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AMMONIUM VANADATE DECREASES VIABILITY AND PROLIFERATION ACTIVITY OF CULTURED VIRUS-TRANSFORMED RAT SARCOMA CELLS

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Abstract

The aim of the study presented was to evaluate the effect of ammonium vanadate (NH_4VO_3) on the viability and proliferation of cultured virus-trasformed rat sarcoma LSR-SF-SR cells. The investigations were performed by MTT test, neutral red uptake cytotoxicity assay, double staining with acridine orange and propidium iodide, the method of Pappenheim and colony-forming technique. The results obtained revealed that applied at a concentration range of 0.1–20 μg/ml NH_4VO_3 expresses significant cytotoxic and/or cytostatic effects that are time- and concentration-dependent.

Key words: ammonium vanadate, virus-transformed cells, cytotoxic/antiproliferative activity, rat sarcoma, Rous sarcoma virus

Introduction. Vanadium (atomic number 23) is a member of period 4, group 5d transition element of the periodic table. As a micronutrient, it is included in the list of 40 essential elements that are required in small amounts for normal metabolism. In recent years, there has been a growing interest in the biological activity of vanadium, which is not surprising. This transition element is known to influence a variety of enzymatic systems, namely phosphatases, ATPases, peroxidases, ribonucleases, protein kinases and oxidoreductases. Among the most intriguing properties of vanadium are its ability to lower cholesterol, triglycerides and glucose levels; insulin-mimetic action; diuretic and

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natriuretic effects; contraction of blood vessels; enhancement of oxygen-affinity of haemoglobin and myoglobin [1–3]. There are data that vanadium compounds have anti-neoplastic potential against chemically-induced rat liver [4–6], mammary [7, 8], and colon [9, 10] carcinogenesis. According to the literature available, the anticancer activity of vanadium compounds in virus-transformed tumour cells has not been clarified yet. The aim of our study was to evaluate the influence of ammonium vanadate on the viability and proliferation of cultured virus transformed rat sarcoma cells.

Materials and methods. ***Chemicals and other materials.*** Dulbecco's modified Eagle's medium (D-MEM) and fetal bovine serum were purchased from Gibco-Invitrogen (UK). Dimethyl sulfoxide (DMSO), neutral red, acridine orange, propidium iodide and trypsin were obtained from AppliChem (Germany); thiazolyl blue tetrazolium bromide (MTT) and purified agar were from Sigma-Aldrich Chemie GmbH (Germany). All other chemicals of the highest purity commercially available were purchased from local agents and distributors. All sterile plastic and syringe filtres were from Orange Scientific (Belgium).

Compound. Ammonium vanadate (Valerus) was dissolved initially in bidistilled water and sterilized by filtration (0.2 µm diameter of pores) and then diluted in culture medium. The concentration of the compound in stock solution was 1 mg/ml.

Cell cultures and cultivation. The cell line LSR-SF-SR established from a transplantable sarcoma in rat induced by Rous sarcoma virus strain Schmidt-Ruppin [11] was used as an experimental model in our study. The cells were grown as monolayer cultures in D-MEM medium, supplemented with 5–10% fetal bovine serum, 100 U/mL penicillin and 100 g/mL streptomycin. The cultures were maintained at 37 °C in a humidified CO_2 incubator (Thermo scientific, Hepa class 100). For routine passages, adherent cells were detached using a mixture of 0.05% trypsin and 0.02% EDTA. The experiments were performed during the exponential phase of cell growth.

Cytotoxicity assays. The cells were seeded in 96-well flat-bottomed microplates at a concentration of 1×10^4 cells/well. After the cells were grown for 24 h to a subconfluent state ($\sim 60-70\%$), the cells from monolayers were washed with phosphate-buffered saline (PBS, pH 7.2) and covered with media modified with different concentrations (0.1, 0.5, 1, 5, 10, 20 µg/ml) of NH_4VO_3. Each solution was applied into 4 to 6 wells. Samples of cells grown in non-modified medium served as controls. After 24 h, 48 h and 72 h of incubation, the effect of the compound on cell viability and proliferation was examined by MTT (thiazolyl blue tetrazolium bromide) test [12] and neutral red uptake cytotoxicity assay (NR) [13]. Optical density was measured at 540 nm using an automatic microplate reader (TECAN, Sunrise^{TM}, Austria). Relative cell viability expressed as a percentage of the untreated control (100% viability) was calculated for each concentration. "Concentration–response" curves were prepared and the effective concentrations

of the compound – CC_{50} (causing a 50% reduction of cell viability) and/or CC_{90} (causing a 90% reduction of cell viability), were estimated (where possible) from these curves.

Evaluation of cytopathological changes. The cells were grown on sterile cover slips in 6-well plates in the presence of the compound tested. Non-treated cells served as controls. After 24, 48 and 72 h of incubation, the cover slips were removed and the ability of the compounds to induce cytopathological changes was assessed using double staining method with acridine orange (AO) and propidium iodide (PI) [14] and the method of Pappenheim (May–Grunwald–Giemsa) according to the standard procedure. The cells were examined microscopically using Leica DM 5000B (Leica Microsystems, Wetzlar, Germany).

Colony-forming assay. Tumour cells (approximately 10^3 cells/well) suspended in 0.45% purified agar in D-MEM medium containing different concentrations of the compounds examined (ranging from 0.1 to 20 µg/mL) were layered in 24 well microplates. The presence/absence of colonies was registered using an inverted microscope (Carl Zeiss, Jena, Germany) during 16-day period.

Statistical analysis. The data are presented as mean standard error of the mean. Statistical differences between control and treated groups were assessed using one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test.

Results and discussion. In our experiments NH_4VO_3 was applied as solutions in water/DMEM at concentrations of 0.1, 0.5, 1, 5, 10, 20 µg/ml for 24, 48 and 72 h. The MTT and NR assays were performed and concentration-response curves were calculated, using GraphPad 4.03 softwear (California, USA). Some of them are presented in Fig. 1. Cytotoxic concentrations 50 and 90 were calculated from these curves and summarized in Table 1. The combined staining

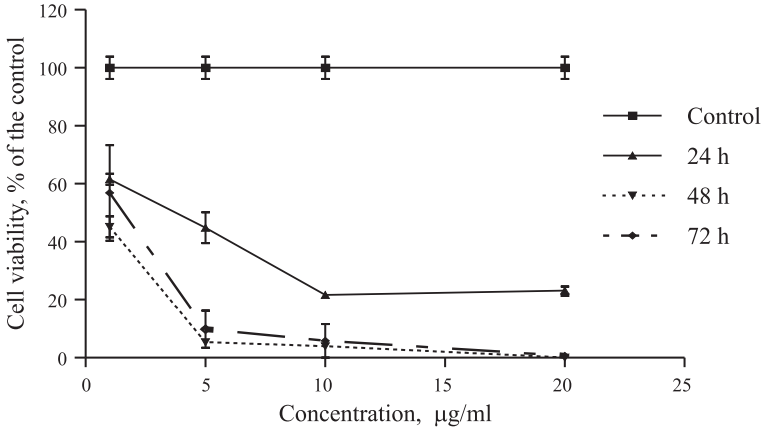

Fig. 1. Effect of NH_4VO_3 on viability and proliferation of LSR-SF-SR cells after 24, 48, and 72 h of treatment. The evaluation was performed by MTT test

Table 1

Cytotoxic activity (CC_{50} and CC_{90}) of NH_4VO_3 in LSR-SF-SR cells

Cytotoxicity assay	MTT			NR
Treatment period	24 h	48 h	72 h	72 h
CC_{50}	2.2	1.0	1.0	2.0
CC_{90}	n.d.	4.9	4.9	4.4

CC_{50} (CC_{90}) – concentrations (µg/ml), that reduce cell viability by 50% (90%)
n.d. – CC_{90} was not determined because at all concentrations administered the cell viability was > 10%.
MTT – MTT test
NR – neutral red uptake cytotoxicity assay

with acridine orange and propidium iodide as well as Pappenheim staining revealed significant cell losses in LSR-SF-SR cells treated with NH_4VO_3 whereas the available cells were swollen with picnotic nuclei and chromatin condensation (Figs 2, 3).

The effective concentration at which ammonium vanadate completely inhibited the colony-forming ability of tumour cells in semisolid medium was found to be ≥ 5 µg/ml.

The results obtained by us indicated that NH_4VO_3 decreased significantly the viability and proliferation of cultured rat sarcoma cells in a time- and concentration-dependent manner. Good correlations between the data coming from short-term tests (NR and MTT, 24–72 h) in monolayer cultures and long-term colony-forming assay (16 days, 3D colonies in semi-solid medium) as well as between MTT (which reflects damage to mitochondria) and NR (indicates damage to lysosomes and Golgi apparatus) methods were observed.

According to the literature available, this is the first report demonstrating the ability of vanadium (ammonium vanadate) to express antitumour activity in cultured virus-transformed cancer cells (LSR-SF-SR). The cell line LSR-SF-SR was established from a transplantable sarcoma in rat induced by the avian Rous sarcoma virus strain Schmidt–Ruppin (SR-RSV) [11] and is a suitable model system for investigations in oncology and oncopharmacology. It is not surprising because the protein tyrosine kinase v-SRC is the transforming product of Rous sarcoma virus, the first identified oncogenic retrovirus. SRC, the cellular counterpart of v-Src, is a member of a multigene family of membrane-associated non-receptor tyrosine kinases that comprise nine additional members. Src can regulate a number of signalling pathways that have impact on the behaviour of tumour cells, including proliferation, differentiation, survival, migration, invasion and angiogenesis. Abnormal expression of these genes has been documented in cancers that arise in the breast, colon, ovary, melanocyte, gastric mucosa, head and neck, pancreas, lung, and brain [15–17].

Recent studies have suggested that vanadium could be considered a representative of a new class of non-platinum antineoplastic agents. The mechanisms underlying the antitumour properties of this metal and its compounds remain unclear. Some of the following actions are probably related to its anticancer properties: 1) protective effect against the induction of DNA strand breaks and chromosome aberrations by potent hepatocarcinogens; 2) inhibition of metabolic activation of the procarcinogen, leading to reduced generation and/or binding of the ultimate carcinogen to DNA; 3) elevated detoxification of the precarcinogen and/or its reactive metabolites through specific induction of activities of some of the xenobiotic biotransforming enzymes; 4) inhibition of DNA polymerasaes, nucleotidyl transferases and phosphotransferas; 5) effect on the immune system [18–22]. Additional experiments are required to clarify better the possible involvement of vanadium in src signalling pathways. Such data could not simply enrich knowledge in this area, but also reveal new molecules for targeted anticancer therapy.

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