Antiproliferative Activity of King Cobra (Ophiophagus hannah) Venom l-Amino Acid Oxidase

Ma Li Lee, Ivy Chong, Shin Yee Fung, M.S. Karthimath and Ngai Hong Tan

1CENAR and Department of Molecular Medicine, Faculty of Medicine, University of Malaysia, Kuala Lumpur, Malaysia and 2Department of Pharmacology, Faculty of Medicine, University of Malaysia, Kuala Lumpur, Malaysia

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Abstract: King cobra (Ophiophagus hannah) venom l-amino acid oxidase (LAAO), a heat-stable enzyme, is an extremely potent antiproliferative agent against cancer cells when compared to LAAO isolated from other snake venoms. King cobra venom LAAO was shown to exhibit very strong antiproliferative activity against MCF-7 (human breast adenocarcinoma) and A549 (human lung adenocarcinoma) cells, with an IC50 value of 0.04 ± 0.00 and 0.85 ± 0.00 μg/ml, respectively, after 72-hr treatment. In comparison, its cytotoxicity was about 3-4 times lower than that of human non-tumorigenic breast (184B5) and lung (NL20) cells, suggesting selective antiproliferative activity. Furthermore, it was more potent against the two cell lines and the overall cytotoxicity of the LAAO was confirmed by flowcytometry (FCM) analysis and annexin-V/ligand of apoptosis (LLV) apoptotic assay, which showed a significant increase in apoptotic cells was observed in LAAO-treated tumor cells than in their non-tumorigenic counterparts. The ability of LAAO to induce apoptosis in tumor cells was further demonstrated using caspase-3/7 and DNA fragmentation assays. We also determined that the enzyme may target oxidative stress in killing of tumor cells, as its cytotoxicity was significantly reduced in the presence of catalase (a H2O2 scavenger). In view of its heat stability and selective and potent cytotoxic activity against cancer cells, king cobra venom LAAO can be potentially developed for treating solid tumors.

LAAOs from various snake venoms have been reported to exhibit cytotoxicity against cancer cell lines [8-12]. Ahn et al. [13] reported the cytotoxicity of king cobra venom LAAO on stomach cancer, dermal melanoma, fibrosarcoma, colorectal cancer and Chinese hamster ovary cell lines. It is believed that the cytotoxic action of the enzyme is mainly mediated by apoptosis [8,9,14-17]. Despite the evidence supporting LAAO's antiproliferative activity in several cancer cell lines, it is unknown whether LAAO cytotoxicity applies to non-tumorigenic cells. The effects of LAAO in non-tumorigenic cells, if any, are critical if this enzyme were to be developed as an anticancer agent. In this study, we investigated the cytotoxic action of king cobra venom LAAO against two human carcinoma cell lines and compared its cytotoxicity and apoptosis induction against their respective non-tumorigenic cell lines. In addition, we also compared its potency as a cytotoxic agent with doxorubicin, a standard anticancer drug.

Materials and Methods

Materials: King cobra (Ophiophagus hannah) venom was obtained from Snake Valley (Sembran, Malaysia). The snakes were identified by one of the authors (N.H. Tan). Human breast adenocarcinoma (MCF-7), human lung adenocarcinoma (A549), human non-tumorigenic breast (184B5) and human non-tumorigenic lung (NL20) cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), cell culture media, RPMI-1640, M199, mammary epithelial cell growth medium (MEGM Bullet Kit) and Ham's F12 medium were purchased from Lonza (Basel, Switzerland). Roswell Park Memorial Institute (RPMI)-1640, M199, mammary epithelial cell growth medium (MEGM Bullet Kit) and Ham's F12 medium were purchased from Lonza (Basel, Switzerland). Roswell Park Memorial Institute (RPMI)-1640, M199, mammary epithelial cell growth medium (MEGM Bullet Kit) and Ham's F12 medium were purchased from Lonza (Basel, Switzerland).