Chemistry of Natural Compounds, Vol. 41, No. 5, 2005

ACTION OF NGF FROM *Naja oxiana* COBRA VENOM ON MURINE MELANOMA KML CELLS *IN VITRO*

M. G. Khafizova, N. K. Kuznetsova, R. S. Salikhov, and D. Kh. Khamidov

UDC 616-006:612.014.4;577.11

Nerve growth factor (NGF) of mammals (mouse NGF) has a broad spectrum of action that includes antitumor activity toward neuroendocrine tumors. It has been shown that NGF under *in vitro* conditions inhibits migration and stimulates adhesion of cancer cells to principal components of the extracellular matrix without affecting cell proliferation [1].

A rich source of NGF is snake venoms. Minor differences in the structures of mammal and reptile NGF have practically no effect on the biological activities of these proteins for nerve tissue. Therefore, NGF from snake venom is expected to possess antitumor activity.

We previously developed a scheme for purifying NGF for preparations from *Naja oxiana* cobra venom that included gel filtration over Sephadex G-100, ion-exchange chromatography over KM-cellulose, and isoelectric focusing in a thin layer of Sephadex G-75 Superfine [2]. In the present work, the purification scheme was limited to two steps, gel filtration over Sephadex G-100 and electrophoresis in PAAG, in order to prepare analytical (up to 1 mg) amounts of NGF. Figure 1 shows as a marked area the protein fraction resulting from gel filtration that contained components with MW 25-30 kDa and NGF activity. This fraction was lyophilized and used for the next purification step. Electrophoresis in PAAG was as successful in separating NGF from accompanying proteins as isoelectric focusing. Two main protein groups were obtained. The electrophoretically mobile proteins had NGF activity.

The anticancer activity of cobra-venom NGF was evaluated using its action on the proliferative activity and cell adhesion of murine melanoma cancer cells. Melanoma produces malignant tumors of the system of neuroendocrine cells distributed throughout an organism. The studied concentration (100 ng/mL) of NGF corresponds to the maximum effective one for manifesting nerve-growth activity of a preparation in a biotest.

It has been found that NGF at 100 ng/mL has essentially no effect on the rate of cancer-cell proliferation. The ability of melanoma cells to attach to IY collagen (the main protein of the extracellular matrix) was investigated in a quick test to estimate the effect of cobra NGF on cellular adhesion. We observed that the number of cells attached to the substrate was statistically higher than in the control [253.57±36.09 (n = 32) and 142.47±35.25 (n = 32), respectively] after incubation of cancer cells for 96 h in a medium containing cobra-venom NGF followed by incubation for 3 h in the same medium over a layer of collagen. Whole cobra venom stimulated adhesion of cancer cells to collagen only insignificantly [171.36±52.43 (n = 32)].

Thus, *in vitro* experiments showed that the nature of action of cobra-venom NGF toward cancer cells is analogous to that of mouse NGF although the intensity of its effects are inferior to it.

Dysintegrins, which inhibit cancer-cell and L-aminooxidase proliferation and stimulate apoptosis in cancer cells, were identified in snake venoms. Our results indicate that NGF can be added to the list of nontoxic snake-venom proteins with antitumor activity.

Venom (100 mg) from the central Asian cobra *Naja oxiana* was dried over $CaCl_2$, fractionated by gel filtration over a column (1.8×87 cm) of Sephadex G-100 with elution by acetic acid (1%) at flow rate 6 mL/h. The resulting fractions were lyophilized. The protein content in the fractions was determined by the Lowry method. Electrophoresis in PAAG (pH 8.3) was carried out in glass tubes at 4-6°C with pretreatment by electrophoresis to remove ammonium persulfate. The gel pieces containing NGF were washed with acetic acid (0.1%) on the glass filters. The resulting solution was dialyzed, concentrated, and lyophilized.

Institute of Biochemistry, Academy of Sciences of the Republic of Uzbekistan, Tashkent, ul. Akad. Kh. Abdullaeva, 56. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 502-503, September-October, 2005. Original article submitted February 8, 2005.

0009-3130/05/4105-0613

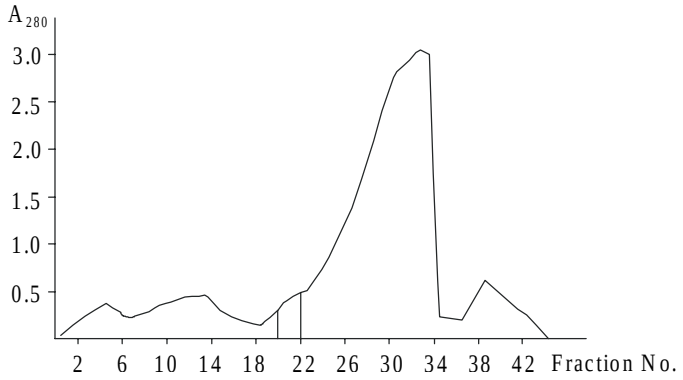

Fig. 1. Gel filtration of *Naja oxiana* cobra venom (100 mg) over Sephadex G-100 with subsequent electrophoresis in PAAG of the cobra venom fraction with NGF activity.

The NGF activity in the fractions was estimated from the growth rate of the neural sheath around spinal ganglia of 8-day chicken embryos after 24-h cultivation at 37°C on glass substrates covered with collagen in Hanks solution containing the studied protein.

Murine melanoma KML cells were cultivated in RPMI 1640 medium containing fetal calf serum (10%), L-glutamine (200 mM), and antibiotics [3].

The proliferative activity was measured by distributing cells (40,000) in vials with nutrient medium (3 mL) containing the studied sample (100 ng/mL) and [^{14}C]-thymidine (0.03 μCi) per vial and cultivating them for 24 h at 37°C in a CO_2 incubator. The culture medium was decanted. The cells were placed on a filter, washed three times with trichloroacetic acid (5%) for 10 min and three times with distilled water for 10 min, and dried. The filter was counted in ZhS-106 scintillant.

Adhesive properties of cancer cells were determined as before [4].

Cells were counted in the microscope field of view at 35×. Eight fields in each dish were analyzed in each of four directions at radii of 0.5 and 2.0 cm. Results were treated statistically by determining the arithmetic mean, its mean-square deviation, and the reliability of the differences using the Student t-criterion.

REFERENCES

1. M. Paez Pereda, C. Missale, Y. Grubler, E. Arzt, L. Schaaf, and G. K. Stalla, *Mol. Cell. Endocrinol.*, **167**, 99 (2000).
2. D. Kh. Khamidov, R. S. Salikhov, M. G. Khafizova, and L. Ya. Yukel′son, *Khim. Prir. Soedin.*, 546 (1985).
3. N. N. Kuznetsova, Z. I. Mardanova, V. B. Leont′ev, Z. S. Khashimova, and A. A. Sadykov, *Byull. Rasmii Akhborotnoma*, 2001, No. 4, Pat. IDP No. 04808.
4. M. Paez Pereda, U. Hopfner, U. Pagotto, and A. Renner, *Br. J. Cancer*, **81**, 381 (1999).