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PROTECTIVE EFFECT OF COBRA VENOM ON STRIATAL DOPAMINE DEPLETION IN A MOUSE MODEL OF PARKINSONISM

A. Al. Asmari[1]*, K. Al. Moutaery[2] and M. Abbas[1]

Research Center[1] and Division of Neurosurgery[2], Armed Forces Hospital, Riyadh (Saudi Arabia)

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ABSTRACT

Venoms of cobras (Family Elapidae) are complex mixtures of toxic proteins and enzymes. These complex mixtures were thought to play key roles incuring neurodegenerative diseases like Parkinson's disease and Alzheimer's disease. This study determined the effect of the cobra (*Naja haje arabica)* venom on MPTP-induced Parkinsonism in mice. Adult male mice (C57 BL), weighing 30 ± 2, were treated with MPTP (30 mg/kg, i.p.) daily for 3 days. Cobra venom (100 ml) was injected (i.p.) in doses of 0, 0.025, 0.05, 0.075 mg/kg daily (three days) 30 min before MPTP in four different groups. Two other groups of mice received either vehicle (control) or a high dose of cobra venom (0.075 mg/kg). Two hours after the last injection of MPTP the mice were killed by decapitation and striata were collected for the analysis of dopamine (DA). Administration of MPTP significantly reduced striatal DA, which was significantly and dose-dependently reversed by cobra venom. Further studies will be of interest to explore interactions between cobra toxin and potential neurodegenerative events.

Key words: Parkinsonism, MPTP, *Naja haje arabica*, Dopamine and Neurotoxicity.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by the selective loss of dopamine neurons from the substantia nigra (SNC) and a concomitant appearance of motor disturbances including tremors, rigidity, and a slowness of movement (akinesia). In PD it is now well established that good clinical results can be obtained (Widner, 1998; Fahn et al., 1999), which correlates with fluorodopa uptake on PET scans (Martin and Perlmutter, 1994; Wenning *et al.,* 1997) and dopaminergic cell survival within the graphs in those came to post mortem (Kordower *et al.,* 1998). Peripheral administration of MPTP in C57 black mouse has been widely used as convenient and acceptable model for the induction of experimental Parkinsonism (Arai *et al.,* 1990). Although cobra venom is notorious for its lethality, certain enzymes from cobra venom may hold the keys towards finding cures for PD and Alzheimer's disease (AD) (Ferrer *et al.,* 2001). Cobra venom also contains nerve growth factor (NGF), a protein that is involved in the maintenance and growth of healthy nerve cells which appears to be distinct from the major toxins (John Evans, 2004). NGF-Tf conjugate also reversed the neurodegenerative changes such as karyopyknosis, chromotolysis and intracytoplasmic inclusion in disease neurons (Li, 2000). The present investigation was conducted to study the effect of cobra venom on MPTP-induced neurotoxicity in mice.

Methods

Animals and Treatment

Eight to 10 weeks-old male C57 BL mice were (30±5 g) used in MPTP studies. All efforts were made to minimize the suffering and number of animals used. Mice were placed in a temperature-controlled room with a 12h light/dark cycles. The animals were housed in groups of 5 to 6 per cage and had free access to food and water. The experimental protocol of this study was approved by the Research and Ethical Committee of Armed Forces Hospital, Riyadh. The mice were randomly divided into six groups of five animals each. One group served as control and received

vehicle only, whereas another group treated with high dose (0.075 mg/kg) of cobra venom *Naja haje arabica* and served as venom alone group (without MPTP). The remaining four groups were treated with MPTP (30 mg/kg, i.p.) daily for 3 days; three of these groups also received i.p. injection of cobra venom (100 ml) in the doses of 0.025, 0.05 and 0.075 mg/kg, 30 min before administration of MPTP (RBI, Natrick, MA, USA). The animals were sacrificed 2 h after the last injection of MPTP. The striata were carefully isolated from the cerebrum and immediately frozen in liquid nitrogen and then stored at -80°C until analyzed for dopamine.

Venom

Naja haje arabica venom was obtained from the National Serpentarium, Riyadh, Saudi Arabia. Professional hunters collected the snakes from the wild throughout the kingdom of Saudi Arabia. The animals were kept in a Serpentarium facility. A specialized team of the venomology unit was responsible for scientific classification, milking of specimens, lyophilization and storage of the venom. The venom was dissolved in saline (final concentration 10 mg/ml) and immediately stored at -20°C until used.

Analysis of Dopamine

The analysis of dopamine in striatum was done according to the procedure of Patrick et al. (1991). The striata were weighed and homogenized for 10s in 300 ml (for mice) of 0.1 M Perchloric acid containing 0.05% EDTA, using Teflon homgenizer. The homogenates were immediately centrifuged at 10,000 rpm at 4°C for 10 min. The supernatants were filtered using 0.45 mm pore filters and analyzed by high performance liquid chromatography (HPLC). The HPLC system consisted of electrochemical detector from Waters (Waters 2465), solvent delivery pump (Water 1515 Isocratic pump), manual injector (Waters), integrator (Waters), computer software and C-18 mBondapak (3.9 ´ 150 mm) column (Waters). The mobile phase consisted of a mixture of 0.1 M citric acid monohydrate, 0.1 M sodium acetate, 7% methanol, 100 mM EDTA and 0.01% sodium octane sulfonic acid. The flow rate of mobile phase was maintained at 1 ml/min and the injection volume was 20 ml.

Statistics

The data were analyzed by ANOVA followed by independent samples t-test. A value of $P<0.05$ was considered as statistically significant.

Results

Administration of MPTP (30 mg/kg, i.p. for 3 days) produced significant depletion of striatal dopamine ($P<0.0001$) in mice, whereas the animals treated with cobra venom alone (0.075 mg/kg, i.p.) did not affect striatal dopamine as compared to control group. Co-treatment with cobra venom significantly and dose-dependently attenuated MPTP-induced striatal dopamine depletion in mice (Fig. -1).

Discussion

The results of this study clearly demonstrated the ability of cobra venom to attenuate MPTP induced depletion of striatal dopamine in a dose dependent manner (Fig. -1). Beneficial effect of cobra venom was observed against a variety of neuropathological condition including experimental stroke (Lew *et al.,* 1999) and ischemic neuronal injury (Rahmy and Hassona, 2004). The mechanism of cobra venoms induced protection against MPTP is far from clear. MPTP exerts its neurotoxicity by selectively degenerating dopaminergic neurons (Shughrue, 2004; Quik *et al.,* 2000). In brain, MPTP is converted to its toxic metabolite MPP^{++} in presence of enzyme monoamine oxidase B (MAO-B). MPP^{+} is actively taken up into nigrrostriatal neurons wherein it inhibits mitochondrial oxidative phosphalation leading to neuronal death (Singer, 1987). Inhibition of MAO-B enzyme has been shown to attenuate MPTP neurotoxicity (Hsu, 1993; Kindt, 1986; Da Prada, 1987). However, elevated MAO-B activity in transgenic mice did not enhance their sensitivity to MPTP suggesting that conversion of MPTP to MPP^{+} by MAO-B is not the only rate limiting factor for MPTP neurotoxicity (Anderson *et al.,* 1994).

Recent findings indicate that PLA_2 activation may play an important role in neurodegenerative process (Bazan et al., 1995; Dorandeu, 1998; Farroqui, 1997; Ross et al., 1997; Stephenson et al., 1996). Quinacrine, a PLA_2 inhibitor has been shown to protect mice against MPTP-induced neurotoxicity (Tariq et al 2001). In contrary attenuation of MPTP-induced neurotoxicity by cobra venom which is a source of PLA_2 toxins (Basavarajappa 1992), points towards the role of some PLA_2 independent mechanism accounting MPTP toxicity. Further works on the Naja haje arabica venom enzymatic and biological activity were tested and speculated by Al Asmari (1996). Cobra (elapidae family) venoms were mainly directed to their neurocardiotoxic effects on experimental animals, due to the nature of the major components present in these venoms.

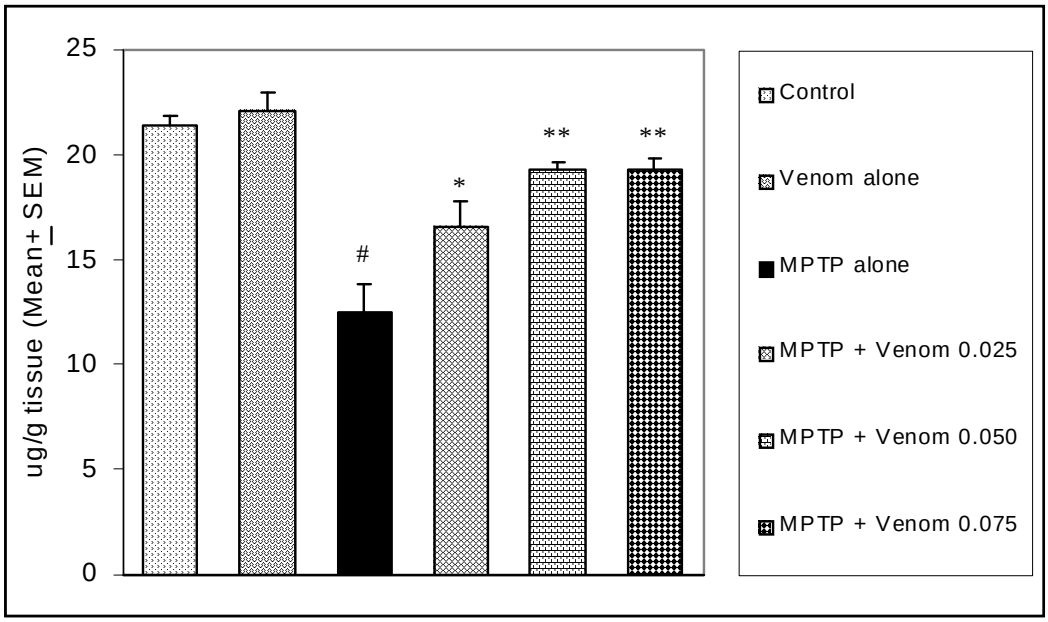

Fig. -1: Effect of *Naja haje arabica* venom on MPTP-induced changes in striatal DA levels in mice. # P < 0.001 vs. control (saline only group); *P <0.01 and ** P< 0.001 vs. MPTP alone treated group.

Different toxic effects produced by cobra venoms were due to their contents of neurotoxin, cardiotoxins, activated complement factors, and enzyme toxins (Tann, 1990).

Despite the wide range of toxicities associated with cobra venom the finding of the present investigation indicates that this heterogenous material might have some useful enzymes that may hold the keys to finding cures for Parkinson's disease and Alzheimer's disease. Further studies are warranted to identify the active component in the cobra venom responsible for the protection of dopaminergic neurons.

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