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A NOVEL BIOCHEMICAL AND PHARMACOLOGICAL AGENT: L-AMINO ACID OXIDASE WITH CORRELATION TO CANCER MANAGEMENT: AN OVERVIEW

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ABSTRACT : *Different types of snake venom including LAAO venom found in different organism which possesses anticancer effect as well as other pharmacological activity. Now a days using medicine for cancer treatment that have a lots of adverse effect such as alopecia, bone marrow depression, vomiting etc., we are focusing on SV-LAAOs to search out the natural bioactive novel agents. King cobra venom LAAO has more stability than other SV-LAAOs which may be correct agent to developed anticancer medicine. These effects mainly produced due to hydrogen peroxide which is generated during catalytic reaction.*

KEYWORDS: *L-amino acid oxidase, cancer, snake venom, H_2O_2, cytotoxic effect.*

I. INTRODUCTION

Snake venom is a complex mixture of different components that include peptides, proteins, enzymes, carbohydrates, and minerals [1]. L-amino acid oxidases (LAAOs) are widely distributed in many different species including insects, fungi, bacteria, and snakes [2] and are found even in plants where one of their catalytic products, ammonia, is used as a nitrogen source in cell metabolism [3, 4]. Experiments have shown that cytotoxic effects displayed by snake venoms are specifically related to the species, genus, and tissue targets. Thus, these findings provide new direction and probable application of snake venom as well as isolated toxins for cancer treatment [5]. L-Amino acid oxidase (L-amino acid:O2 oxidoreductase, E.C. 1.4.3.2.) is a flavoenzyme that catalyzes the oxidative deamination of an L-amino acid to form the corresponding α-ketoacid, hydrogen peroxide and ammonia. The detailed chemical equation of the mentioned reaction is shown below:

$$R\text{-}CHNH_2\text{-}COOH\ (\text{L-amino acid}) + O_2 + H_2O \xrightarrow{\text{L-AAO}} R\text{-}CO\text{-}COOH\ (\alpha\text{-keto acid}) + NH_3 + H_2O_2$$

L-Amino acid oxidase (LAAO) occurs widely in nature [6] and snake venoms are perhaps the richest sources of the enzyme. Snake venom LAAOs are generally very active and have been used widely in preparation of α-keto acids because of their chemo- and stereospecificity[7,8]. It represents 1-9% of total venom protein [2]. Almost all LAAOs described to date are flavoproteins of dimeric structure, with each subunit presenting a non-covalent bond with flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD). Flavins present in LAAOs are responsible for the characteristic yellow color of many snake venoms and contribute to their toxicity because of the oxidative stress that results from the production of H_2O_2[9]. Recently, snake venom LAAO has become an interesting object for biomedical studies because of its antimicrobial, anti-HIV, anticoagulant, platelet aggregation-inducing and inhibiting, apoptotic-inducing as well as anti-cancer activities. Snake venom LAAO is recognized as a multifunctional protein with promising biomedical application. Several reviews on snake venom L-amino acid oxidases have been published in many journals. [2, 6, 10-14]. The individual LAAOs differ in their substrate specificity: snake venom L-amino acid oxidases (SV-LAAOs) which constitute upto 30% (by weight) of the crude venom, possess a clear preference for hydrophobic amino acids [15].

The king cobra (*Ophiophagus hannah*) venom LAAO, with a molecular weight of 130 kDa, has an unusual thermal stability which is different from other venom LAAOs. At pH 7.4, the enzyme retained 100% activity after incubation at 37 °C for 5 days. Unlike other snake venom LAAO, king cobra venom LAAO was stable at alkaline condition and was not inactivated by freezing [16]. Because of these favourable stability properties, king cobra venom LAAO has a greater potential, when compared to other venom LAAOs, to be

developed as a cancer therapeutic agent. Earlier studies also showed that the king cobra venom enzyme exhibited extremely potent anti-proliferative activities in comparison with other venom LAAOs, and that its cytotoxicity was selective against tumorigenic cells [17]. Nowadays, treatment of cancer is a major challenge to the medical world. Present methods of treatment are very costly and have numerous side effects. Patient has to suffer physically, mentally as well as economically. Some of the components of snake venom cause retardation of growth of cancerous cells. Due to its therapeutic activity, potency and availability, snake venom may be a vital nominee for the medicine in the future for many diseases and disorders [18]. Snake venoms are recognized as useful sources of bioactive substances showing a wide range of biochemical & pharmacological activities. The purpose of this article is to review recent finding regarding therapeutic potential of snake venom L-amino acid oxidase with correlation to cancer management.

II. A POTENT BIOCHEMICAL ANALOGUE

Many studies have been described that the SV-LAAOs are a potent biochemical analogue showing different biological activities such as edematogenic, hemorrhagic, anticoagulant, platelet aggregation inducing and inhibiting activities. Hydrogen peroxide not alone is responsible for these biological activities of SV-LAAOs; that is, other mechanisms are probably triggered and cause a potent biological response [19]. Some studies have been conducted to determine the true mode of action of LAAOs in the induction of edema, that revealed that the action of these compounds is related to the stimulation and release of inflammatory mediators such as histamine, prostaglandin, kinins, and serotonin [25]. The edematogenic activity of LAAOs enzymes do not lose their activity in the presence of antihistamines but the activity of this enzyme was completely suppressed when treated with glutathione, indicating that edema induced by LAAO is directly related to the presence of hydrogen peroxide. The hemorrhagic induction of snake venoms mediated by degradation of extracellular matrix proteins of vascular endothelium. Souza et al. [20] proposed that SV-LAAOs trigger a process of apoptosis in vascular endothelial cells, causing rupture of the endothelium and concomitant leakage of blood to the interstice. SV-LAAO purified from Agkistrodon halys blomhoffii venom possesses anticoagulant activity [21].The enzyme significantly delayed the onset and progress of blood coagulation, prolonged the activated partial thromboplastin time but had little effect on the prothrombin time. It is interferes primarily with the intrinsic blood coagulation pathway, and further studies indicated that the anticoagulant effect of LAAO is due to its inhibitory action on clotting factor IX. SV-LAAOs activities on platelets is still controversial, with a variable potential of these enzymes to inhibit or induce platelet aggregation. Some investigation reported that LAAOs induce platelet aggregation, whereas others reported that LAAOs have an inhibitory action on platelet aggregation [2]. Catalase, a H2O2 scavenger, inhibited both platelet aggregation inducing and inhibiting effects, indicating that both effects are due primarily to the action of H2O2 produced by the enzyme during the oxidation. LAAOs from venoms of *C. Durissus cascavella, E. macmahoni, B. alternatus, B. pirajai, O. hannah* induce platelet aggregation [22-26]. On the other hand, LAAOs from *A. h. blomhoffi, V. lebetina* and *N. naja kaouthia* dose-dependently inhibited both agonist-induced platelet aggregation and shear induced platelet aggregation [27-29]. LAAO isolated from Ophiophagus hannah dose dependently, induces platelet aggregation in the concentration range of 10-60 mg/ml, and inhibit platelet aggregation in the concentration range of 4.8-48 mg/ml(IC50 0.15 mM) [30].

III. A POTENT PHARMACOLOGICAL AGENT

SV-LAAOs is a potent pharmacological agent have become an interesting object for biomedical studies because of its bactericidal, leishmanicidal, antiviral, apoptotic, cytotoxic, and other physiological effects. These effects are mediated by the chemically very reactive hydrogen peroxide generated in the oxidation process, because H2O2 scavenger such as catalase neutralizes the effects. Sometimes the toxic effects cannot be attributed to H2O2 liberated alone and direct interactions between LAAO and the target cells may play an important role [31]. The antibacterial property of snake venom, in particular, has gathered increasing scientific interest due to antibiotic resistance. Infections of microorganism may be difficult to treat due to developing resistance to the current available antimicrobial agents. Examples of bacterial species that have developed resistance to conventional antibiotics are *Pseudomonas, Klebsiella, Enterobacter, Acinetobacter, Mycobacterium, Salmonella, Staphylococcus, Enterococcus* and *Streptococcus* [32, 33]. Therefore, we are searching new biochemical sources for the development of novel antimicrobial agents. King cobra venom was most efficient against *Escherichia coli* and was least effective against *Pseudomonas aeruginosa* [34]. A recent study describe that the minimum inhibitory concentrations (MIC)of the Siamese Russell's viper LAAO enzyme against *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 higher than the MIC of king cobra LAAO [19]. It is also shown antiviral effect which has been well explored. Zhang *et al.* reports the possible inhibition of HIV-1 infection and replication by an LAAO isolated from Trimeresurus stejnegeri venom, called TSVLAO [31]. This activity was mediated by a reduction in a protein p24 production as well as decrease in syncytium formation. The antiviral potential of LAAOs was

also studied against DENV-3 virus strains, the etiological agent of dengue, with BjarLAAOI isolated from *Bothrops jararaca* snake venom [35]. The treatment's efficiency was demonstrated due to reduction of viral load in previously infected C6/36 cells exposed to the toxin when compared to controls of unexposed infected cells. SV-LAAOs are also possesses Leishmanicidal activity [26, 36], as the H2O2 generated by the enzyme was a strong inducer of apoptosis in promastigotes of *Leishmania ssp.* cells. The first-line drugs for the treatment of Leishmaniasis are pentavalent antimonials, which have serious side effects and to which the target parasites have shown clinical resistance. Thus, the study of new Leishmanicidal compounds from different sources, including those with antileishmanial activity, is needed for drugs development. An LAAO isolated from *B. moojeni* venom showed leishmanicidal activity against promastigote forms of *Leishmania amazonensis* five times higher than that of the crude venom [37, 38].

IV. RECENT UPDATE ON CANCER MANAGEMENT

Cancer is characterized by uncontrolled cell division, cell transformation, and escape of apoptosis, invasion, angiogenesis and metastasis [39]. The anti-cancer property of snake venom, in particular, has gathered increasing scientific interest due to its potential natural-based cancer therapeutic agents that exhibit highly selective cytotoxicity against cancer cells over the normal cells. Recently ML. Lee et al. [40] reported that LAAO isolated from king cobra venom Induces Apoptosis in prostate adenocarcinoma (PC-3) Cells and Suppresses PC-3 Solid Tumor Growth in a Tumor Xenograft Mouse Model but did not shows any abnormality on the normal organ tissue. Previously discussed that the king cobra venom (LAAO) enzyme also exhibited extremely potent anti-proliferative activities in comparison with other venom LAAOs, and that its cytotoxicity was selective against tumorigenic cells (breast cancer and lung cancer cells) [17]. The cytotoxic effect of SV-LAAOs on normal cells is still controversial, some of groups suggesting it is harmless to normal cells while other mentioning its having cytotoxic effect on normal cells. A large number of studies demonstrated the *in-vitro* anti-cancer action but very few studies that demonstrated the *in vivo* anti-cancer action of snake venom LAAOs on tumors. Induction of apoptosis is the most important mechanism of many anticancer agents. Many research groups describe LAAOs as apoptosis inducers, in human embryonic cells (293T) [41], human promyelocytic leukemia cells (HL-60) [42, 43], human monocytic cells (MM6) [48], rat lymphocytic leukemia cells (L1210), and human leukemia T cells [25]. Apoptosis is an important pathway of LAAOs of exerting cytotoxicity activity selectively against cancer cells, it is reported many previous reports [37, 43-47]. Several of the LAAOs isolated have been considered cytotoxic, including APIT to Jurkat T cells [48], *Vipera berus berus* LAAO to HeLa and K562 cells [49], BpirLAAOI to S180 cells and macrophages [50], ABU-LAO to human monocytes and T cells [43], BmooLAAO-I to Ehrlich ascites tumor cells [23], BatroxLAAO to HL-60, Jurkat, B16F10, and PC12 cells [49], ACTX-8 to HeLa cells [37], BjarLAAO-I to Ehrlich ascites tumour [45], BF-LAAO to A549 cells [35], BpLAAO to SKBR3 breast carcinoma and Jurkat leukemia cells [49], BmarLAAO to macrophages [51], Bl-LAAO to LL- 24, RKO, HUTU, and MKN-45 cells [52], and LmLAAO to AGS cells-gastric adenocarcinoma and MCF-7 cells-breast adenocarcinoma [53]. Cura et al. [54] reported that Ophiophagus hannah LAAO venom decreases thymidine uptake in fibrosarcoma, colorectal cancer and Chinese hamster ovary cell line as well as also showed reduction in cellular proliferation. Also, LAAO isolated from *Agkistrodon acutus* snake venom showed accumulation of tumor cell at sub-G1 phase of cell cycle and induced apoptosis via Fas pathway in A549 cells (human alveolar epithelial cell line) [55]. Ande *et al.* [56] and Samel *et al.* [57], using Jurkat and K562 (human chronic myeloid leukemia) cells, respectively, reported that at low concentration LAAO induced apoptosis, but caused necrosis at higher concentrations of LAAO. The factors contributing to apoptosis are: (i) generation of toxic intermediates from fetal calf serum and (ii) binding and internalization of LAAO, which appears to be mediated by the glycan moiety of the enzyme, as desialylation of the enzyme reduces cytotoxicity [56]. In 1999, Souza et al. [58] showed the cytotoxicity level of an LAAO from *Agkistrodon contortrix laticinctus* through the fragmentation of DNA onHL-60 cultures hybrid cells. We can say that SV-LAAOs isolated from different snakes species showing antitumor effect at a different concentration by different mechanism. Different concentration and treatment time were obtained through several assays regarding their cytotoxic effect upon cell cultures and animal models, as well as the mechanisms involved and reactions able to explain these effects are given in the table1.

Table -1 showing various LAAOs venom and their effect.

LAAOs Venom Protein name	Snakes	Cellular target/mechanism	Concentration and treatment time	reference
LAAO	*Ophiophagus hannah*	Demonstrated potent cytotoxicity against PC-3, stomach cancer, murine melanoma, fibrosarcoma, and colorectal and ovary cell lines.	0.05-2 μg/mL for 72 h	[40, 59]
B1-LAAO	*Bothrops leucurus*	Cytotoxicity in the stomach cancer MKN-45, adenocarcinoma HUTU, colorectal RKO, and human fibroblast LL-24 cell lines.	0.1–20 μg/mL for 24 h	[54]
ACTX-8	*Agkistrodon acutus*	Induces apoptosis in human cervical cancer Hela cell line.	20 μg/mL for 12–48 h	[49]
BmooLAAO-I	*Bothrops moojeni*	Cytotoxicity and apoptosis	8–16 μg/mL for 12 h	[60]
LAAO	*Vipera berus berus*	Induces apoptosis in HeLa and K562 cell lines.	2.5–10 μg/mL for 7–24 h	[43]
LAAO	*Trimeresurus flavoviridis*	Antitumor activity in RBR17T and C6 cell	2.5 and 5 μg/mL for 24 h	[47]
LAAO	*Eristicophis macmahoni*	Apoptosis	25–100 μg/mL for 18 h	[26]
LAAO	*Agkistrodon contortrix laticinctus*	Caused DNA fragmentation in HL-60 cells.	2.5–100 μg/mL for 16 h	[20]
LmLAAO	*Lachesis muta*	Cytotoxicity in the LL-24, AGS, MCF-7, and HUTU	1.17–75 μg/mL for 24 h	[55]

V. CONCLUSION AND FUTURE PROSPECTS

Many experiments have shown that cytotoxic effects displayed by snake venoms LAAOs are specifically related to the species, genus, and tissue targets for cancer treatment. SV-LAAOs is a flavoenzyme that catalyzes the oxidative deamination of an L-amino acid to form the corresponding α-ketoacid, hydrogen peroxide and ammonia. A lots of biomedical studied described that snake venom LAAOs as an interesting object because of its antimicrobial, anti-HIV, leishmanicidal, anticoagulant, platelet aggregation-inducing and inhibiting, apoptotic-inducing as well as anti-cancer activities. All these above pharmacological effect are produce mainly by hydrogen peroxide which is produce during catalytic oxidative reaction of LAAOs.

Unlike other venom LAAOs, king cobra (*Ophiophagus hannah*) venom LAAO has an unusual stability as well as selective cytotoxicity against tumorgenic cells. Because of these favourable stability properties, king cobra venom LAAO has a greater potential, when compared to other venom LAAOs, to be developed as a cancer therapeutic agent. SV-LAAOs which isolated from different species of snakes are effective against different cancer cell lines both in-vitro as well as in-vivo condition at different concentration. The effect of SV-LAAOs only check out against cancer cell lines but do not check out against chemical induced cancer. So snake venom LAAOs may be open the doors to investigate against chemical induced cancer. Some SV-LAAOs also effective against viruses. So these venom may be a future prospect for the treatment of Ebola virus because no one's check out the effect against Ebola virus. These venom open a gate to check out the effect of SV-LAAOs against Ebola virus.

CONFLICTS OF INTERESTS The authors hereby declare that there is no conflict of interests.

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