

MULTIPLE SCLEROSIS & IT'S TREATMENT WITH ALPHA-COBRATOXIN: A REVIEW

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ABSTRACT: Venom is a protein secreted by various reptiles, spiders, & other nocturnal animal which can cause damage to a living tissue. Snake venom is a complex mixture of numerous bioreactive proteins, such as toxins, enzymes, hormones and growth factors. Snake venom derived proteins have importance in medicine, pharmacology and in various diagnostic procedures. The derived proteins may helpful for treating a pathogenic state, so venom can be a possible tool for therapy of various diseases and disorders in present & in future. Cobra venom contains numerous proteins & novel toxins, which are used in the treatment of conditions like multiple sclerosis, infections caused by herpes viruses, retroviruses and many other uses are still studied on. The Russell's viper (*Daboia russelli*) venom in particular gaining early use in the treatment of hemophilia due to presence of potent haemostatic agents. The alpha-neurotoxin from *Naja naja* and/or *Naja kaouthia* is administered for the treatment of chronic pains associated with advanced cancer. The present review reports the isolation, characterization and pharmacological importance of the proteins such cobratoxins from the venom of few varieties of snakes.

KEYWORDS: Multiple Sclerosis, Alpha Cobratoxin, *Naja naja siamensis*.

INTRODUCTION

ONSET OF SNAKE VENOM THERAPY:¹

In the early part of the last century, cobra venoms were established products mainly for the treatment of severe pain, but also for rheumatism, trigeminal neuralgia, asthma, ocular therapy, and neuroses. Cobra venom has been widely used for decades in China for the treatment of rheumatoid arthritis and cancer. Prior to use, it is partially denatured by heating.² This process will inactivate many of the venom enzymes, although the neurotoxins can retain their toxicity. Such modified venoms proved to be 80% effective in the clinic for the treatment of headache and arthritis pain. One feature of this venom product is its slow onset of analgesic activity. In the United States, two cobra venom products, Cobroxin (Hynson, Westcott and Dunning) and Nyloxin (Lederle), were marketed up to 1972 for the Treatment of pain and arthritis.³ With the new FDA drug registration requirements in that year, these products were abandoned over production issues and in favor of newer drugs.

The demise of the accepted therapeutic use of diluted solutions of native cobra venoms apparently commenced when it was applied to the treatment of Multiple sclerosis (MS). In the mid 1970s, a Miami

physician, Ben Sheppard (1903-1980), was suffering with rheumatoid arthritis (RA). Dr. Sheppard, a pediatrician and a Dade County medical examiner, tried Venagen/PROveo- a raw cobra, krait and viper venom mixture, a version of Cobratoxin produced by the Miami Serpentarium. Being impressed with his responses to the venom product, he founded the Ben Sheppard Foundation through which he attempted to develop treatments for multiple sclerosis and arthritis using snake venom. He established clinics and administered Venagen to an estimated 7000 people. Many with MS, RA, and shingles. Interviews with physicians and subjects that used Venagen indicated that a positive effect was observed in pain associated with shingles, consistent with its historical use, and some benefits were reported by subjects with MS such as increased endurance, strength, and improved continence. The study program was initiated without FDA approval and had no clinical controls. With concomitant manufacturing quality failings for the product (specifically characterization and sterility), and following the death of Dr. Sheppard, the FDA closed down the clinics. It ruled that the drug had not been properly tested or licensed for human use.

COBRA-VENOM

Naja naja siamensis: INDOCHINESE SPITTING COBRA⁴⁻⁵

Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata
Class: Reptilia
Order: Squamata
Suborder: Serpentes
Family: Elapidae
Subfamily: Elapinae
Genus: Naja

Figure 1: Cobra-Naja naja siamensis

HABITAT:

It lives in dense highland forests. The snake has a preference for living in areas dotted with lakes and streams. Cobra populations have dropped in some areas of its range due to the destruction of forests, but despite this the snake is not listed by the World Conservation Union (IUCN) as in danger of becoming extinct. It is, however, listed as an Appendix II Animals within Convention on International Trade in Endangered Species (CITES)

VENOM:

Cobra's venom, which is composed mostly of proteins and polypeptides, is produced in specialized salivary glands (as is the case with all venomous reptiles) just behind the animal's eyes. When biting its prey, venom is forced through the snake's half-inch (1.25 cm) fangs and into the wound. Although its venom is not the most toxic one, a Cobra's size enables it to inject larger quantities of venom than most other species. On a single bite, it injects as much as 6 to 7 ml of venom. The large amount of venom in a single bite allows the Cobra to kill faster.

The Cobra's venom is primarily neurotoxic and thus attacks the victim's central nervous system and quickly induces severe pain, blurred vision, vertigo, drowsiness, and paralysis. In one to two minutes, cardiovascular collapse occurs, and the victim falls into a coma. Death soon follows due to respiratory failure. A protein component of the venom Ohanin causes hypo locomotion and hyperalgesia in mammals other components have cardiotoxic, cytotoxic and neurotoxic effects. There are two types of antivenin viz. Snake Antivenin I.P. & Monovalent Cobra Antivenom Serum⁶ made specifically to treat

Cobra poisoning. The Red Cross in Thailand manufactures one, and the Central Research Institute in India manufactures the other, however both are made in small quantities, and are not widely available.

 α -COBRATOXIN:⁷**STRUCTURE:**

The crystal structure of the "long" α -neurotoxin α -cobratoxin was refined with a Resolution factor of 19.5% to 2.4-Å⁰ resolution. The polypeptide chain forms three loops, I, II, III, knotted together by four disulfide bridges, with the most prominent, loop II, containing another disulfide close to its lower tip. **Loop I** is stabilized internally by three hydrogen bonds. There are two runs of a short β -pleated sheet, between Cys and Lys, and type II β -turn t_1 at its tip, formed by residues Ile-Thre-Pro-Asp. **Loop II** consists of a narrow hairpin with a bulgy tip that is stabilized in its structure by disulfide Cys-Cys. The peptide forming the tip, segment 27-35, is folded into two distorted, right-handed helical turns stabilized by four main chain hydrogen bonds. **Loop III** is stabilized at both "ends" by disulfide Cys-Cys and, at its lower tip, by type II β -turn t_6 (Lys-Tyr-Gly-Val). One of its legs is involved in β -sheet formation with loop II, and the other leg is bonded to it by hydrophobic interactions between the side chains of Val, Val, and Ile. Loop II and one strand of loop III form an antiparallel triple-pleated β -sheet, and tight anchoring of the Asparagine (Asn) side chain fixes the tail segment.

In the crystal lattice, the α -cobratoxin molecules dimerize by β sheet formation between strands 53 and 57 of symmetry-related molecules.

Because such interactions are found also in a cardiotoxin and α -bungarotoxin, this could be of importance for interaction with acetylcholine receptor. The three-dimensional structure of α -cobratoxin consists of three hairpin type loops, two minor ones with sequences 1-17 (loop I) and 43-67 (loop III) and a major one with sequence 18-42 (loop II), and a tail with sequence 5-71.

ISOLATION:

MATERIALS & METHODS:

α -Cobratoxin was isolated and purified from *N. naja siamensis* venom and crystallized, by microdialysis of a 1.5%w/w aqueous solution of protein against 75% 2-methyl-2,4-pentanedial and 25% buffer containing 0.05 M glycine Hydrochloride, pH 2 (giving a final pH of 2.83). Crystals grow as hexagonal needles in space group with the unit cell dimensions. The crystallographic data is shown in **Table 1**

PHARMACOLOGICAL APPROACH TOWARD

A-COBRATOXIN:¹

The active antiviral agents in the venom to be associated with the neurotoxic fractions were discovered. The major component of interest was identified as cobratoxin, having a molecular weight averaging 8000 and being highly basic. Cobratoxin (CTX) targets the nicotinic acetylcholine receptor (NAChR) in nerve and muscle tissue where it functions by preventing depolarization of postsynaptic membranes through the regulation of sodium channels. α -bungarotoxin (having an identical pharmacological activity to its homologue, CTX) has found great utility as a molecular probe in the study of neuromuscular transmission and ion channel function. So far, nine different types of NAChRs α - subunits have been identified with variable pharmacology profiles. BTX (α -bungarotoxin isolated from Indian krait of the genus *Bungarus*) has the highest affinity for NAChRs containing α - 1, 7, 8, and 9 subunits. Also, it has been found that some NAChRs can conduct calcium ions, which has direct effects on neurotransmission and secretion. It is also notable that neuronal excitability can induce an increase in the number of BTX sites after exposure of the cultures to nicotinic antagonists. In the CNS, the best functional evidence points to a role for these BTX-sensitive receptors in the release of other transmitters such as Dopamine, Gamma Amino Butyric Acid (GABA), and Noradrenaline. Based on the above, it is clear that the NAChRs have different effects depending upon the cells on which they are expressed and the functions of those cells. The identification of acetylcholine like receptors, including those identified by the binding of BTX a wide variety of cell types (including non neuronal, non muscular), has caused some intrigue as to the role of these receptors in such a diverse array of cell types. NAChRs

and BTX binding has been measured in lymphocytes, thymus, and even sperm cells. Recently, BTX has also been found to be a potent inhibitor of ATP-gated calcium channels, P2X, in neuronal cells. Therefore, it seems that receptors for α -neurotoxins may be widespread, may not necessarily be NAChRs, and may provide a communication link between the CNS and the immune and endocrine systems.

The toxicity of neurotoxins is based on their relative affinity for the NAChR receptor, which far exceeds that of acetylcholine. Many studies have demonstrated various methods for the chemical modification of CTX by oxidation with substances such as hydrogen peroxide, formate, and ozone, which alters its affinity for the NAChR with an associated reduction in or loss of toxicity. For the majority of the studies described below, the method of chemical detoxification employed hydrogen peroxide as the reactive species. The safety of new drugs is always a great concern and now even more so following the difficulties experienced with the use of Natalizumab (Tysabri) and the induction of progressive multifocal leukoencephalopathy (PML), a rare but deadly viral infection of the central nervous system (CNS) associated with immunosuppression. Modified Neurotoxin (MN) has an excellent safety record over years of use in human studies and modified cobratoxin (MCTX) has not revealed any measurable toxicity. MCTX injections of 5 g/Kg into mice (650,000 times a human dose) reveal no adverse effects and in numerous preclinical studies no animals have ever died from MCTX administration even when given intracerebrally. Preliminary human clinical investigations with MCTX revealed no significant concerns.

MULTIPLE SCLEROSIS & ITS TREATMENT WITH A-COBRATOXIN:⁹⁻¹⁰

MULTIPLE SCLEROSIS

Multiple Sclerosis (abbreviated MS, also known as *disseminated sclerosis* or *encephalomyelitis disseminata*) is an autoimmune condition in which the immune system attacks the central nervous system, leading to demyelination. Disease onset usually occurs in young adults, and it is more common in women. It has a prevalence that ranges between 2 and 150 per 100,000. MS was first described in 1868 by Jean-Martin Charcot.

MS affects the areas of the brain and spinal cord known as the white matter, destroying a fatty layer called the myelin sheath, which wraps around nerve fibers and electrically insulates them. When myelin is lost, the axons of neurons can no longer effectively conduct action potentials. The name *multiple sclerosis* refers to the scars (scleroses – better known as plaques or lesions) in the white matter. Although much is known about the mechanisms involved in the disease process, the cause remains

unknown. Theories include genetics or infections. Different environmental risk factors have also been found. Almost any neurological symptom can appear with the disease, and often progresses to physical and cognitive disability. MS takes several forms, with new symptoms occurring either in discrete attacks (relapsing forms) or slowly accumulating over time (progressive forms). Between attacks, symptoms may go away completely, but a permanent neurological problem often occurs, especially as the disease advances.

The prognosis is difficult to predict, it depends on the subtype of the disease, the individual patient's disease characteristics, the initial symptoms and the degree of disability the person experiences as time advances. Life expectancy of patients is nearly the same as that of the unaffected population

PATHOPHYSIOLOGY:

MS AS AN AUTOIMMUNOLOGICAL DISEASE:¹²



Figure 1: MRI FLAIR sequence showing bright spots (plaques) where multiple sclerosis has damaged myelin in the brain¹¹.

Damage is believed to be caused by the patient's own immune system. The immune system attacks the nervous system, possibly as a result of exposure to a molecule with a similar structure to one of its own. The cause of MS is unknown, but genetics, an infectious agent, a faulty immune system, or a combination of these factors appears to play a role in why a person contracts the disease. While there is no evidence that MS is directly inherited, relatives of those with MS have a slightly increased risk of developing the disease. Researchers have identified two genes [IL2RA & IL7RA] that appear to be associated with multiple sclerosis. Both genes contain the instructions for producing interleukin receptor proteins on the surface of immune-system cells.

Epidemiological studies have shown that MS is more prevalent in people who spend their first 15

years of life in a temperate climate than in those who live their first 15 years in a tropical climate. Many believe that the second factor is an infectious agent, such as a virus or bacterium. Some experts propose that MS is not triggered by a single infectious culprit, but instead by the way a person's immune system reacts to an infection.

Studies suggest that MS is an autoimmune disease, in which the immune system attacks the body's own tissue. Certain bacteria or viruses have been found to contain proteins that make them resemble the cells of body tissues, including those of the nervous system. In MS, the immune system may be activated to attack both the invaders and the tissues they resemble

LESIONS:

The name *multiple sclerosis* refers to the scars (scleroses – better known as plaques or lesions) that form in the nervous system. MS lesions most commonly involve white matter areas close to the ventricles of the cerebellum, brain stem, basal ganglia and spinal cord; and the optic nerve. The function of white matter cells is to carry signals between grey matter areas, where the processing is done, and the rest of the body. The peripheral nervous system is rarely involved.¹³

More specifically, MS destroys oligodendrocytes, the cells responsible for creating and maintaining a fatty layer—known as the myelin sheath—which helps the neurons carry electrical signals. MS results in a thinning or complete loss of myelin and, as the disease advances, the cutting (transection) of the neuron's extensions or axons. When the myelin is lost, a neuron can no longer effectively conduct electrical signals. A repair process, called remyelination, takes place in early phases of the disease, but the oligodendrocytes cannot completely rebuild the cell's myelin sheath. Repeated attacks lead to successively fewer effective remyelinations, until a scar-like plaque is built up around the damaged axons.¹⁴

CAUSES:

MS likely occurs as a result of combination of both environmental and genetic factors.

INFECTIOUS CAUSES:

Genetic susceptibility can explain some of the geographic and epidemiological variations in MS incidence, like the high appearance of the disease among some families or the risk decline with genetic distance, but does not account for other phenomena, such as the changes in risk that occur with migration at an early age.¹⁵

An explanation for this epidemiology finding could be that some kind of infection, produced by a widespread microbe rather than a rare pathogen, is the

origin of the disease.¹⁵ Different hypotheses have elaborated on the mechanism by which this may occur. The *hygiene hypothesis*-the science of health & the study of ways of preserving it, particularly by promoting cleanliness- proposes that exposure to several infectious agents early in life is protective against MS. MS would be an autoimmune reaction triggered in susceptible individuals by multiple infective microorganisms, with risk increasing with age at infection.^{15,16,17}

Evidence for viruses as a cause includes the presence of oligoclonal bands in the brain and cerebrospinal fluid of most patients, the association of several viruses with human demyelinating encephalomyelitis, and induction of demyelination in animals through viral infection. Human herpesviruses are a candidate group of viruses linked to MS; *Varicella zoster* virus has been found at high levels in the cerebrospinal fluid of MS patients. Other agents that have also been related with MS are human endogenous retroviruses and *chlamydia pneumoniae*.¹⁸

NON-INFECTIOUS ENVIRONMENTAL RISK FACTORS:¹⁹

MS is more common in people who live farther from the equator. Decreased sunlight exposure has been linked with a higher risk of MS. Decreased vitamin D production and intake has been the main biological mechanism used to explain the higher risk among those less exposed to sun.

Severe stress may also be a risk factor although evidence is weak; parents who lost a child unexpectedly were more likely to develop MS than parents who had not. Smoking has also been shown to be an independent risk factor for developing MS. Association with occupational exposures and toxins-mainly solvents-has been evaluated, but no clear conclusions have been reached. Vaccinations were also considered as causal factors for the disease; however, most studies show no association between MS and vaccines.

Gout occurs less than would statistically be expected in people with MS, and low levels of uric acid have been found in MS patients as compared to normal individuals. This led to the theory that uric acid, which can protect against oxidative stress from substances such as peroxynitrite, protects against MS. Although some of these risk factors, including infection, are partly modifiable, only further research—especially clinical trials—will reveal whether their elimination can help prevent MS

TREATMENT:

Although there is no known cure for multiple sclerosis, several therapies have proven helpful. The primary aims of therapy are returning function after an attack, preventing new attacks, and preventing disability. As with any medical treatment, medications

used in the management of MS have several adverse effects. Alternative treatments are pursued by some patients, despite the paucity of supporting, comparable, replicated scientific study.²⁰

MANAGEMENT OF ACUTE ATTACKS²¹⁻²²

During symptomatic attacks, administration of high doses of intravenous corticosteroids, such as methylprednisolone, is the routine therapy for acute relapses. The aim of this kind of treatment is to end the attack sooner and leave fewer lasting deficits in the patient. Although generally effective in the short term for relieving symptoms, corticosteroid treatments do not appear to have a significant impact on long-term recovery. Potential side effects include osteoporosis and impaired memory, the latter being reversible.

DISEASE-MODIFYING TREATMENTS

The earliest clinical presentation of relapsing-remitting MS (RRMS) is the clinically isolated syndrome (CIS). Several studies have shown that treatment with interferons during an initial attack can decrease the chance that a patient will develop clinical MS.

The six disease-modifying treatments have been approved by regulatory agencies of different countries for RRMS. Three are interferons: two formulations of interferon β -1a (*Avonex* and *Rebif*) and one of interferon β -1b [*Betaseron* (U.S.), *Betaferon* (Europe & Japan)]. A fourth medication is Glatiramer acetate (*Copaxone*). The fifth medication, Mitoxantrone, is an immunosuppressant also used in cancer chemotherapy, approved only in the USA and largely for secondary progressive MS. The sixth is Natalizumab (*Tysabri*). All six medications are modestly effective at decreasing the number of attacks and slowing progression to disability, although their efficacy rates differ, and studies of their long-term effects are still lacking. Comparisons between immunomodulators (all but mitoxantrone) show that the most effective is Natalizumab, both in terms of relapse rate reduction and halting disability progression; it has also been shown to reduce the severity of MS. Mitoxantrone is generally not considered as a long-term therapy, as its use is limited by severe cardiotoxicity. The interferons and Glatiramer acetate are delivered by frequent injections, varying from once-per-day for glatiramer acetate to once-per-week (but intra-muscular) for *Avonex*. Natalizumab and mitoxantrone are given by IV infusion at monthly intervals.

Treatment of progressive MS is more difficult than relapsing-remitting MS. Mitoxantrone has shown positive effects in patients with secondary progressive and progressive relapsing courses. It is moderately effective in reducing the progression of the disease and the frequency of relapses in patients in short-term

follow-up. No treatment has been proven to modify the course of primary progressive MS.

ADVERSE EFFECT OF DISEASE MODIFYING AGENTS:

As with any medical treatment, these treatments have several adverse effects. One of the most common is irritation at the injection site for Glatiramer acetate and the interferon treatments. Over time, a visible dent at the injection site, due to the local destruction of fat tissue, known as lipoatrophy, may develop. Interferons produce symptoms similar to influenza; some patients taking Glatiramer experience a post-injection reaction manifested by flushing, chest tightness, heart palpitations, breathlessness, and anxiety, which usually lasts less than thirty minutes. More dangerous are liver damage from interferons and mitoxantrone, the immunosuppressive effects and cardiac toxicity of the latter; and the putative link between Natalizumab and some cases of progressive multifocal leukoencephalopathy.

MANAGEMENT OF THE EFFECTS OF MS

Disease-modifying treatments reduce the progression rate of the disease, but do not stop it. As multiple sclerosis progresses, the symptomatology tends to increase. The disease is associated with a variety of symptoms and functional deficits that result in a range of progressive impairments and disability. Management of these deficits is therefore very important. Both drug therapy and neurorehabilitation have shown to ease the burden of some symptoms, though neither influences disease progression. As for any patient with neurologic deficits, a multidisciplinary approach is key to limiting and overcoming disability; however, there are particular difficulties in specifying a 'core team' because people with MS may need help from almost any health profession or service at some point. Similarly, for each symptom there are different treatment options. Treatments should therefore be individualized depending both on the patient and the physician.

IMMUNOMODULATORY ACTIVITY OF ALPHA COBRATOXIN:²³

Hudson et al first demonstrated that an iodoacetamide derivative of CTX could protect guinea pigs from the induction of experimental acute encephalomyelitis (EAE) with myelin basic protein, without any reports of toxicity, and that the majority of the immune suppressive activity is associated with the N-terminal section of the protein. Both hydrogen peroxide and carboxy-methylated CTX have demonstrated antiviral activity. A second study is undertaken to confirm Hudson's observations and verify that the method of detoxification by hydrogen peroxide did not adversely impact the immune modulating activity of the modified protein in the rat

model of MS. MCTX at the same dose (0.2mg) is compared for efficacy to MCTX and it was found that MCTX is significantly more effective in both acute and chronic relapsing studies. These studies served to verify that MCTX is the primary immunosuppressive component in the modified venom. This result contrasted with antiviral experiments where MN was an equivalent or more potent inhibitor. In animals without functional deficit, histology revealed that no lymphocytic invasion was apparent around the brain vasculature in contrast to animals that displayed symptoms of the disease. This Immunomodulatory activity would support the prior use of MN in rheumatoid arthritis studies. The conclusions drawn suggested that MCTX may be at least as therapeutically effective as the α -interferons. Nicotinic receptors have been detected on T lymphocytes, and, when blocked by BTX, prolonged carbamoylcholine-induced proliferation ensued. This result would seem counterproductive in EAE or may reflect the activity of alpha-neurotoxins on particular lymphocyte subsets. It is interesting to note that Yourist et al. reported that the treatment of mice with cutaneous herpes lesions with MCTX resulted in an 80% reduction in scarring over controls, an activity of significance in a sclerosing condition like MS, and suggestive that MCTX influences the wound-healing process. Immune modulating activity is central to the current approaches to the treatment of MS, an activity clearly demonstrated by MCTX.

NEUROPROTECTIVE EFFECTS OF A-COBRATOXIN:^{24,25}

The loss of neurons through apoptotic processes underscores the increasing disability experienced by subjects with MS. A novel neurotoxic mechanism in addition to the well-established excitatory amino acid receptor pathway revolves around the prolonged opening of Na^+ channels inducing the neuronal death of cerebellar cells. It has also been reported that the blockade of α -7 NACHR containing receptors inhibits the release of glutamate, a known trigger of cell apoptosis. Several studies have reported that people with Amyotrophic Lateral Sclerosis (ALS) have a high level of glutamate circulating in the CNS. In stroke victims, the hypoxic state triggers a large outpouring of glutamate killing the postsynaptic neuron. Excitotoxic neuronal death mediated by N-methyl-D-aspartate (NMDA) glutamate receptors can contribute to the extended brain damage that often accompanies trauma or disease. Nicotine protection to NMDA intoxication was mediated through a BTX-sensitive receptor. When co-applied, neuroprotection to NMDA by nicotine was abolished but could be recovered with BTX. The study suggested that BTX-sensitive nicotinic neurotransmitter receptors, presumably α -7 NACHRs, confer neuroprotection through potentially antagonistic pathways. Numerous studies with α -

neurotoxins in the CNS of developing chick embryos have demonstrated that BTX can provide beneficial effects when applied directly to the CNS or administered to embryos. It was interesting to note that α -1-type NACHRs binding activity was required for neuronal protection. Therefore, it seems that nicotinic agonists and antagonists can have conflicting effects on certain nerve cells while also displaying similar pharmacological effects in other aspects such as analgesic effects. Regardless, it is the mainly postsynaptic α -1 and presynaptic α -7 receptors that have been identified in these protective pathway receptors, which are particularly sensitive to a-neurotoxins, and the majority of studies suggest that inactivation or blockade of the receptor is the primary protective mechanism.

NEUROMODULATORY & ANALGESIC EFFECT OF A- COBRATOXIN:¹

The neuroactive properties of MCTX were demonstrated through competitive binding assays conducted with purified α -1 NACHR from electroplax tissue. MCTX-NACHR binding in vitro is determined by a method derived from an enzyme immunoassay (EIA) developed by Stiles for the detection of postsynaptic neurotoxins. Cobratoxin binds strongly and specifically to the NACHR. Recently, CTX and MCTX have been demonstrated to have analgesic activity in three animal models of pain, namely, hot-plate, tail-flick, and writhing tests, although native CTX was more potent, due most likely to its higher affinity for the NACHR. MCTX was administered intraperitoneally and intracerebroventricularly. The involvement of muscarinic and the opioid peptidergic systems in MCTX induced analgesia were examined by pretreatment of animals with atropine or naloxone, and they were found not to be involved. The effect of MCTX on motor activity was also tested using the Animex test in mice to ensure that neuromuscular inhibition did not impact the responses observed in the test animals. The effects of the drug were seen 2-3 hours after its administration.

In the rat tail-flick assay, the intracerebroventricular administration of MCTX was not toxic and it produced marked analgesic effects with 1/160th dose as that of the systemic dose (10 mg/kg). This antinociceptive activity is suspected to have been mediated through the Peripheral Nervous System (PNS) and may explain the lag period to the onset of analgesia, although the CNS may also

contribute to MCTX's analgesic effects because i.e. administration was effective. This presumption is based on the pharmacokinetics of such neurotoxins, which do not appear to penetrate the CNS, although the blood-brain barrier may be compromised in an inflammatory disease such as MS. Pain is a common symptom in MS, although not always reported by the patient, and antinociceptive activity would be a useful activity for any drug used to treat this disease. Its peripheral route of administration certainly gives it advantages over other toxin-based drugs such as the conotoxin SNX111 (Prialt), which is given intravenously. Cobratoxin, which exhibits similar analgesic activity & is used clinically in China both parenterally and orally. Recent studies reveal it to have potent and long-lasting analgesic effects in postoperative and cancer patients. This data supports the historical use and identifies the active components in cobra venom used early in the last century for the treatment of severe pain.

CURRENT MS TREATMENT:¹

The primary drug for the treatment of MS is β -interferon (Avonex, Rebif, Betaseron), followed by Glatiramir acetate (Copaxone), Natalizumab (Tysabri), and Mitoxantrone (Novantrone), all functioning primarily as immune suppressants. Both Mitoxantrone and the interferons have antitumor activity, while the interferons also have antiviral activity. Frampridine (4-aminopyridine, 4-AP) is a drug that blocks the voltage-gated potassium channels in neurons. This effectively improves the transmission of nerve impulses down damaged axons. It does not replace damaged myelin, but users of 4 amino pyridine report dramatic improvement in a number of symptoms, especially paresthesia. It also alters the course of Experimental Acute Encephalomyelitis (EAE) in rats. **Table 2** compares the pharmacological effects of current MS drugs to that of MCTX. Other potassium channel neurotoxins have been proven to prevent the onset of EAE by their action on T-cell proliferation. A pentapeptide from Thymopoietin (Thymopentin), in spite of the apparent differences in composition, has been reported to have structural homology to long-chain neurotoxins and similar clinical activity to MCTX, in addition to having a good safety profile. Despite positive clinical reports, the development of Thymopentin, an immunomodulators with suspected NACHR affinity, appears to have lost momentum in the early 1990s.

Table 1: Crystallographic data for α -Cobratoxin⁸

Parameters	
Space group	P 6 ₅ 22
Cell constants	$a = b = 76.61(2) \text{ \AA}$, $c = 42.76(2) \text{ \AA}$
X-ray source	Elliott, GX20, rotating anode generator operated at 40 kV, 70 mA (2.8 kilowatts); focal size, 0.2 X 2.0 mm
Wavelength used	1.54182 \AA , CuK α /graphite monochromator
Detector	films, Ceaverkean, Cea-Reflex 25
Crystal to film distance	55.5 mm
Maximum resolution	2.3 \AA
Total rotation for a data set	32° around the c axis
Rotation for each exposure	2.0°
Time for each exposure	4000 s/degree, 2.2 h, 10 oscillations
Total number of reflections	12,612
Number of unique reflections in the range of 2.4-10.0 \AA used in structure calculations	3,271
Overall merging R(I) factor	7.4%

Table-2: Comparison of the Activities of MS Drugs to MCTX

Activity	Interferon	Copaxone	Tysabri	Novantrone	Frampridine	MCTX
Immunomodulation	+	+	+	+	+	+
Antiviral Activity	+	-	-	-	-	+
Neuromodulation	-	-	-	-	+	+
Analgesic	-	-	-	-	-	+
Side Effects	-	+	-	-	-	+

+: Activity, -: No Activity

Table 3: Animal Toxins under Therapeutic Development or Use

Company	Product	Source	Application
Abbots Labs	Ancord	Malayan pit viper	Stroke
Abbots Labs	Epibatitide	Poisonous frog	Pain
Amylin Pharm.	Extendin 4	Gila monster	Diabetes
Astra Zeneca	Exanta	Cobra	Atrial fibrillation
Bristol Myers Squibb	Captopril	Bothrops	Antihypertensive
Celtic Biotech	Crotoxin Cardiotoxin	Rattlesnake Cobra	Cancer Cancer
Cognetix	Conotoxin M1	Conus snail	Pain/Stroke
COR/Schlering	Intergilin	Pygmy rattlesnake	Cancer
Elan Pharmaceuticals	Ziconitide	Conus snail	Pain

Kunming Institute	Cobrotoxin	Cobra	Drug addiction and pain
Merck	Aggrastat	Saw-scaled viper	Coronary application
PentaPharm	Defibrase Haemocoagulase	Bothrops Bothrops	Acute cerebral infarction Hemorrhaging
ReceptoPharm	Receptin Pepton Cobratoxin	Cobra Cobra Cobra	MS Antiviral Pain
Transmolecular Vernalis	Chlorotoxin Marimastat	Scorpion Viper	Cancer Cancer
	Disintegrin Escozul	Copperhead Scorpion	Cancer Cancer

CONCLUSION

MS is a painful autoimmune disease, possibly induced or worsened by viral infection, causing reduced nerve conduction through demyelination in addition to neuronal damage and loss. Current therapies seek to prevent the progression of the disease, but the most widely used drugs inflict significant side effects. Tolerance issues are also described for the Neuromodulatory 4-AP. Notwithstanding the anecdote clinical reports with cobra venoms, the preclinical data demonstrating that MCTX has antiviral, immunomodulatory, and neuromodulatory activity, would strongly suggest that

it is a prime candidate for controlled clinical studies in subjects with MS where all these facets contribute to the disease process. Supported by an excellent safety profile in MCTX, the medication has been well tolerated in clinical studies to date, with almost no side effects. So it has been said that the Modified Cobratoxin can a possible therapy of a long-lasting Multiple Sclerosis.

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