Vanadium pentoxide induces the secretion of CXCL9 and CXCL10 chemokines in thyroid cells

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Abstract. Vanadium is a grey metal, existing in different states of oxidation, whose most common form in commercial products is vanadium pentoxide (V2O5). All vanadium compounds have been considered toxic. A carcinogenic role of vanadium on the thyroid has recently been proposed. However, no in vivo or in vitro studies have evaluated thyroid disruption in humans and/or animals after exposure to vanadium. In the present study, we evaluate the effect of V2O5 on proliferation, and chemokine secretion in normal thyrocytes. Our study demonstrated that V2O5 has no effect on thyroid follicular cell viability or proliferation, but it is able to induce the secretion of T-helper (Th1) chemokines into the thyroid, synergistically increasing the effect of important Th1 cytokines such as interferon (IFN)γ and tumor necrosis factor (TNF)α. Through this process, V2O5 promotes the induction and perpetuation of an inflammatory reaction in the thyroid. Further studies are necessary to evaluate thyroid function, and nodules, in subjects occupationally exposed, or living in polluted areas.

Introduction

Vanadium is a grey metal that exists in a number of different states of oxidation: -1, 0, +2, +3, +4 and +5. The most common form in commercial products is vanadium pentoxide (V2O5). All vanadium compounds have been considered toxic. The maximum amount of human V2O5 exposure has been established by the Occupational Safety and Health Administration of the US Department of Labor as 0.05 mg/m3 for dust and 0.1 mg/m3 for fumes, in workplace air for an 8 h workday/40 h work week (1). Exposure to a 35 mg/m3 dose of vanadium is considered life-threatening and could provoke serious and perpetual health issues including death, as determined by The National Institute for Occupational Safety and Health (1).

The respiratory system is the most vulnerable system to the toxic effect of vanadium whereas there is inconsequential action on the gastrointestinal system due to the minimal gut absorption rate of the substance (2-4). Unfortunately, we lack satisfactory data to determine the reference range of a subchronic or chronic inhaled dose. The effects resulting from an oral or inhaled vanadium exposure on serum parameters (5,6), the liver (7), nervous system (8) and development of other tissues have been described in rat models (9).

Recently, the increase in the incidence of thyroid cancer in areas of volcanic activity suggests a carcinogenic effect of volcanic pollution. In the Mount Etna volcanic area, the incidence of thyroid cancer was higher than that in control areas (18.5 and 9.6/105 inhabitants, respectively). In volcanic areas, various trace elements are increased (with respect to control areas) in both lichens and drinking water, indicating atmospheric and water pollution. The amounts of trace elements are significantly increased, among them vanadium which was increased 8 times, and its possible carcinogenic role on the thyroid has been hypothesized (10,11).

However, no in vivo or in vitro studies have evaluated thyroid/endocrine disruption in humans and/or animals after exposure to vanadium. In the present study, we evaluated the effect of V2O5 on the proliferation and chemokine secretion of normal thyrocytes.

Materials and methods

Thyroid follicular cells (TFCs). We collected 10 specimens of thyroid tissue from 10 euthyroid patients (mean age, 41 years, range 24-61; 5 females, 5 males) (8 undergoing parathyroidectomy, 2 laryngeal intervention). We obtained informed consent from all participants, and the local ethics committee of the University of Pisa provided approval for the study. Thyrocytes were prepared as previously described (12-14).
The digestion of tissue samples was carried out by collagenase (Roche Diagnostics GmbH, Mannheim, Germany; 1 mg/ml) with RPMI-1640 (Whittaker Bioproducts, Inc., Walkersville, MD, USA) for 1 h at 37°C. The semi-digested follicles were removed and sedimented for 2 min; they were then washed and cultured with RPMI-1640 medium in the presence of fetal bovine serum (FBS) 10% (Seromed Biochrome, Berlin, Germany), 50 mg/ml penicillin/streptomycin, 2 mM glutamine, with 5% CO₂ at 37°C.

Cell viability and proliferation assay. Cell viability and proliferation were evaluated using the WST-1 (Roche Diagnostics, Almere, The Netherlands) assay (which uses 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, in the MTT assay) (15-17). TFCs were seeded at a density of 35,000 cells/ml (in a final volume of 100 µl) in each well of 96-well plates. To determine how V₂O₅ affects TFC proliferation, the cells were treated (24 h) with increasing concentrations of V₂O₅ (1, 10 and 100 nM). For each cell preparation, the experiments were performed in triplicate. Cells were plated and treated for 24 h with V₂O₅ or with its vehicle alone.

Proliferation assay: Cell counting. Cell number counting was also used to evaluate the proliferation of TFCs as previously described (15-17). Chemokine secretion assays. To successfully perform the chemokine (C-X-C motif) ligand (CXCL)9, or CXCL10 secretion assays, 30,000 cells/ml were seeded (in 96-well plates) in a final volume of 100 µl/well, in growth medium, which was then removed (after 24 h). Cells were then washed with phosphate-buffered saline (PBS) and incubated (for 24 h) in serum and phenol red-free medium with interferon (IFN)γ (500, 1,000, 5,000 and 10,000 IU/ml; R&D Systems, Minneapolis, MN, USA) and tumor necrosis factor (TNF)α (10 ng/ml) (R&D Systems), in combination (13) or alone. The TNFα concentration was chosen to obtain the highest secretion in preliminary experiments. The supernatant was then obtained (after 24 h), and kept frozen at -20°C (until chemokine assay).

To understand how V₂O₅ affects the chemokine secretion induced by IFNγ; the cells were treated (for 24 h) with increasing concentrations of V₂O₅ (1, 10 and 100 nM), in the presence/absence of IFNγ (1,000 IU/ml) and/or TNFα (10 ng/ml). CXCL9 and CXCL10 in the supernatants were measured by ELISA. The experiments were conducted 3 times with each different cell preparation.

ELISA for CXCL9 and CXCL10. CXCL9 or CXCL10 was assessed in the supernatants from cell cultures by commercially prepared kits (R&D Systems). The minimum (mean) detectable doses were 1.5 or 1.2 pg/ml, for CXCL9, or CXCL10 (respectively). The intra- and inter-assay coefficients of variation were for 3.5 and 6.4%, respectively, for CXCL9, while these coefficients for CXCL10 were 4.5 and 7.3%, respectively. Quality control pools of normal, low and high concentrations were also included in each assay.

Data analysis. For normally distributed variables, the values are expressed in the text as mean (± SD), or mean (± SEM) in figures, otherwise as median [and interquartile range]. Mean group values were compared by using one-way analysis of variance (ANOVA); the Bonferroni-Dunn test was used for post hoc comparison; P<0.05, for all comparisons.

Results

Cell proliferation. Cell viability and proliferation assay WST-1 demonstrated that V₂O₅ (1, 10 and 100 nM) did not alter the viability or proliferation of TFCs [mean group values are compared by using one-way analysis of variance (ANOVA); the Bonferroni-Dunn test was used for post hoc comparison of normally distributed variables.

CXCL9. CXCL9 levels were not detectable in supernatants gathered from primary thyrocyte samples, whereas the CXCL9 concentration was increased after dose-dependent induction of IFNγ (0, 61±27, 136±34, 196±41 and 262±67 pg/ml; with IFNγ 0, 500, 1,000, 5,000 and 10,000 IU/ml, respectively; P<0.001 by ANOVA). TNFα alone was not able to promote any impact on CXCL9 (remaining undetectable), while the synergy of IFNγ plus TNFα elicited a significant influence on CXCL9 release (CXCL9, 8976±1456 vs. 142±34 pg/ml with IFNγ alone, P<0.0001 by ANOVA).

Following the treatment of thyrocytes with V₂O₅ (1, 10 and 100 nM), CXCL9 secretion was dose-dependently stimulated (ANOVA, P<0.0001) (Fig. 2A). Following the treatment of thyrocytes with V₂O₅ (1, 10 and 100 nM), together with TNFα, CXCL9 secretion was not significantly altered with respect to V₂O₅ alone (data not shown).

Following the treatment of thyrocytes with V₂O₅ (100 nM), together with IFNγ, CXCL9 release was synergistically increased (ANOVA, P<0.0001) (Fig. 3A).

Following the treatment of thyrocytes with V₂O₅ (100 nM), together with IFNγ and TNFα, CXCL9 secretion was synergistically increased (ANOVA, P<0.0001) (Fig. 4A).
CXCL10. CXCL10 was not detectable in the supernatants from primary thyrocyte cultures in basal condition. IFNγ induced CXCL10 secretion dose-dependently (0, 45±22, 111±35, 213±27, 254±64 pg/ml, with IFNγ 0, 500, 1,000, 5,000 and 10,000 IU/ml; respectively; P<0.001 by ANOVA). TNFα only was not able in this case to carry out any impact on CXCL10, while the synergy of IFNγ plus TNFα elicited a significant influence on CXCL10 secretion (2,011±154 vs. 108±26 pg/ml with IFNγ alone; P<0.0001, by ANOVA).

Following treatment of thyrocytes with V2O5 (1, 10 and 100 nM), CXCL10 secretion was stimulated dose-dependently (P<0.0001 by ANOVA) (Fig. 2B). Following treatment of thyrocytes with V2O5 (1, 10 and 100 nM), together with TNFα, CXCL10 secretion was not significantly changed with respect to V2O5 alone (data not shown).

Following treatment of thyrocytes with V2O5 (100 nM), together with IFNγ, CXCL10 release was synergistically increased (P<0.0001 by ANOVA) (Fig. 3B).

Following treatment of thyrocytes with V2O5 (100 nM), together with IFNγ plus TNFα, CXCL10 secretion was synergistically increased (P<0.0001 by ANOVA) (Fig. 4B).
several autoimmune diseases, for example in the thyroid in Graves’ disease, or in autoimmune thyroiditis. This process has been hypothesized to be involved in the initiation and/or the perpetuation in several autoimmune disorders (12-21), and it can be applied also to the thyroid.

Our results agree with those of other researches in different cell types. V2O5 exposure is a cause of occupational bronchitis, and a study evaluated gene expression profiles in human lung fibroblasts (in cultures) after exposure in vitro to V2O5 in order to identify genes that may play a role in the bronchial inflammation, repair and fibrosis in the pathogenesis of bronchitis. Approximately 12 genes were overexpressed following exposure to V2O5, including chemokines (CXCL9, CXCL10 and interleukin-8) (22). In a second study, it was shown that fibroblasts respond to vanadium oxidative stress by producing IFNβ and activating STAT-1, that led to increased CXCL10 levels (23), playing a role in the innate immune response.

Importantly, vanadium was able to increase chemokine secretion in a dose range, from 1 to 100 nM. It was observed that normal blood levels of vanadium range from 0.45 to 18.4 nM, and that 100 nM is a dose that may mimic an abnormally high exposure (24).

The mechanisms by which V2O5 induces lung cancer have been investigated in mice in numerous studies. Experts agree that in vivo and in vitro research suggests that cancers are induced by secondary mechanisms (probably not genotoxic effects) (25).

Thus, we hypothesized that, also for the thyroid, the induction and perpetuation of an inflammatory reaction into this gland, and the variety of involved candidate genes could predispose to the appearance of thyroid cancer (as recently demonstrated for the association of papillary thyroid cancer and autoimmune thyroiditis), and could be at the basis of V2O5-induced effects after occupational and environmental exposure.

In conclusion, the present study showed that V2O5 is able to induce the secretion of Th1 chemokines into the thyroid, synergistically increasing the effect of important Th1 cytokines such as IFNγ and TNFα, leading to the induction and perpetuation of an inflammatory reaction in the thyroid. Further studies are necessary to investigate the mechanism of action by which chemokines are secreted, and to evaluate thyroid function, and nodules, in subjects occupationally exposed, or living in polluted areas.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors’ contributions

PF, RF, AC, AA and SMF made substantial contributions to conception and design, and to acquisition of data; PF, RF, GE, FR, AP, SB, AC, AA and SMF analysed the data; PF, AA and SMF have been involved in drafting the manuscript; AA revised it critically for important intellectual content. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Informed consent from all participants was obtained, and the local ethics committee of the University of Pisa provided approval for the study.
Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References


