Beneficial Effects of a Vanadium Complex with Cysteine, Administered at Low Doses on Benzo(a)Pyrene-Induced Leiomyosarcomas in Wistar Rats

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Abstract. Background: Vanadium is a potent environmental and body metal, possessing remarkable antitumor and anti diabetic properties. Vanadium salts and complexes have been widely investigated for their antitumorigenic properties in experimental carcinogenesis. In the present study the antitumor effects of a new vanadium complex with cysteine in relation to identical doses of vanadyl sulfate and cysteine, in tumor bearing rats, were investigated. Materials and Methods: Male wistar rats were injected with benzo(a)pyrene and divided into four groups of 21 rats each. Control group was treated only with BaP. The first group (TR-1) was treated by vanadyl sulfate per os at daily doses of 0.5 mg of V/kg b.w per day. The second (TR-2) by cysteine at doses of 4.5 mg/kg b.w per day and the third group (TR-3), by the complex V(III)-cysteine at daily doses of 0.5 mg/ml/kg b.w (containing cysteine at concentrations of 4.5 mg/ml/kg). Treatment was started when tumors were developed (evidenced by palpable mass at the site of BaP injection) and went on till death. Routinological tests were performed in 27 rats divided into a control group and two test groups: TR-1 administered with vanadyl sulfate at daily doses of 18.5 mg/kg b.w and TR-2 group with V(III)-cysteine complex at daily doses of 18.5 V/kg b.w, for 9 weeks. Mean survival time, death rate, tumor growth rate, the carcinogenic potency of BaP, and the antitumorigenic potency in relation to histological findings in each treatment group were calculated in each group in order to evaluate the antitumor effects of the substances used. Results: Vanadyl sulfate, cysteine and V(III)-cysteine exerted antitumor effects on leiomyosarcomas bearing Wistar rats. However, V(III)-complex exerted much more potent effects than the other treatments, significantly prolonging mean survival time, retarding tumor growth rate and decreasing the carcinogenic potency of BaP in the TR-3 group, in comparison to the control and the TR-1 and TR-2 groups. Moreover V(III)-cysteine complex resulted in complete remission of 4 (19.7%) of the tumor bearing rats. Blood, urine, biochemical routine tests as well as autopsy did not reveal any toxic effects either of vanadyl sulfate or V(III)-cysteine complex. Conclusions: Vanadyl sulfate, cysteine and V(III)-cysteine complex exerted antitumor effects in tumor bearing rats. The V(III)-cysteine complex, however, exerted much more potent effects, as evident from the results of the present study. These beneficial effects of the above complex, in combination with its low toxicity provide evidence suggestive of its possible application in the treatment of human malignant diseases.

Vanadium (V) is considered a trace metal possessing antitumor effects when administered at the correct concentrations in experimentally-induced tumors (1-3). Various effective antitumor complexes of vanadium have been so far investigated, the most active of which are the bis(cyclopenta-dienyl)dichloro-V(IV), and the peroxovanadates(V) (4-7). Compounds of vanadium with aminoacids have not, however, widely used for the prevention or treatment of experimentally-induced malignant diseases (8).

In the present study, results concerning the therapeutic effects of low dose administration of a new organic complex of vanadium (III) with the amino acid L-cysteine, in comparison to vanadyl sulfate and cysteine, on experimentally-induced malignant tumors in rats, are investigated.

Materials and Methods

Treatment study. Male wistar rats, 10-12 weeks, weighing 190-200g, were divided into groups and were s.c injected with 10.0 mg of benzo(a)pyrene (BaP), dissolved shortly before injection in 1 ml of tricaprylin. Previous investigations have shown that this amount of BaP induces 100% development of malignant tumors in the animals (8-9).

A group of 21 rats, injected with BaP alone, was used as control (C-G). The rest of the animals were divided into three treatment groups (TR-G): The first group, 21 rats, were orally administered with 0.5 mg/kg b.w per day of Vanadium (IV) as vanadyl sulfate (VOSO4) (TR-1 group), the second group comprised 21 rats, orally administered with 4.5

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mg/kg b.w of cysteine (TR-2 group), and the third group, comprised 21 rats given 0.5 mg/kg b.w of V(III) as a complex with cysteine (4.5 mg/kg b.w) (TR-3 group).

The animals of the treatment groups received VOSO4, cysteine and V-
cysteine complex in water solutions prepared fresh daily. The
administration of the substances began after the appearance of a
palpable subcutaneous tumor (about 12 weeks after BaP injection)
(8,10) and treatment was continued until death.

Animals of all groups were followed-up till death. Then autopsies
were performed. Tumors were carefully excised and weighed and, along
with the lungs, liver, stomach, spleen, intestine and kidneys, they were
subjected to histological examination.

The percentage of the animals manifesting complete tumor remission,
the mean survival time (days from BaP injection till death), the
histological type and the grade of the tumors as well as the tumor growth
rate (TGR) were estimated. TGR is a quotient of the tumor weight and
the survival time of each animal, as follows:

Tumor growth rate (TGR) = \frac{\text{weight of tumor (gr)}}{\text{survival time (days)}}

Two other indices, the Carcinogenic Potency of BaP(CP BaP) and the
Anticarcinogenic Potency of the substance administered (AP abut.)
were calculated in each group as follows:

CP BaP = \frac{\text{percentage of tumor induction}}{\text{Mean Survival Time of animals}} \times 100

AP abut. = \frac{\text{CP BaP control}}{\text{CP BaP TR-group}}

Toxicological study. Male Wistar rats of the same age and weight as
above were divided into three groups of 9 rats each for toxicological
studies:

The first group (T1-control), was administered with tap water
(50 ml/day), the second (T2-test) was orally administered with 15 mg/
body of V(IV) as vanadyl salicylate(VOSO4), in tap water, and the thi
(T3-test), was orally administered with 15.5 mg/kg b.w day of V as
the cysteine complex, in tap water. All substances were prepared fresh
daily and administered to the animals for nine weeks. Then animals were
anesthetized, midline thoracotomy was performed and blood withdrawn
for hematocrit, RBC, WBC, platelet count, uric acid, creatinine, SGOT/SGPT, ALP and γ-GT. Urine specimen collected from rats placed in metabolic cages was also as well analyzed. Lungs, liver, spleen, stomach, and kidneys were also carefully excised and subjected to histological examination.

The results obtained from the treatment and toxicological group
were compared to the control group and were statistically evaluated
using the student's t-unsquared test.

Preparation of the V-cystine complex(7): VII=[V(III)Cp2(μ2-PR)]2. 1/2 MeOH
One portion solid cysteine(1.32 g) (4.62 g · mol⁻¹) was added to
to stirred solution of V(III) chloride(2.00 g, 12.71 mmol) in methanol
(50ml). After 6 hours of stirring, the solution changed color to dark
green, and when cysteine was completely dissolved a brown precipitate
was formed. The solid was filtered off, washed with methanol (10 ml)
and diethyl-ether (2 × 20 ml) and dried in vacuum. Yield 3.00 g (57%)
Vanadium calculated for [VII(μ2-PR): 1/2 MeOH 11.86%, 11.92%
Vanadium found 11.79%. Two possible complexes of Vanadium with
cysteine were thus formed (scheme 1).

Physicochemical characteristics of the complex were also investigated.

Results

Physicochemical characteristics of V(III)-cystine complex
The vanadium complex has a magnetic moment of 2.70 μB, a
expected for a d⁷ system. Its UV-Vis spectral data (λmax, nm

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The Carcinogenic Potency of BaP(CP,BaP) in the control group was found to be 58.3 units, in the TR-1 group 45.3 units, in the TR-2 group 45.0 units, and in the TR-3 group 32.9 units. The Anticarcinogenic Potency (AP) of the vanadyl sulfate (TR-1 group) was calculated as 13 units, the cysteine (TR-2 group) 13.3 units whereas the AP of the V(III)-cysteine complex (TR-3 group) was 25.4 units (a 45.6% reduction of the CP of BaP in this group).

The tumor growth rate (TGR) was found to be 1.4 ± 0.4 g/day for the control group, 1.1 ± 0.4 g/day for the TR-1 group, 1.0 ± 0.2 g/day for the TR-2 group and 0.7 ± 0.3 g/day for the TR-3 group. The tumor growth rate of the TR-3 group was significantly lower than the control group (p<0.001) and the TR-1 and TR-2 groups (p<0.05). In contrast the TGR of the TR-1 and TR-2 groups was not different than the control group (p>0.05).

Toxicological studies performed by the per os administration of 18.5 mg/kg b.w of V either as vanadyl sulfate (T2 group) or as V(III)-cysteine complex, for 9 weeks showed no alterations in blood and biochemical tests, compared to the control group (T1 group). There was only a slight, but not significant, increase in body weight in V(III)-cysteine treated group in comparison to control (T1) and a non significant loss in body weight in vanadyl sulfate group (T2). Autopsy of the various organs of sacrificed rats revealed no macroscopic alterations. Detailed histology is not yet available. Metastases were not found in the animals of all groups.

Discussion

In the present study the organic complex of vanadium(III) with the amino acid L-cysteine(V(III)-cysteine complex), in comparison to similar doses of V as vanadyl sulfate and the amino acid cysteine, were administered at low doses in rats bearing experimentally-induced malignant tumors (leukemia) and were compared for their anticarcinogenic effects.

Our data suggest that vanadyl sulfate and cysteine possess, at the doses administered, moderate antitumor effects, since some of the parameters used for the evaluation of their action
on experimental leiomyosarcomas were beneficially modified by the substances, in comparison to the control group.

V(III)-Cysteine complex. In contrast, at identical vanadium and cysteine concentrations to vanadyl sulfate and cysteine respectively, exhibited a remarkable beneficial effect on tumor bearing rats, significantly increasing the mean survival time of the animals, inducing a complete remission of tumors developed in four of the animals (19.5%), decreasing the carcinogenic potency (CP) of BaP by 43.6% and retarding significantly the tumor growth rate, in comparison to the control vanadyl sulfate (TR-1) and Cysteine (TR-2) group. V(III)-cysteine complex also exhibited an antineoplastic potency (AP) of 25.4 units, which is almost the sum of the antineoplastic potencies exhibited by vanadyl sulfate (TR-1 group) and cysteine (TR-2 group) (13.0 and 13.3 units, respectively).

The mechanisms underlying the antitumor effects of vanadium and its complexes remain unclear. Some of the following actions of vanadium are probably related to its antitumor properties.

Vanadium(V) has been shown to inhibit as well as to enhance, depending upon its concentration in the media, DNA synthesis in vitro (11). DNA polymerases and nucleotidy transferases as well as phosphotransferases and phosphorylases are also inhibited by vanadium (12-14).

Orthovanadate is considered to generate hydroxyl vanadium radicals intracellularly, exerting cytotoxic effects on proliferating cells, but not on non proliferating cells (5). Vanadium uptake by the cancer cells is, in addition, higher than that of the normal cells (15). Cancer cells differ from normal cells in a number of ways including lower pH; larger free radical character and higher water content. The above properties of cancer cells comprise excellent conditions for interaction with specific vanadium complexes. Unique heteroligand vanadium co-ordination spheres, could form in the cancer cells vanadium polyhedra distinguished from those formed in the normal cells where pH, water content and free radical character are not identical, exerting cytotoxic actions (6). It has been found in the present study that V(III)-cysteine complex is slowly oxidized to in water to V(V) species (see results). Whether such a reaction takes place in cancer cells in vivo or is facilitated by the cancer cell environment remains to be investigated. This low oxidation of V(III)-cysteine complex to cytotoxic free radical species in vitro, indicates that solutions of the complex, for per os administration, have to be prepared fresh daily in order to avoid cytotoxic effects on normal cells.

The toxicological study did not, however, reveal any toxic effect, either of V(III)-cysteine complex or of Vanadyl sulfate administered per os at significantly higher doses of V (18.5 mg kg b.w. per day) for 9 weeks, in non-tumor bearing Wistar rats, than those administered for the treatment of tumor bearing animals.

An increasing number of publications support the antitumor activity of vanadium and its compounds. Vanadium 0.5 ppm in drinking water exerted inhibitory effects on rat liver carcinogenesis (2) and ammonium monovanadate, 0.2 and 0.5 ppm, inhibited altered liver cells and persistent nodule growth induced by diethylnitrosamine hepatocarcinogenesis in rats (3). Vanadyl sulfate 5 ppm administered for 9 days inhibits small MDAY-D2 solid tumors at 80-100%, an effect which is augmented by pretreatment with N-acetylcysteine (5). Dietary vanadyl sulfate 4 ppm at concentration of 25 p.p.m remarkably inhibited chemically-induced mammary carcinogenesis in rats (1). Vanadocene dichloride exhibited in vivo marked activity against fluid and solid Ehrlich ascites tumors, as well as against other experimental tumor systems such as intraperitoneally growing mouse mammary tumor (6).

Ammonium monovanadate has also been found to have anticarcinogenic effect in host mice bearing a transplantable ascitic lymphoma (16).

It has recently been reported that the concentration of sodium orthovanadate that could be tolerated in the drinking water without significant toxicity for rats, is 0.2 mg/ml (17).

The range of the antitumor activity dose of peroxovanadate compounds is considered to be 5-12 mg V/kg, while toxicity ranges between 6 and 16 mg V/kg (6). In our experimental model vanadium was orally administered at daily doses of 0.5 mg V/kg/day, significantly lower than the therapeutic ones. Nevertheless, at this low dose the V(III)-cysteine complex seems to exert a remarkable beneficial effect on tumor bearing animals, compared to the control group and to anticarcinogenic effects exhibited by vanadyl sulfate and cysteine alone, administered at similar to the complex concentrations of V and cysteine.

It is possible that vanadium existing on trivalent state, V(III), in the organic complexes used in our study, and slowly oxidized in water to a cytotoxic free radical species, to exhibit antitumor effects at lower concentrations than those of V(V/IV) administered in other studies.

In conclusion, the vanadium (III) complex with the amino acid L-cysteine seems to exert, even at the low daily doses of 0.5 mg kg b.w a significant antitumor effect on experimentally-induced leiomyosarcomas in rats. The antitumor activities of this V(III)-cysteine complex, first reported by us, and its low toxicity, may, in relation to further studies, establish it as a possible candidate for human cancer treatment.

References


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