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Immunomodulatory Activities of Zygacyne Isolated from *Veratrum nigrum*

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Veratrum species are well known for their pharmacological properties. Some of them reduce high blood pressure, inhibit Sonic hedgehog (Shh) signaling during the gastrulation-stage of embryonic development and provoke malformations in several animal species. We tested zygacine isolated from *Veratrum nigrum* on murine bone marrow colony formation and two tumor lines K-562 and LSCC-SF(Mc29) It was found that zygacine activated bone marrow precursor cells and had different dose dependent effect on tumor lines.

Key words: zygacine, lymphocyte proliferation, bone marrow cells, tumor lines.

Introduction

Veratrum species are well known for their pharmacological properties. Extracts of several *Veratrum* plants have been used for treatment of various health disorders as toothache, herpes and hypertension. The steroidal alkaloids cyclopamine and jervine are shown to be primarily responsible for the malformations in several animal species. They are potent teratogens that inhibit Sonic hedgehog (Shh) signaling during gastrulation stage of embryonic development [1]. The steroidal alkaloid cyclopamin acts as inhibitor of P-gp-mediated drug transport and multi drug resistance [2]. Rubijervine possesses antimicrobial and antifungal activity [3]. Here we report for isolation of zygacine from *Veratrum nigrum* and first examine its immunomodulatory activity in mouse.

The aim of the present study was to explore the in vitro influence of this steroidal alkaloid on the formation of bone-marrow cells cultures and its activity on cells of two tumor line K-562 and LSCC-SF(Mc29).

Material and Methods

Plant material

Roots and rootage of *Veratrum nigrum* L. (Liliaceae) were collected in September 2005 from the Vratsa mountain, Bulgaria. Zygacine was isolated according the meth-

ods proposed of Atta - Ur - Rahman and preparative thin layer chromatography. The structure of alkaloid was elucidationed on the basis of NMR assay.

Bone-marrow agar cultures

Bone-marrow was isolated from femur bone by washing the internal bone cavity with RPMI 1640 containing 10% fetal calf serum. Cells were adjusted to 5×10^5 cells/ml and were distributed to plastic Petri dishes. The alkaloid was added in concentrtations 8, 16 and 33 μg/ml. After 7 days of incubation 37°C, 5% CO_2, 98% humidity the samples were dried and stained with Giemsa. The number of the colonies were observed under light microscope. Supernatant from fibroblast cell line 3T3 served as a positive control.

Proliferation of K-562 and LSCC-SF(Mc29) cell lines.

Veratrum alkaloid was added at concentrations 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39 and 0.19 μg/ml to 1×10^4 cells. The samples were incubated for 24 h at 37°C, 5% CO_2, 98% humidity. For the last 16-18h of the culture 1 μCi [^{3}H]-thymidine was added to each culture. The cells were harvested with cell harvester. The cells were count in scintillation counter (Beckman). The [^{3}H]-thymidine incorporation was expressed as counts per minute (cpm). All samples were in triplicates and the results are shown as average value for each triplicates.

Results and Discussion

Until now the alkaloid zygacyne was not reported about *Veratrum nigrum*. Its immunomodulatory activity was not investigated.

In Figs. 1 and 2 bone marrow agar cultures from mice treated with zygacine and a positive control / supernatant from fibroblast cell line 3T3/ are presented.

They show that the alkaloid have a stimulatory effect on bone-marrow progenitory cells and stimulate formation of bone-marrow colony compared with the control /supernatatant from fibroblast cell line 3T3/.

Some Veratrum alkaloids have been identified for their anticancer effect. We tested zygacine's effect toward two tumor lines K-562 and LSCC-SF(Mc29)

The results are presented in Figures 3 and 4.

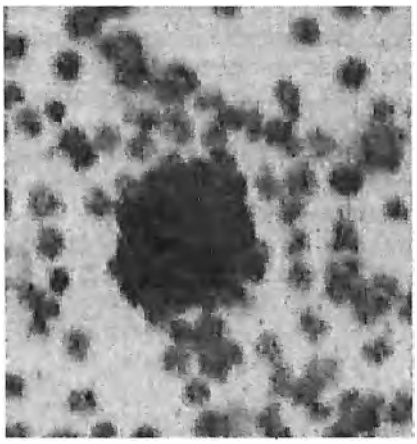

Fig. 1

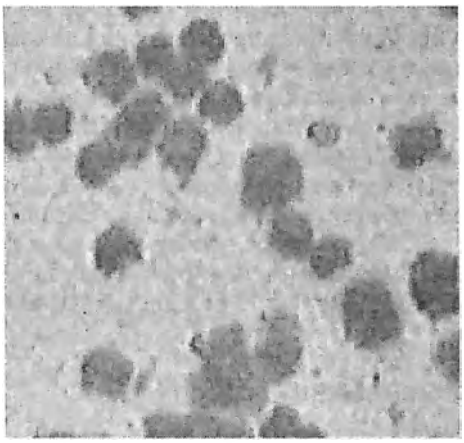

Fig. 2

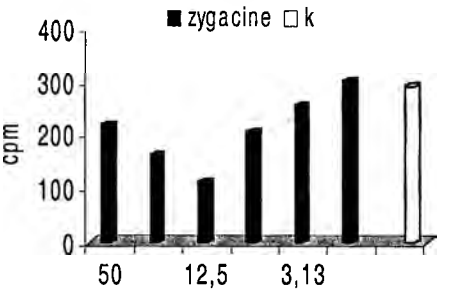

Fig. 3

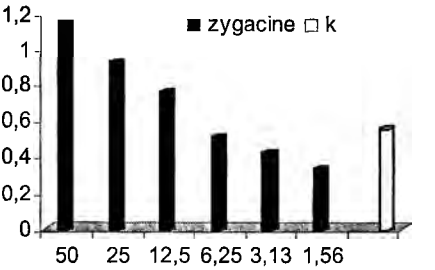

Fig. 4

They show that the zygacine with the exception of concentration 0.19 mg/ml was toxic toward the cells of K-562. It decreased thymidine incorporation into tumor cells in all used concentration. In the case of LSCC-SF(Mc29) the alkaloid's activity is dose-depended. In high concentration zygacine stimulated proliferation of tumor cells but in low concentration it suppress cells proliferation.

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