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Inhibitory effect of *Abrus* abrin-derived peptide fraction against Dalton's lymphoma ascites model

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Abstract

Peptides derived from larger molecules that are important modulators in cancer regression are becoming leads for development of therapeutic drugs. It has been reported that *Abrus* abrin, isolated from the seeds of *Abrus precatorius*, showed *in vitro* and *in vivo* antitumor properties by the induction of apoptosis. The present study was designed to evaluate the *in vivo* therapeutic effectiveness of abrin-derived peptide (ABP) fraction in Dalton's lymphoma (DL) mice model. The lethal dose (LD_{50}) of ABP was found to be 2.25 mg/kg body weight and further the acute toxicity was determined with sublethal doses in normal mice. The acute toxicity like body weight, peripheral blood cell count, lympho-hematological and biochemical parameters remained unaffected till 200 μg/kg body weight of ABP. The sublethal doses of ABP showed very significant growth inhibitory properties *in vivo* DL mice model. There were 24%, 70.8% and 89.7% reductions in DL cell survival in 25, 50 and 100 μg/kg body weight of ABP, respectively. Analysis of the growth inhibitory mechanism in DL cells revealed nuclear fragmentation, and condensation with the appearance of the sub-G_0/G_1 peak is indicative of apoptosis. Further, the Western blotting showed that apoptosis was mediated by the reduction in the ratio of Bcl-2 and Bax protein expression, and activation of caspase-3 through the release of cytochrome *c* in DL cells. Kaplan–Meier survival analysis showed an effective antitumor response (104.6 increase in life span (ILS) %) with a dose of 100 μg/kg body weight.

Keywords: Abrin-derived peptide (ABP); *Abrus precatorius*; Dalton's lymphoma (DL); Apoptosis

Introduction

Abrus abrin, isolated from the seeds of *Abrus precatorius*, is a hetero-dimeric glycoprotein of 63-kDa molecular weight, composed of two nonidentical polypeptide chains (A- and B-chain) cross-linked through a single disulfide bond (Tahirov et al., 1995). It belongs to the type II ribosome inactivating protein family (RIPII) with a protein synthesis inhibitory concentration (IC_{50}) of 10 ng/ml and a lethal dose (LD_{50}) of 20 μg/kg body weight in mice (Hegde et al., 1991; Stirpe et al., 1992).The abrin binds to the cell-surface receptors containing terminal galactose through the B subunit, enter cells by receptor-mediated endocytosis and A subunit inhibits the protein synthesis by modification of the ribosomal subunits of the cells. In addition, abrin induces apoptosis followed by the inhibition of protein synthesis. The apoptosis is triggered through intrinsic mitochondrial pathway by caspase 3-activation involving mitochondrial membrane potential damage and reactive oxygen species production (Narayanan et al., 2004, 2005). Abrin is 10–100 times more toxic to some transformed cell lines than to normal cells. The selective antiproliferative properties of abrin are attractive as a

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potential anticancer agent (Moriwaki et al., 2000; De Mejía and Prisecaru, 2005). The antitumor properties of abrin have been reported in different models (Lin et al., 1982; Ramnath et al., 2002). The *in vivo* tumoricidal property of abrin against Dalton's lymphoma (DL)-and Ehrlich's ascites carcinoma-induced tumors is thought to mediate through apoptosis (Ramnath et al., 2007).

Peptides derived from larger molecules that are important modulators in cancer regression are becoming leads for the development of therapeutic drugs (Bhutia and Maiti, 2008). In our previous study, we have reported that *Abrus* agglutinin-derived peptide fraction named 10kMPP induced apoptosis in cervical cancer cells through the intrinsic pathway (Bhutia et al., 2008). Here we have explored the antitumor activity of a peptide fraction isolated from toxic lectin abrin present in the seed of *Abrus*. The study was designed to evaluate the *in vivo* antitumor properties of abrin-derived peptides (ABP) fraction obtained from 10 kD molecular weight cut-off membrane permeate of tryptic-digested abrin in a DL mice model. Further, we have investigated to determine the possible mechanisms of cell death elicited by ABP on DL cells.

Material and methods

Reagent

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium), dimethylsulfoxide (DMSO), propidium iodide (PI), RNase A, trypsin, rhodamine123, dihydrorhodamine 123 (DRH123), and agarose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Amicon Ultra (10 kDa) was purchased from Millipore, India. Fetal bovine serum (FBS) was from Hyclone, and RPMI-1640 media, was from Invitrogen, India.

Enzymic hydrolysate of abrin and isolation ABP

The ABP was isolated and characterized as previously reported (Bhutia et al., 2008). Briefly, trypsin (10×10^3 BAEE unit/mg of protein) was added at a ratio of 1:50 to abrin solution (1.0 mg protein/ml in 0.01 M phosphate buffer saline [PBS]). Trypsinization was carried out at a temperature of 37 °C overnight. ABP was isolated using a 10-kDa molecular weight cut-off membrane (Amicon Ultra, Millipore). The peptide fractions were lyophilized and the concentration was quantified using Fluorescamine with glycine as standard (Udenfriend et al., 1972). MALDI-ToF mass spectrum was obtained on a Voyager-DE^{TM} PRO (Applied Biosystem) mass spectrometer equipped with a nitrogen laser operating at 337 nm. Mass spectra were recorded in a linear mode in positive ion detection using α-cyano-4-hydroxycinnamic acid (10 mg/ml) as the matrix.

MTT assay for DL cell viability

DL cells were harvested and the cell concentration was adjusted to 1×10^5 cells/ml and cells were plated in 96-well flat-bottom culture plates and incubated with various concentrations of ABP. All cultures were incubated for 24, 48 and 72 h at 37 °C in a humidified incubator, which maintained a constant 5% CO_2. Cell concentration was checked by MTT assay (Mosmann, 1983).

Animal experiment

Mice and tumor system

Female Swiss albino mice (20 ± 2 g, 6–8 weeks old) were used for acute toxicity and anticancer study. Mice were housed in open-top cages and maintained on food and water ad labium. Room temperature was maintained at 22 ± 2 °C with light and dark cycle of 14/10 h. All animal experiments were performed according to the rules of "Committee for the purpose of control and supervision of experiments on animals, Ministry of Environment and Forests, government of India" and Institutional Animal Ethics Committee, Indian Institute of Technology, Kharagpur, PIN-721302. DL is maintained in ascetic form by serial transplantation in Swiss albino mice or *in vitro* cell culture system by serial passage. Irrespective of whether the cells are obtained from *in vitro* culture or from ascetic fluid, they exhibited typical phenotypic features.

Assay of acute toxicity

The LD_{50} of ABP was determined by the method of Litchfield and Wilcox (Litchfied and Wilcoxon, 1949) and LD_{50} was found to be 2.25 mg/kg body weight. Later, acute toxicity assay was performed with sublethal doses according to the method described in Ghosh et al. (2006). Female mice (5–7 weeks old, 20–22 g) were randomly divided into 5 groups with a control and 5 in mice each group. Different sublethal doses of ABP in the range of 25–500 μg/kg body weight were given intraperitoneally (i.p.) everyday to each group till 10 days and the control mice received PBS. The body weight of mice was taken daily to monitor the toxicity effect. On the 11th day, all mice were sacrificed and different parameters of toxicity like blood cell count, total hemoglobin content and serum enzymes were measured. To examine the effect of ABP administration on spleen and thymus, these organs are harvested and the number of nucleated cells determined with a hemocytometer.

Evaluation of antitumor activity in DL-bearing mice model

The tumor growth inhibition study was carried out in a DL-bearing mice model (Ghosh and Maiti, 2007).