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Review Article

Neurological Implications of Dendrotoxin: A Review

Anil Kumar* and Varun Gupta

Pharmacology Division, University Institute of Pharmaceutical Sciences (UGC-Centre of Advanced Studies), Panjab University, Chandigarh, India

***Corresponding Author:** Anil Kumar, Pharmacology Division, University Institute of Pharmaceutical Sciences (UGC-Centre of Advanced Studies), Panjab University, Chandigarh, India.

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Abstract

Dendrotoxin, the most potent component of Mamba snake venom is considered to be majorly responsible for the exceedingly high rate of morbidity and mortality observed in Mamba snake victims. Studies suggest that in addition to inducing toxicity in the brain and other organs, the footprints of dendrotoxin can also be seen in various other neurological and physiological conditions. The understanding of dendrotoxin has been further deepened by its applications in neurophysiology. Studies have been done on the binding of dendrotoxin at various sites in the brain, therefore suggesting a pathological connection. In this review, authors highlight the involvement of dendrotoxin in diverse neurological conditions either in the induction of direct neurotoxicity or having the potential to be involved in the pathology of various neurological disorders. The authors also explain here the molecular mechanisms associated with dendrotoxin along with other components of mamba venom. Need for the search of a potent, yet safer drug candidate, which can be effective in cases of delayed medical assistance in mamba snake victims, is also urged upon.

Keywords: *Dendrotoxin; Mamba Snake Venom; Neurological Implications*

Introduction

Snakebite related morbidity and mortality is a serious public health concern in many parts of the world, majorly in rural areas where appropriate and cost effective treatment is not available. It is estimated that every year, at least 421,000 envenomings and 20,000 deaths occur due to snakebite globally. Since many cases of snakebites remain unreported, the actual number can be 3 times more [1]. Amongst those who survive after a venomous bite generally suffer from permanent tissue damage caused by venom, leading to disability [2]. Most snake envenoming and fatalities occur in South Asia, Southeast Asia, and sub-Saharan Africa, with India reporting the most snakebite deaths of any country [1].

Of the 3000 known species of snakes, meager 15% of these are believed to be venomous [3]. Mamba snakes hold a major repute in the class of venomous snakes because of their unmatching attributes. The symptoms of a mamba snake bite include progressive unconsciousness, diaphoresis, flaccid paralysis, respiratory paralysis, sustained hypotension, neuronal hyper-excitability leading to convulsions and direct cardiotoxicity leading to death [4]. Mamba Snake venom has various components such as dendrotoxins and fasciculins which render it toxic; however recently discovered mambalgins from mamba venom have been reported to possess central analgesic activity. The presence of binding sites for these toxins in the brain and direct toxic actions are the major reasons for various neuropathological conditions induced after the mamba snake bite. The use of antivenom therapy against Mamba venom is associated with various adverse effects and therefore research needs to be envisaged to understand the neuropathological involvement of this toxic venom and the possible solutions to them.

Biology

Mambas are fast-moving, terrestrial, venomous snakes of the genus *Dendroaspis* in the family Elapidae with four species viz. *Dendroaspis angusticeps* (eastern green mamba), *Dendroaspis viridis* (western green mamba), *Dendroaspis polylepis* (black mamba), *Dendroaspis jamesoni* (Jameson's mamba). The word *Dendroaspis* literally refers to tree snake, more accurately describing green mamba species since black mamba is not a tree dweller [4].

Venom of Mamba

All mambas are highly venomous consisting mostly of neurotoxins (Figure 1) which exhibit potent effects attributed to the presence of specific polypeptides. High activity of hyaluronidase present in the venom is believed to facilitate the spread of venom. Mamba snake venom lack hemolytic, hemorrhagic and procoagulant activities [5].

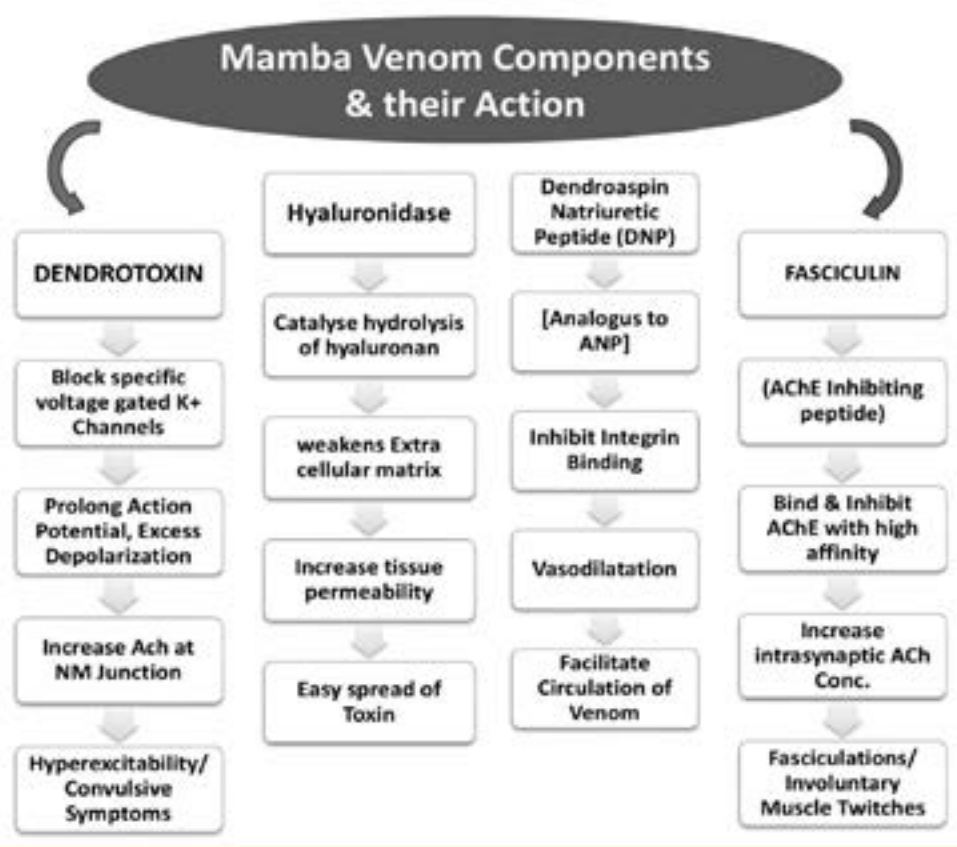

Figure 1: *Components of Mamba Snake Venom with their actions.*

The most prominent neurotoxin present in the mamba snake venom is known as dendrotoxin. Dendrotoxins are a class of presynaptic neurotoxins (having 57 - 60 amino acid residues cross-linked with di-sulfide bridges) that block particular subtypes of voltage-gated potassium channels in neurons, thereby enhancing the release of acetylcholine at neuromuscular junctions leading to convulsions [6].

Another constituent of Mamba snake venom referred to as fasciculins, that have been isolated from the venom of eastern green mamba are acetylcholinesterase (AChE) inhibitor peptides and are the only known peptide AChE inhibitors. They have high affinity for binding to the enzyme and lead to increased intrasynaptic acetylcholine concentrations leading to severe fasciculations in mice [7-9].

Recently, two more polypeptides namely Dendroaspin natriuretic peptide (DNP) and Calciseptine have been isolated from various *Dendroaspis* venoms. Dendroaspin has been reported to inhibit the binding of integrins to an integrin receptor [10]. DNP is an analogue of atrial natriuretic peptide (ANP) having similar activity facilitating the circulation of venom through a vasodilatory action [11]. However, calciseptine is an L-type calcium channel blocker that lowers blood pressure without inducing significant cardiac effects in rats making it relevant to find applications in the treatment of cardiac and vascular diseases [12,13].

Another recently found toxin component from Mamba venom is Mambalgin. It has two isopeptides, mambalgin-1 and mambalgin-2 both of them comprising 57 amino acidic chain with 8 residues of cysteine [14]. Mambalgins have been reported to have a potent analgesic effect in both central and peripheral nerves, with a better potency and safety as compared to morphine because of lesser induced tolerance and respiratory distress. Unlike morphine, which acts through the opioid pathway for analgesic action thereby causing undesired effects such as interference in cognitive functions, addiction, headache and emesis, mambalgins reduce pain by inhibiting centrally and peripherally present acid-sensing ion channels (ASIC) thus interfering with the signaling of pain sensation from the pain site to the brain. These peptides can therefore be potential candidates for analgesic treatment of patients with chronic respiratory diseases since they are unlikely to produce respiratory distress and other unwanted effects associated with morphine treatment [15].

Discovery of Dendrotoxin

Dendrotoxins were discovered after a series of work was carried against venom from mamba snakes in Africa. For the first time, toxin from Eastern green mamba snake, *Dendroaspis angusticeps*, was reported to potentiate the effects of acetylcholine [16]. This work was supported by the findings of Barret and Harvey [17] who showed that green mamba venom increases the twitch height of isolated nerve-muscle preparations as a consequence of a prejunctional action that facilitates the release of acetylcholine. Finally, Harvey and Karlsson [18] demonstrated that the facilitatory effect for acetylcholine release is induced by a small protein that they isolated from the venom and called dendrotoxin.

Dendrotoxin binding sites in brain

Radioligand binding studies have shown high densities of dendrotoxin acceptor sites in synaptic regions and also along fiber tracts. In this context, ^{125}I-labelled dendrotoxins have been used to study the dendrotoxin binding sites distribution in the brain [19-21]. Binding sites for ^{125}I-α-dendrotoxin have been successfully reported in monkey and human brain sections. Loss of dendrotoxin and toxin K binding sites was observed in hippocampus samples of patients who had died from Alzheimer's disease. Significant binding of dendrotoxin was reported in demyelinated plaques from postmortem samples of patients with multiple sclerosis (Figure 2). A similar study demonstrated changes in various regions of the rat brain with ageing. Dense labeling has been observed in synapses rich areas and seizure prone regions such as the neocortex and hippocampal formation [22].

Figure 2: *Neurological outreach of Dendrotoxin according to reports on its binding in brain.*

Neuropharmacology of Dendrotoxin

In laboratory conditions, dendrotoxin induces epileptiform activity, clonic seizures and death when administered intracerebroventricularly (i.c.v.) in rats and mice [22,23]. Significant neuronal loss along with seizures in the CA1, CA3, CA4 and DG regions were reported after dendrotoxin injection in the brain [24] which was not blocked by glutamate receptor antagonists [25]. However, the induced epileptiform activity in CA1 and CA3 regions of the hippocampus was found to be decreased by non-NMDA receptor antagonists [26]. The convulsant activity of the peptide is believed to be due to blockade of voltage-dependent K+ channels with a consequent facilitation of synaptic neurotransmitter release which is one of the first identified effects of dendrotoxin [27]. Dendrotoxin has been previously reported to block potassium current in cell bodies of rat sympathetic ganglia which is responsible for enhancing neuronal excitability and facilitation of transmitter release [28]. Though dendrotoxins have been found to be homologous to Kunitz serine protease inhibitors such as aprotinin or bovine pancreatic trypsin inhibitor but they are very weak inhibitors of trypsin [29,30]. Similar to the synaptic actions of 4-aminopyridine (4-AP), which is also a selective blocker of K^+ channels, K^+ channel blockade induced by dendrotoxin presumably facilitates both inhibitory and excitatory neurotransmission, and in the same context, it has been reported to enhance the release of GABA and perhaps also glutamate from cortical synaptosomes [31-33].

Dendrotoxin consists of several components, with different targets (Figure 3):

- Dendrotoxin 1, which inhibits the pre and post-synaptic K^+ channels in the intestinal smooth muscle. It also inhibits Ca^{2+}-sensitive K^+ channels from rat skeletal muscle, incorporated into planar bilayers [34].
- Dendrotoxin 3, which inhibits acetylcholine M4 receptors.
- Dendrotoxin 7, commonly referred to as muscarinic toxin 7 (MT7) inhibits acetylcholine M1 receptors
- Dendrotoxin K, structurally homologous to Kunitz-type proteinase inhibitors [35] acts as a selective blocker of voltage-gated potassium channels [36].

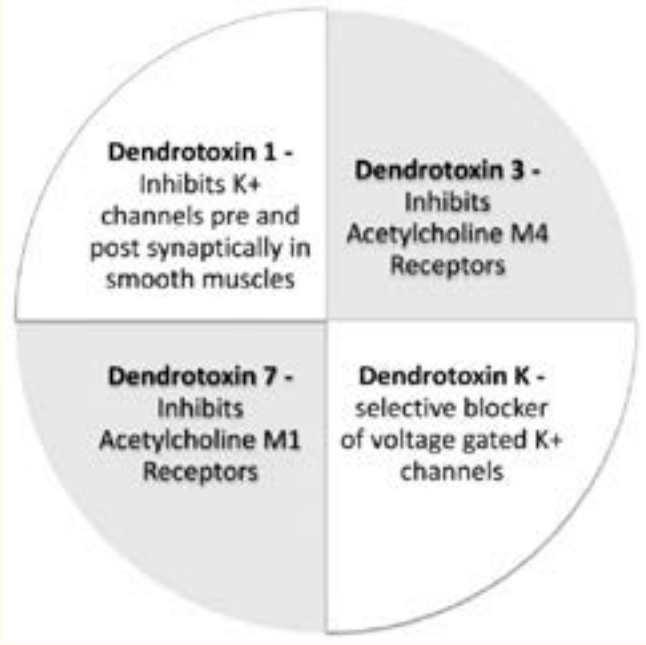

Figure 3: *Major Components of Dendrotoxin with their targets.*

Summarily, dendrotoxins have been shown to block voltage-gated potassium channel subtypes (Kv1.1 and Kv1.2) in the neuronal tissue [36]. Voltage-gated K^+ channels control the excitability of nerves and muscles by controlling the resting membrane potential and by repolarizing the membrane during action potentials. Dendrotoxin binds to the nodes of Ranvier of motor neurons thereby blocking the activity of potassium channels [37]. In this way, dendrotoxin prolongs the duration of action potentials leading to increased acetylcholine release at the neuromuscular junction thereby causing hyper-excitability and convulsive symptoms. Progressive hyperexcitability further induces tetanic contraction of respiratory muscles leading to respiratory paralysis and death.

Current Treatments

- Many antivenoms have been developed to cope with the clinical emergency due to snake bites. Equine immunoglobulin F(ab')2 anti-venoms are currently used as an antivenom therapy against dendrotoxin.
- Anticonvulsants namely phenytoin, valproate, phencyclidine and felbamate have been reported to be effective in reversing the convulsive symptoms induced by dendrotoxin whereas diazepam, tiagabine, MK801, ethosuximide, nimodipine and 7-dichlorokynurenic acid have been found ineffective against such convulsions [6,22].
- Also, the convulsant actions of dendrotoxin-I have been found to be partially blocked by riluzole [38], but not by the K^+ channel opener lemakalim [39].
- Different plant species (ethanolic/methanolic/aqueous extracts of plant parts) belonging to diverse plant resources have been found to neutralize snake venoms of various venomous snakes.

Shortcomings of current anti-venom therapy:

Although anti-venom therapy is available against dendrotoxin but anti-venom therapies have been generally related with many adverse effects such as anaphylactic reaction (discomfort in breathing and swallowing; hives; itching of the limbs; reddening of skin, especially around ears; swelling of eyes, face, or nasal mucosa), serum sickness (enlargement of the lymph glands; fever; inflammation of joints) and pyrogen reaction [40].

Conclusion

Numerous Mamba snakebites are estimated to occur every year, of which many people who survive bites suffer from permanent tissue damage caused by venom, leading to disability. The mechanisms of Dendrotoxin induced neurotoxicity appear to primarily revolve around potassium channel blocking activity, however there are various other pathways involved. Also, the combined toxicity induced at various organ levels by dendrotoxin leading to all-round damage is a concern towards high mortality in Mamba victims. The presence of anti-venom therapy has been useful in the recovery of patients who receive immediate attention. But most of the victims receive late medical attention due to geographical inaccessibility. This is a major reason for high mortality rate of mamba victims. Therefore, research is needed to be envisaged on pharmacological agents that can find their applicability in providing a therapeutic assistance in the late stages also.

Conflict of Interest

The authors report no conflicts of interest.

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