AMMONIUM VANADATE DECREASES VIABILITY AND PROLIFERATION OF CULTURED RETROVIRUS-TRANSFORMED CHICKEN HEPATOMA CELLS

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Abstract


Vanadium compounds have been reported to possess anti-neoplastic potential especially against chemically induced liver, mammary, and colon carcinogenesis in rodents. According to the literature available, the anticancer activity of vanadium compounds in virus-transformed tumor cells has not been clarified yet. The aim of the study presented was to evaluate the effect of ammonium vanadate (NH₄VO₃) on viability and proliferation of cultured LSCC-SF-Mc29 cell line established from a transplantable chicken hepatoma, induced by the myelocytomatosis virus Mc29. The investigations were performed by thiazolyl blue tetrazolium bromide (MTT) test, neutral red uptake cytotoxicity assay (NR), colony-forming method (CFM) and double staining with acridine orange and propidium iodide (PI/AO). The results obtained revealed that applied at a concentration range of 0.1–20 μg/ml for 24–72 h NH₄VO₃ expresses significant cytotoxic and/or cytostatic effects that are time- and concentration dependent. Administered at concentrations ≥ 5 μg/ml this compound completely inhibit the colony-forming ability of chicken hepatoma cells.

Key words: ammonium vanadate, cell culture, chicken hepatoma, cytostatic/cytotoxic activity, myelocytomatosis virus

Introduction

Vanadium (atomic number 23) is a metal of physiological, environmental and industrial importance. 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 is a growing interest in biological behavior of vanadium, especially due to its insulin-mimetic and anti-cancer properties (Alexandrova, 1999; Evangelou, 2002; Mukherjee et al., 2004; Kostova, 2009; Bishayee et al., 2010; Korbecki et al., 2012). According to the literature available, the antineoplastic activity of vanadium compounds in virus-transformed tumor cells has not been clarified yet. It has been found in our previous investigations that ammonium vanadate (NH₄VO₃) decreases significantly the in vitro growth of virus-transformed rat sarcoma cells (Abudalleh et al., 2013). The aim of the study presented was to evaluate the influence of NH₄VO₃ on viability and proliferation of cultured chicken hepatoma cells transformed by the myelocytomatosis retrovirus virus Mc29.

Materials and Methods

Chemicals and other materials

Dulbecco’s modified Eagle’s medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco-Invitrogen (UK). Dimethyl sulfoxide (DMSO), neutral red, 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 filters were from Orange Scientific (Belgium).

**Compound**

Ammonium vanadate (Valerus) $\text{NH}_4\text{VO}_3$ was dissolved initially in bidistilled water and sterilized by filtration (diameter of pores 0.2 $\mu$m) 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 LSCC-SF-Mc29 established from a transplantable chicken hepatoma, induced by the myelocytomatosis virus Mc29 (Alexandrova, 2008) was used as an experimental model in our study. The cells were grown as monolayer culture in DMEM medium, supplemented with 5-10% FBS, 100 U/mL penicillin and 100 g/mL streptomycin. The cultures were maintained at 37 ºC 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 $1\times10^4$ cells/well. After the cells were grown for 24 h to a subconfluent state (~ 60-70%), the culture medium was removed and changed with media modified with different concentrations (0.1, 0.5, 1, 5, 10 and 20 μg/ml) of $\text{NH}_4\text{VO}_3$. 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 (Mossman, 1983) and neutral red uptake cytotoxicity assay (NR) (Borenfreund and Puerner, 1985). Optical density was measured at 540 nm using an automatic microplate reader (TECAN, SunriseTM, Austria).

**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 and Origin 6.1TM.

**Results**

Short-term (MTT, NR, AO/PI, 24–72 h) and long-term (16 days) experiments were performed in order to study the influence of ammonium vanadate on viability and proliferation of cultured chicken hepatoma LSCC-SF-Mc29 cells. The obtained data are summarized in Figures 1 and 2 and Table 1. They reveal that applied at concentrations of 0.1 – 20 μg/ml for 72 h $\text{NH}_4\text{VO}_3$ decrease in a time- and concentration-dependent manner the viability and proliferation of the treated cells. At concentrations ≥ 5 μg/ml this compound completely inhibit the colony-forming ability of LSCC-SF-Mc29 cells.

**Discussion**

In this study we demonstrate for the first time the ability of ammonium vanadate to suppress the in vitro growth of chicken hepatoma cells transformed by the myelocytomatosis virus Mc29. A positive correlation between the data
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Fig. 1. Effect of NH₄VO₃ on viability and proliferation of LSCC-SF-Mc29 cells. The compound was applied at concentrations of 0.1, 0.5, 1, 5, 10 and 20 μg/ml for 24 h, 48 h and 72 h. The investigations were carried out by MTT (A) and neutral red uptake cytotoxicity assays (B) received by MTT, NR and AO/PI assays (short-term experiments with monolayer cultures) and colony forming method revealing the inhibitory effect of the compound on 3D colony formation of tumor cells in semi-solid medium was observed.

LSCC-SF-Mc29 cells have been proved to carry v-myc oncogene that is specific for Mc29 virus. It is well known that myc (c-myc, L-myc, N-myc) proto-oncogenes regulate key processes involved in many if not all aspects of cell life. There are data that myc genes are deregulated in several human and animal malignancies as a result from genetic and epigenetic alterations (Albihn et al., 2010; Dang, 2012).

Despite the accumulated data demonstrating the anticancer properties of vanadium, its mechanism of action is not fully understood. The antitumor activity of this element could be due to some of the following actions: i) Protective effect against the induction of DNA strand breaks and chromosome aberrations by potent hepatocarcinogens; ii) Inhibition of metabolic activation of the procarcinogen, leading to reduced generation and/or binding of the ultimate carcino-

gen to DNA; iii) Elevated detoxification of the precarcino-
gen and/or its reactive metabolites through specific induction of activities of some of the xenobiotic biotransforming enzymes; iv) Inhibition of DNA polymerases, nucleotidyl transferases and phosphotransferases; v) Effect on the immune system (Alexandrova et al., 2002; Evangelou, 2002; Alexandrova and Alexandrov, 2004; Kostova, 2009; Bishayee et al., 2010).

The anticarcinogenic effects of vanadium, in combination to its low toxicity, established also, by its administration in humans, suggest vanadium as a candidate antineoplastic agent against human cancer (Kostova, 2009). Additional experiments are required to clarify better the possible involvement of vanadium in myc signaling pathways. Such data will
not only enrich our knowledge of the biological behavior of this element, but will help us to evaluate its putative application in targeted anticancer therapy.

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