear whether the metal participates in normal human biochemistry and physiology, exposure to vanadium-containing compounds can impact health. The respiratory tract is the most frequent target of such exposure. Lung injury associated with vanadium follows occupational exposures of workers engaged in the maintenance of oil-fired boilers in power generating stations (i.e. boilermakers' or vanadium bronchitis) [3,4]. Such individuals exposed to vanadium provide a history of cough, dyspnea, wheezing, and, infrequently, symptoms consistent with pneumonitis. In addition, there can be losses in pulmonary function [4,5]. Symptoms, signs, and pulmonary function decrements can resolve within a few days or weeks of cessation of the exposure [4]. However, workers can also die from inhalational exposures to vanadium with the lungs of these individuals demonstrating inflammation, congestion, and destruction [6]. Mechanistic pathways underling such inflammatory injury following exposure to vanadium-containing compounds are not defined.

There are numerous similarities between the chemistries and biochemistries of vanadium and iron. Those agents which are most effective in chelating vanadium are ligands that offer either oxygen or nitrogen donors (i.e. hard bases) comparable to those effective in chelating iron. As a result, both vanadium and iron can be complexed by many of the same substances. In living systems, an impact of vanadium on iron homeostasis is suggested as exposures to vanadium-containing compounds increase iron concentrations [7,8].

In this study, we tested the postulate that the *in vitro* biological effect of vanadium can result from its impact on iron homeostasis (Fig. 1). Specifically, we tested the postulate that vanadium can reduce concentrations of iron in a cell leading to its immediate deficit, an increased import of this metal by divalent metal transporter 1 (DMT1) with storage in ferritin, oxidant generation, transcription factor activation, and release of pro-inflammatory mediators.

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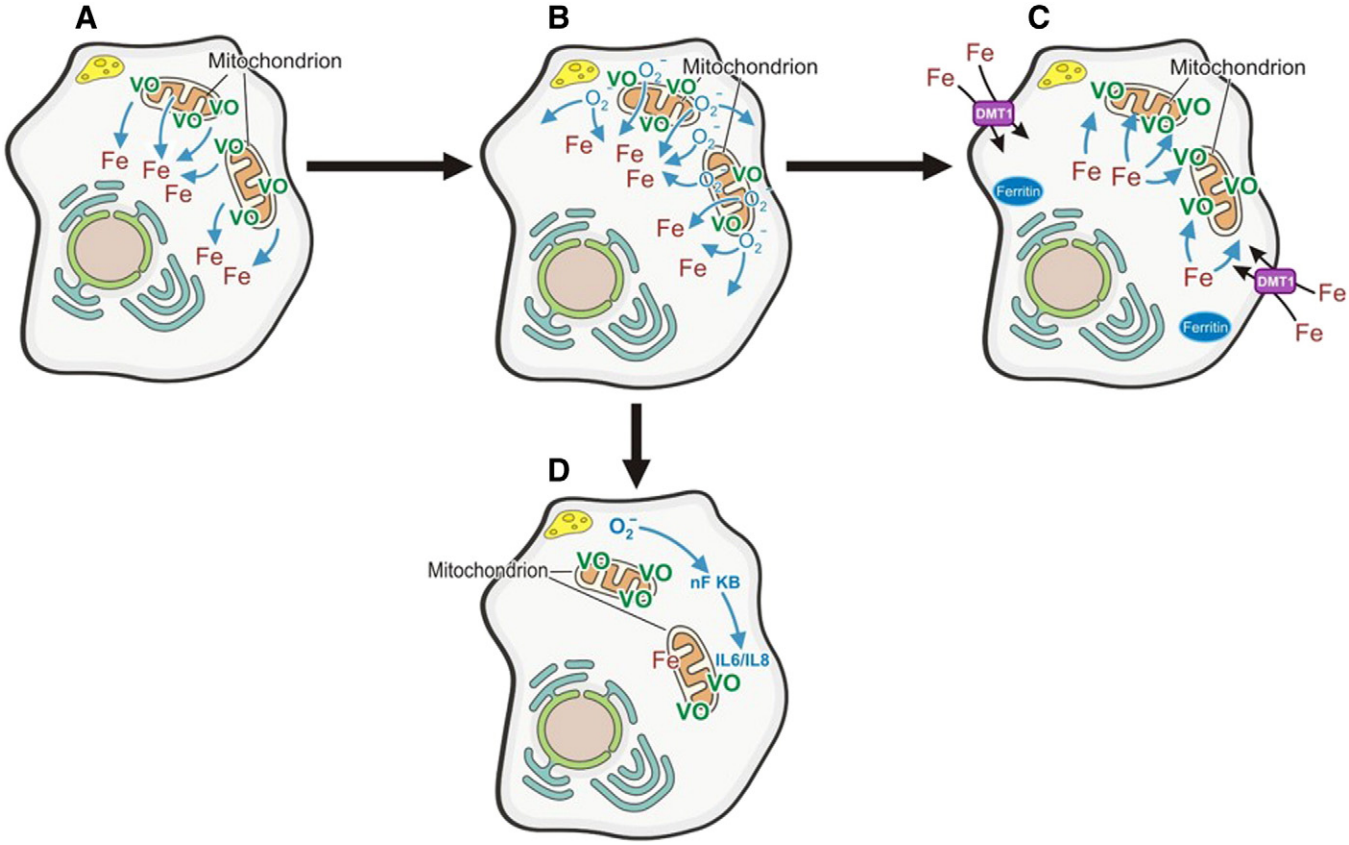

Fig. 1. Schematic for changes in iron homeostasis, oxidant generation, and biological effect following cell exposure to vanadyl sulfate. (A) Vanadyl cation displaces cell iron from intracellular cites. (B) In response to a reduction in intracellular iron essential for function, the cell generates superoxide which can function as a ferri-reductant. (C) Iron homeostasis is altered with upregulation of importers (e.g. DMT1) and storage (ferritin) proteins in an attempt to reacquire essential iron. An accumulation of iron results in the cell. (D) If adequate iron concentrations are not re-established, protracted oxidant generation activates transcription factors resulting in a release of pro-inflammatory mediators and inflammation.

2. Materials and methods

2.1. Materials

All reagents were from Sigma Co. (St. Louis, MO) unless specified otherwise.

2.2 . Cell culture

The protocol for acquiring human bronchial epithelial (HBE) cells was approved by the University of North Carolina School of Medicine Committee on Protection of the Rights of Human Subjects and by the U.S. Environmental Protection Agency. Subjects were informed of the procedure and its potential risks, and each signed an informed consent. Airway epithelial cells were obtained from healthy individuals through bronchoscopy with bronchial brushings. Cells were expanded to passage-3 in bronchial epithelial growth medium (BEGM; Clonetics, San Diego, CA) and plated at a density of 1×10^5 cells/well into 12 well culture plates (Trans-CLR, Costar, Cambridge, MA). HBE cells were maintained in BEGM with supplements.

For those studies requiring transfection, BEAS-2B cells were employed. This is an immortalized line of normal human bronchial epithelium derived by transfection of primary cells with SV40 early-region genes. Cells were grown to 90–100% confluence on uncoated plastic twelve-well plates in keratinocyte growth medium (KGM; Clonetics) which is essentially MCDB 153 medium supplemented with 5 ng/mL human epidermal growth factor, 5 mg/mL insulin, 0.5 mg/mL hydrocortisone, 0.15 μM calcium, bovine pituitary extract, 0.1 μM ethanolamine and 0.1 μM phosphoethanolamine.

2.3. Cytotoxicity

Cytotoxicity of $VOSO_4$ was quantified using both measures of the release of lactic dehydrogenase and trypan blue exclusion.

2.4 . Cell vanadium and iron concentrations

HBE cells were grown in 12-well plates and exposed to vanadyl sulfate ($VOSO_4$), ferric ammonium citrate (FAC), and both metal compounds. After incubation, the media and exposure were removed and the cells were washed with PBS (phosphate buffered saline) and scraped into 1.0 mL 3 M HCl/10% trichloroacetic acid (TCA). After hydrolysis at 70° C for 24 h with precipitation of heme in the TCA, vanadium and non-heme iron concentrations in the supernatant were determined using inductively coupled plasma optical emission spectroscopy (ICPOES; Model Optima 4300D, Perkin Elmer, Norwalk, CT) operated at $\lambda = 238.204$ nm (for Fe) and 292.403 nm (for V).

2.5 . Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated using a Qiagen kit (Qiagen, Valencia, CA) and reverse transcribed to generate cDNA using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Oligonucleotide primer pairs and fluorescent probes for DMT1 and GAPDH were designed using a primer design program (Primer Express, Applied Biosystems) and obtained from Integrated DNA Technologies (Coralville, IA). Quantitative fluorogenic amplification of cDNA was performed using the ABI Prism 7500 Sequence Detection System (Applied Biosystems), primer/probe sets of interest, and TaqMan Universal PCR