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Antitumor effects of snake venom chemically modified Lys49 phospholipase A_2-like BthTX-I and a synthetic peptide derived from its C-terminal region

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Abstract

The present work evaluates both *in vitro* and *in vivo* antitumor activity of BPB-modified BthTX-I and its cationic synthetic peptide derived from the 115–129 C-terminal region. BPB-BthTX-I presented cytotoxicity of 10–40% on different tumor cell lines, which were also susceptible to the lytic action of the synthetic peptide. Injection of the modified protein or the peptide in mice, 5 days after transplantation of S180 tumor cells, reduced 30 and 36% of the tumor size on day 14th and 76 and 79% on day 60th, respectively, when compared to the untreated control group. Thus, these antitumor properties might be of interest in the development of therapeutic strategies against cancer.

Keywords: BthTX-I; *Bothrops jararacussu*; Chemical modification; BPB; Synthetic peptide; Antitumor activity

1. Introduction

Development of new drugs in oncology represents one of the most promising lodes of the pharmaceutical industry. The use of snake venoms in the treatment of some diseases has begun about sixty years ago in the folk medicine. In spite of their toxicological effects, several isolated snake venom proteins and peptides have practical applications as pharmaceutical agents. Thus, the biological and toxicological mechanisms involved in snakebite led physicians to study new methods on the isolation of venom components, as well as to understand how these compounds could help in medicine.

Snake venoms are complex mixtures of pharmacologically active proteins, with phospholipases A_2 (PLA_2s) as one of their main components [1]. Two groups (I and II) of PLA_2 have been distinguished in these venoms based on their primary structure and disulfide bridge pattern. Group I includes the Elapidae family and group II, Viperidae and Crotalidae families [2,3]. Myotoxins isolated from these venoms belong to group IIA and may be subdivided into two subgroups: myotoxins with enzymatic activity, denominated

Abbreviations: BthTX-I, bothropstoxin-I from *Bothrops jararacussu* venom; BPB, *p*-bromophenacyl bromide; BPB-BthTX-I, His48 bromophenylated BthTX-I; pepBthTX-I, synthetic peptide derived from the 115–129 C-terminal region of BthTX-I; S180, sarcoma 180; IR%, inhibition rate (%); PLA_2, phospholipase A_2; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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Asp49 PLA_2, and myotoxins without hydrolytic activity, denominated Lys49 PLA_2 homologues [3,4].

BthTX-I is a basic ($M_r \cong 13{,}869$) Lys49 PLA_2 homologue, catalytically inactive, first isolated from *Bothrops jararacussu* snake venom by Homsi-Brandeburgo et al. [5], which shows several biological effects, including myonecrosis, paw edema in mice and irreversible neuromuscular blockade [2,6–10]. It also displays high cytotoxic activity upon tEnd, C2C12 and NB41A3 cell lines [8].

The region of Lys49 PLA_2 homologues responsible for the cytotoxic effects was identified near their C-terminus [11], and synthetic peptides representing this region can mimic their toxic, bactericidal and antitumor activities [12–15]. His48 residue is highly conserved in the catalytic site of PLA_2s, and when alkylated by *p*-bromophenacyl bromide (BPB), a loss in the enzymatic activity is observed. The modification of His48 residue in Lys49 PLA_2 homologues decreases effects as edema induction and myotoxicity, due to conformational changes in their three dimensional structures [8,16,17].

Chemical modification by BPB was used in this study to verify the presence of cytotoxic effects of the Lys49 PLA_2s-like after conformational changes in their catalytic site and three dimensional structure, since the myotoxic activity, which is also related to the C-terminal region, is partially inhibited by the alkylation of the His48 residue [17]. The results of this evaluation could justify the synthesis of a peptide based on this region with high cytotoxic effects on tumor cells and low myotoxicity [15]. Thus, this article describes the effects of BPB-modified BthTX-I and of a synthetic peptide derived from its C-terminal region on *in vitro* cytotoxicity using different cell lines and *in vivo* antitumor activity on the development of S180 solid tumor implanted in mice.

2. Material and methods

2.1. Obtainment of BPB-BthTX-I

BthTX-I was purified by ion-exchange chromatography from *B. jararacussu* snake venom [8]. Modification of His48 with BPB was carried out as previously described [18], using ammonium bicarbonate buffer instead of Tris–HCl. The excess of reagent was removed by ultrafiltration through 0.22 μm membranes; the remaining solution was washed with water or 0.05 M ammonium bicarbonate, pH 8.0, and then lyophilized.

2.2. Peptide synthesis

Peptides (10 mg) were synthesized by Fmoc chemistry, with native endings by a commercial provider (Chiron Mimotopes, Victoria, Australia or PepMetric Technologies Inc., Vancouver, Canada). Their estimated molecular masses were in agreement with corresponding calculated values, with final purity levels of at least 95% by RP-HPLC analysis. Peptides were kept dry at −20 °C, and dissolved in 0.12 M NaCl, 40 mM sodium phosphate (PBS), pH 7.2, immediately before being tested for their activities. Peptides were derived from the C-terminal region 115–129 of myotoxic Lys49 PLA_2 BthTX-I homologue (pepBthTX-I = ^{115}KKYRYHLKPFCKK^{129}).

2.3. Myotoxic activity

The myotoxic effect of native BthTX-I, BPB-BthTX-I and pepBthTX-I (25 and 50 μg) in mice was evaluated 3 h after injection in the right gastrocnemius muscle of male Swiss mice (25–30 g, $n = 5$). Controls received PBS alone. Blood from mice was collected into heparinized capillary tubes. Plasma creatine kinase activity was determined using the Kit UV (Bioclin, Brazil) and the myotoxic activity was measured and expressed in U/L [19].

2.4. Maintenance of tumor cells

The mice melanoma cells (B16F10), human breast adenocarcinoma cells (SKBR3) and human lymphoblastic T cell leukemia (Jurkat) were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were cultured in RPMI 1640 medium (pH 7.2–7.4) supplemented with 100 U/mL penicillin G, 100 μg/mL streptomycin, 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate and 10% fetal calf serum (FCS) (all reagents obtained from Gibco, Grand Island, NY) at 37 °C, 5% CO_2 and humidified atmosphere. B16F10 and SKBR3 tumor cells were routinely sub-cultured using 2.5 g/L trypsin–ethylenedinitrile tetraacetic acid (EDTA) solution (Sigma–Aldrich, St. Louis, MO). Mice sarcoma 180 tumor cell line was gently provided by Prof. Dr. José R. Mineo (Laboratório de Imunologia, Instituto de Ciências Biomédicas da Universidade Federal de Uberlândia). The S180 tumor was maintained by intraperitoneal injection of tumor in Swiss mice, which was collected and re-transplanted weekly to naive mice.

2.5. In vitro cytotoxic assay

The cytotoxic activity was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [20]. The logarithmically growing B16F10 cells and SKBR3 cells were detached using 2.5 g/L trypsin–EDTA solution, washed 2 times with supplemented RPMI medium at 400 ×*g*/15 min/10 °C and plated at a density of 5×10^4 cells/well into a flat-bottomed 96-microwell plates (Nalge-Nunc, Rochester, NY) and cultured for 24 h. Then, the medium was removed and fresh medium was added. The Jurkat and S180 cells were collected at the day of experiments, washed 2 times, counted and plated at a density of 1×10^5 cells/well into a 96-well plate. Peritoneal macrophages were obtained from male Swiss mice (25–30 g) that received i.p. injections of 4 mL of cold PBS and the peritoneal exudates were collected with syringe under sterile conditions. Cells were washed twice at 400 ×*g*/15 min/4 °C, counted, suspended in complete RPMI medium and plated as described above. Afterwards, cells were incubated for 30 min at 37 °C and non-adherent cells were removed, suspended in complete RPMI medium and used in experiments.