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Both neurotoxin II from venom of *Naja naja oxiana* and its endogeneous analogue induce apoptosis in tumor cells

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Abstract

Both neurotoxin II (NT II from venom of *Naja naja oxiana*) and 20–30 kDa proteins partially purified from pig brain (NTlm) cross-reacting with antibodies to NT II were cytotoxic for L929 and K562 tumor cells at concentrations of 10^{-6}–10^{-8} M. Modification of Cys residues significantly reduced cytotoxicity of NT II. Both NT2 and NTlm induced apoptosis in L929 and K562 cells.

Key words: Cytotoxicity; Neurotoxin II; Apoptosis

1. Introduction

The involvement of neurotrophic substances in cytotoxic cell-mediated immunity was demonstrated. Substance P [1], as well as opioid peptides [2], were shown to regulate the activity of cytotoxic cells. At the same time, cytotoxic proteins were shown to be localized in nerve tissue [3]. The involvement of these proteins in CNS-mediated processes is supported by the fact that behavioral [4,5], as well as electrophysiological [4,6] and neuroendocrine [4] actions, were demonstrated for TNF, LT, IL-1. These data have allowed us to propose the involvement of brain-derived proteins in tumor lysis as well as the direct regulation of CNS-mediated processes by cytotoxic proteins.

2. Materials and methods

2.1. Target cells culturing and cytotoxicity determination

L929 and K562 cells culturing and determination of cytotoxic activity was performed according to [7]. DNA fragmentation was determined as described [8].

2.2. Purification of NTlm

Pig brain tissue was homogenized in 50 mM Tris-HCl (pH 7.4), the pellet was removed and supernatant was subjected to size exclusion chromatography on a HW-50 column. The fraction containing NT II-like immunreactivity (proteins with M_w 20–30 kDa) was subjected to chromatography on CM-Toyopearl (Fig. 1). SDS-PAGE and immunoblotting with antibodies to NT II was performed according to [9,10]. NT II was carboxymethylated with iodoacetate after reduction with dithiothreitol as described [11].

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Abbreviations: NT II, neurotoxin II; NTlm, Neurotoxin-like material; TNF, tumor necrosis factor; CNS, central nervous system; SIRS, soluble immune response supressor; IL-1, interleukin 1; LT, lymphotoxin; TGF, transforming growth factor.

3. Results

Using antibodies to NT II we demonstrated the presence of NTlm in brain tissue. Two stages of chromatographic separation of the proteins of brain extract allowed us to obtain fractions that reacted specifically with antibodies to NT II (Fig. 1). The presence of several immunoreactive proteins with M_w of 20–30 kDa was detected (not shown). We proposed that NTlm would be cytotoxic to transformed cells, so we determined the cytotoxic activity of the fractions, obtained after chromatographic separation, on L929 tumor cells. The maxima of cytotoxic activity correlated with zones of NT-like immunoreactivity detected by dot-blot analysis (Fig. 1). To determine whether NT II is also cytotoxic for transformed cells we compared the cytotoxic activity of NT II and NTlm against L929 and K562 cell lines. Both NT II and NTlm were cytotoxic for L929 and K562 tumor cells (Fig. 2A,B). NT II was cytotoxic for both tumor lines at a concentration of 10^{-7} M. Modification of the Cys residues of NT II significantly reduced NT II cytotoxicity (Fig. 2A), since the conformation of NT II turned out to be essential for its cytotoxicity. K562 and L929 cells differed in their sensitivity to NTlm-mediated cytolysis: NTlm was cytotoxic at a concentration of 10^{-7}–10^{-8} M for L929 cells at a concentration of 10^{-6}–10^{-7} M for K562 cells (Fig. 2A,B). To determine whether the mechanism of target cell cytolysis is specific, we studied the ability of NT II and NTlm to induce DNA frag-

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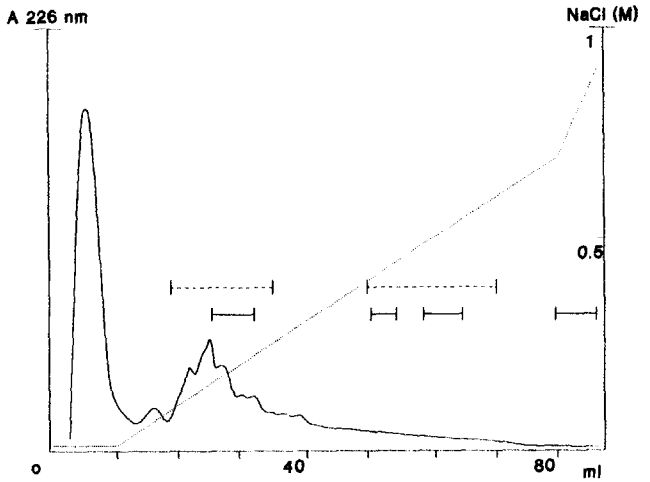


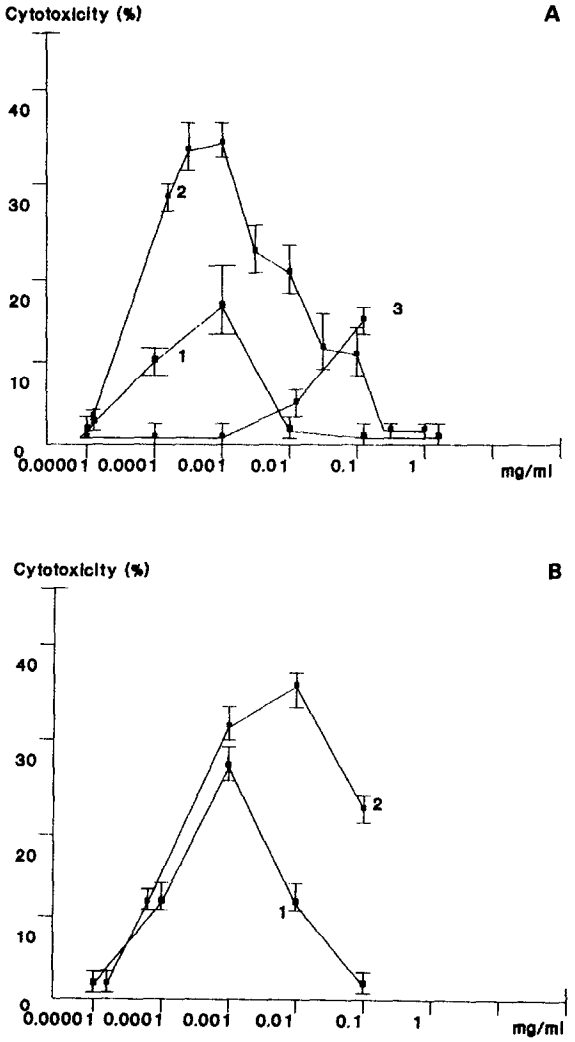
Fig. 1. Chromatographic separation of NTlm on CM-Toyopearl. The column was equilibrated with 20 mM ammonium acetate, pH 5.5. The proteins were eluted by a NaCl gradient (0–1 M). Fractions: (——) reacting with antibodies to NT II; (– – –) cytotoxicity.

mentation in target cells. Fig. 2C shows that both NT2 and NTlm induced apoptosis in L929 and K562 (not shown) cells.

4. Discussion

Endogeneous analogues of neurotoxins from snake venom have been shown to present in the class of Mammalia. Entothelins, the endogeneous analogues of sarafotoxins (carditoxins inducing smooth muscle cells concentration), are distributed widely in different tissues of mammals and display a wide spectrum of biological activities [12]. SIRS was demonstrated to have 80% homology with the N-terminal amino acid sequence of short neurotoxins from snake venom. SIRS has a M_w of 10 kDa and is produced mainly by $CD8^+$ supressor T-lymphocytes. This protein inhibits cytotoxic activity of cytolytic lymphocytes, and induces proliferation of normal and transformed cells [13]. The immunoregulatory activity of SIRS is similar to the activity of proteins of TGFβ family. The conformation of neurotoxin molecules (rigid structure with several disulfide bonds) is similar in conformation to the proteins of TGFβ family. Cytotoxic activity (in particular, ability to induce apoptosis in transformed cells) was demonstrated for TGFβ [14,15]. Specific localization of NT-like immunoreactivity in brain structures turned out to be analogous to distribution of TGFβ2 [16].

We have detected NTlm in brain tissue. The presence of immunoreactivity was demonstrated in several brain structures: in cerebellum (Purkinje cells), cerebral cortex, olfactory bulb, red nucleus, reticular formation. Localization of NTlp correlates with distribution of GABA-ergic neurones. We have demonstrated that NT II and



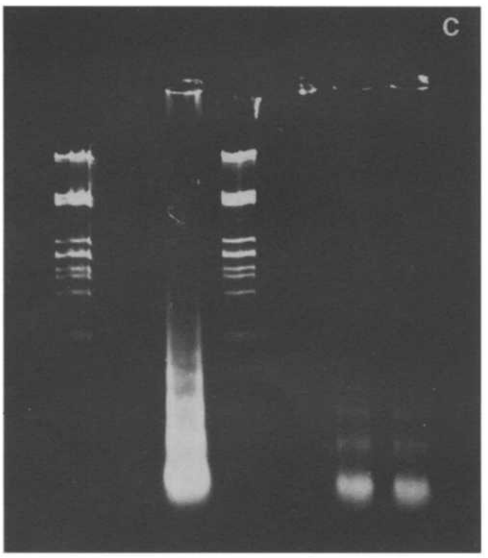


Fig. 2. Cytotoxic activity of NT II (1) and NTlp (2), and carboxymethylated NT II (3) against L929 (A) and K562 (B) cells. (C) DNA fragmentation induced by NT II and NTlm in L929 cells: lane 1, λ *Pst* M_w standards; lane 2, TNF; lane 3, λ *Pst* M_w standards; lane 4, untreated cells; lane 5, NT II (0.001 mg/ml); lane 6, NTlm (0.001 mg/ml).

NTlm are cytotoxic for tumor cells. The cytotoxicity of both substances against the L929 cell line was comparable with TNF (10^{-7} M, not shown). Their action was highly specific with respect to both target specificity and ability to induce apoptosis. Modification of NT II results in a decrease in its activity, so rigid conformation of the toxin is essential for cytotoxicity. Based on these data we propose that NTlm is involved both in elimination of tumor cells and CNS-mediated processes. Further study of the mechanisms of neuroregulation and cytotoxic action of NTlm will be useful for establishing the principles of direct interactions between nervous and immune systems.

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