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Evaluation of genotoxicity of oral exposure to tetravalent vanadium *in vivo*

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Abstract

The trace element vanadium interacts with living cells, in which it exerts a variety of biological effects depending on its chemical form and oxidation state. Tetravalent vanadium was shown to affect several genotoxicity end-points *in vitro*, but its genotoxic potential *in vivo* is not elucidated.

In this study, the genotoxic effects induced *in vivo* by subacute oral exposure to vanadyl sulphate ($VOSO_4$), a tetravalent vanadium salt, were investigated. To this aim male CD1 mice were administered with $VOSO_4$ in drinking water over the dose range 2–1000 mg/l for 5 weeks. The incidence of micronucleated blood reticulocytes was measured along treatment period. At the end of treatment, micronuclei in both blood reticulocytes and bone marrow polychromatic erythocytes were determined; in addition, DNA lesions detectable by comet assay were assessed in marrow and testicular cells. Tissue distribution of vanadium at sacrifice was determined by atomic absorption spectrometry.

Comet assays and the analysis of micronuclei in polychromatic erythrocytes did not reveal treatment related effects. A slight increase in micronucleated reticulocytes, with no relationship with the administered dose, was observed in some treated groups. The determination of vanadium content in kidney, liver, spleen, bone, stomach, small intestine and testis highlighted low internal exposure, especially in soft tissues. Overall, data indicate scarce bioavailability for orally administered tetravalent vanadium, and lack of significant genotoxic potential *in vivo*.

Keywords: Vanadyl sulphate; *In vivo*; Genotoxicity; Comet; Micronucleus; Mouse

1. Introduction

Vanadium is a trace element present in the natural and occupational environment. It can be found in water, rocks and soil at low concentrations as well as in fossil fuels at relatively high concentrations. Vanadium exists in different oxidation states, the most common being +3, +4, and +5, which modulate its toxicity (WHO, 1988).

Food is the main source of exposure to vanadium for the general population, with an estimated daily dietary intake of a few tens of micrograms (WHO, 1996). Drinking water contributes to a lesser extent, even though relatively high vanadium concentrations have been recorded in some water supplies, notably in groundwater from volcanic areas (WHO, 1988; Farias et al., 2003). Another important source of vanadium exposure

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is from the use of dietary mineral supplements, which can provide more than 10 mg vanadium/day (Barceloux, 1999; EFSA, 2004).

Vanadium is poorly absorbed from the gastrointestinal tract, thus ingested vanadium compounds are mainly eliminated in feces. On the other hand, urine is the predominant route of elimination of absorbed vanadium. The distribution of vanadium via blood circulation is rapid: the highest concentrations of vanadium initially appear in kidney, liver and lungs, while long-term storage sites are bone and muscles (Aragon and Altamirano-Lozano, 2001; Ivancsits et al., 2002).

Several evidences indicate that vanadium is essential for different organisms, although the conclusive demonstration of its essentiality for humans is still lacking (Poggioli et al., 2001). Among the biological actions of vanadium, an increase of glucose transport and metabolism in different tissues and cell types has been noted. This activity prompted its pharmacological utilization as an alternative therapy in diabetic patients, and as dietary supplement among bodybuilders. On the other hand, depending on blood level, vanadium may also cause several adverse effects in mammals, such as haemopoietic changes, nephrotoxicity, reproductive and developmental toxicity (Llobet and Domingo, 1984; Domingo, 1996; Aragon and Altamirano-Lozano, 2001; Poggioli et al., 2001; Ivancsits et al., 2002). As far as carcinogenicity is concerned, no conclusion can be drawn from the limited oral carcinogenicity studies performed, while the positive findings from inhalation studies with particles of vanadium pentoxide (IARC, 2003) are of scarce relevance for evaluating potential risks from oral exposure.

Considerable attention has attracted in recent years the assessment of the genotoxicity of vanadium compounds. Adverse effects of pentavalent and tetravalent vanadium compounds on chromosome integrity and segregation have been observed *in vitro*. In particular, pentavalent vanadium compounds have been reported to induce micronuclei, DNA strand breaks, sister chromatid exchanges (SCE) and chromosomal aberrations in different *in vitro* systems (Owusu-Yaw et al., 1990; Migliore et al., 1993, 1995; Rojas et al., 1996; Ramirez et al., 1997; Ivancsits et al., 2002). Also vanadium tetravalent compounds proved to be genotoxic in several *in vitro* systems, inducing micronuclei and chromosomal aberrations in human peripheral blood cells (Migliore et al., 1993, 1995; Rodrìguez-Mercado et al., 2003), and causing guanosine hydroxylation and DNA strand breaks through the generation of free radicals (Shi et al., 1996; Bay et al., 1997; Wozniak and Blasiak, 2004). These positive findings were confirmed in some *in vivo* studies in mice using acute intraperitoneal or intragastric administrations (Ciranni et al., 1995; Altamirano-Lozano et al., 1996, 1999; Mailhes et al., 2003). However, the results from these *in vivo* studies can be considered of limited use for hazard characterization of low dose repeated oral exposures, as typically experienced by humans. To fill this gap, in a previous study the genotoxic activity of pentavalent vanadium was evaluated in mice treated with a wide range of doses of sodium *ortho*-vanadate in drinking water for 5 weeks (Leopardi et al., 2005). In the present study an identical experimental approach has been applied to evaluate the genotoxic potential *in vivo* of the tetravalent compound vanadyl sulphate. Effects on chromosome integrity and segregation, visualized as micronuclei, and primary DNA lesions detectable by comet assay, have been assessed in somatic and germ cells, and evaluated also with respect to tissue concentrations of the element at the end of the exposure period.

2. Materials and methods

2.1. Animals

CD-1 male mice aged 5–6 weeks were obtained from Harlan s.r.l. (Udine, Italy), and acclimatized 1 week before treatment. Animals were housed under standard environmental conditions ($22 \pm 2\,^{\circ}C$, $55 \pm 15\%$ relative humidity, on a 12 h light/dark cycle), with drinking water and laboratory rodent diet *ad libitum*. Experiments were carried out in compliance with the ethical provisions enforced by the European Union and authorized by the National Committee of the Italian Ministry of Health on the *in vivo* experimentation.

2.2. Chemicals

Vanadyl sulphate hydrate ($VOSO_4 \cdot 5H_2O$), purity 99.99%, MW 253, CAS 123334-20-3 was from Sigma–Aldrich (Milan, Italy). Stock solutions of the chemical were prepared daily by dissolving the blue crystalline powder in distilled water. Their actual concentrations were determined by ICP-OES spectrometry as described below. Tap water was used to dilute $VOSO_4$ stock solutions and administered as such to control animals.

2.3. Treatments and organ sampling

From eight to ten male mice were randomly assigned to each treatment group. In the first experiment, $VOSO_4$ was administered in drinking water for 5 weeks at the concentrations of 10, 100, 500 and 1000 mg/l. The top dose was selected as maximum tolerated dose on the basis of the results of a preliminary range-finding experiment and of literature data (Ciranni et al., 1995). A repeat experiment with 10 and 2 mg $VOSO_4$/l was performed to check the effect of the low dose of vanadyl sulphate on MnRETs observed in the first trial.