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A heat stable protein toxin (*dr*CT-I) from the Indian Viper (*Daboia russelli russelli*) venom having antiproliferative, cytotoxic and apoptotic activities

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Abstract

A heat stable 7.2 kDa protein toxin (*dr*CT-I) has been purified and crystallized from Indian *Daboia russelli russelli* venom (Roy Choudhury et al., 2006. Acta Cryst. F Struct Biol Cryst Commun, 62(Pt. 3), 292). The *N*-terminal (first 20) amino acid sequence of *dr*CT-I was LKCNKLVPLFYKTCPAGKNL, which showed sequence homology to cytotoxins isolated from *Naja* venom. *dr*CT-I has been evaluated for anticancer activity against EAC cells in vivo and human leukemic cells (U937, K562) in vitro. *dr*CT-I (125 μg/kg, i.p/day for 10 days) significantly decreased EAC cell count, cell viability ($p<0.001$) and significantly increased the survival time of tumour bearing mice (T/C% 178.64, $p<0.01$) in comparison to untreated tumour bearing control. *dr*CT-I, produced dose and time-dependent inhibition of U937 and K562 cell growth and had an IC50 of 8.9 and 6.7 μg/ml respectively after 24 h treatment. The reduced MTT values after *dr*CT-I treatment indicated its cytotoxic nature, which supported its antiproliferative action. Scanning electron microscopy and confocal microscopy in U937 and K562 cells after *dr*CT-I treatment indicated certain features of apoptosis such as membrane blebbing, perforations, nuclear fragmentation. The induction of apoptosis was further confirmed by phosphatidylserine externalization observed using annexinV-FITC/PI staining and flow cytometric analysis. *dr*CT-I brought about apoptosis by G1 phase arrest of the cell cycle. The effect of *dr*CT-I on normal human peripheral blood mononuclear cell (PBMNC) viability and cytotoxicity was studied in culture and was found to be lower than that on U937 and K562 cells. Thus both in vivo and in vitro experimental results suggested that *dr*CT-I possessed anticancer potential.
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Keywords: *Daboia russelli russelli*; Viper venom; Heat stable protein toxin; Cytotoxin; Cytotoxic activity; Antiproliferative activity; Apoptotic activity

1. Introduction

The search for snake venom as antitumour agent dates back to 1933 when Calmette et al., reported an antitumour effect of *Naja naja* venom

Abbreviations: EAC, Ehrlich Ascites Carcinoma; T/C, Treated/Control; IC50, Median inhibitory concentration

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on adenocarcinoma cells (Calmette et al., 1933). Later antineoplastic activity of venoms of different snake species have been reported (Yoshikura et al., 1966; Braganca et al., 1967; Braganca, 1976; Markland, 1986; Chaim-Matyas and Ovadia, 1987; Lipps, 1998; Silva et al., 1996; da Silva et al., 2002; Sun et al., 2003; Kim et al., 2004, Yang et al., 2005, Tsai et al., 2006). There was no report on antitumour agent from Indian Russell's viper venom. A purified lethal toxin from Burmese Russell's viper (*Daboia russelli siamensis*) showed cytotoxicity on Hela cells (Maung-Maung et al., 1995). Mady (2002) reported antitumour activity of *Echis coloratus* snake venom of Viperidae family. Abu-Sinna et al. (2003) isolated a lethal and a non-lethal protein fraction from *Cerastes cerastes cerastes* snake venom of Viperidae family, which have anti-tumour activity on EAC cell line. From the present laboratory, a heat stable protein toxin (*dr*CT-I) purified from Eastern India *Daboia russelli russelli* venom has been crystallized and diffraction data indicated *dr*CT-I to be a three finger toxin structurally homologous to Naja venom cytotoxins (Roy Choudhury et al., 2006). In this communication, we describe in vivo and in vitro anti-tumour effect of the heat stable protein toxin (*dr*CT-I).

2. Materials and methods

2.1. Chemicals

Annexin-V antibody (Sigma, USA), Ara-C (Sigma, USA), DMSO (SRL, India), Ethidium bromide (Sigma, USA), Fetal bovine serum (GIBCO, BRL, USA), 5-Fluorouracil (5FU) (Sigma, USA), Gentamicin (Nicholas, India), Glutaraldehyde (Merck, India), Heparin (Sigma, USA), Histopaque (Sigma, USA), Hoechst 33342 (Sigma, USA), Imatinib mesylate (IM) (Natco, India), MTT (Sigma, USA), Osmium tetraoxide (Sigma, USA), Penicillin (Sigma, USA), Phytohemagglutinin (Sigma-Aldrich, USA), Polylysine (Sigma, USA), Propidium iodide (PI) (Sigma, USA), RNase A (Sigma, USA), RPMI (GIBCO, BRL, USA), Sodium cacodylate (SRL, India), streptomycin (Sigma, USA), Trypan blue (SRL, India).

2.2. Animals

Male Swiss albino mice (20 ± 2 g) were used for in vivo experiments. They were given synthetic pellet diet (Ashirwad Industries, Chandigarh, India) and clean tap water ad libitum and maintained at 24 ± 1 °C with 55 ± 5% relative humidity and day & night cycles of 12 h each. Institution animal ethics committee clearance was availed before the experiments.

2.3. Ascites tumour

Ehrlich ascites carcinoma (EAC) cells were maintained intraperitoneally in Swiss albino mice in the ascitic form. Tumour cells were collected by aspiration with a syringe aseptically, centrifuged for 5 min at 2000 rpm, washed and diluted with 0.9% saline and tumour cell number was adjusted to 1×10^6 cells/ml by counting the number with a haemocytometer using a phase contrast microscope (Olympus, CK40). Cell viability was evaluated by the trypan blue dye exclusion assay and only cell suspensions that presented more than 95% viability were used. Aseptic condition was maintained throughout the transplantation procedure.

2.4. Human myeloid leukemic cells and culture

Human leukemia cell lines U937 and K562 cell line were obtained from National Facility for Animal Tissue and Cell Culture, Pune, India. The cells were cultured in RPMI 1640, supplemented with 10% heat inactivated fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml), gentamicin (100 μg/ml) and maintained in a humidified incubator containing 5% CO_2 and 95% air at 37 °C. Cells were subcultured weekly at an initial density of 1×10^6 cells/ml and maintained.

2.5. Snake venom collection and preparation of *dr*CT-I

Lyophilized Indian Russell's viper venom collected from adult captive snakes (both sexes) was purchased from Calcutta Snake Park, Kolkata, India. Eastern India Russell's viper (*Daboia russelli russelli*) venom solution was subjected to heat treatment (80 °C) for 30 min, centrifuged and subjected to CM-cellulose ion exchange chromatography, to elute a pure heat stable toxin *dr*CT-I, which was crystallized (Fig. 1) (Roy Choudhury et al., 2006) and further characterized.

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