Venom and Purified Toxins of the Spectacled Cobra (Naja naja) from Pakistan: Insights into Toxicity and Antivenom Neutralization

Kin Ying Wong, Choo Hock Tan,* and Nget Hong Tan*

Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Abstract. Geographical variations of snake venoms can result in suboptimal effectiveness of Indian antivenoms that are currently used in most South Asian countries. This study investigated the toxicity and neutralization profile of the venom and toxins from Pakistani spectacled cobra, Naja naja, using VINS polyvalent antivenin (VPAV, India), Naja kaouthia monovalent antivenom (NKMAV, Thailand), and neuro bivalent antivenom (NBAV, Taiwan). Cation-exchange and reverse-phase high-performance liquid chromatography fractionations followed by toxin identification through liquid chromatography-mass spectrometry (MS) indicated that the venom comprised mainly of postsynaptic neurotoxins (NTXs) (long neurotoxins [LNTXs], 26.3%; short neurotoxins [SNTXs], 8%), cytotoxins (CTXs) (31.2%), and acidic phospholipases A2 (12.3%). NKMAV is the most effective in neutralizing the lethal effect of the venom (potency = 1.1 mg venom/mL) and its LNTX (potency = 0.5 mg toxin/mL), consistent with the high content of LNTX in N. kaouthia venom. VPAV was effective in neutralizing the CTX (potency = 0.4 mg toxin/mL), in agreement with the higher CTX abundance in the Indian cobra venom. All the three antivenins were weak in neutralizing the SNTX (potency = 0.03–0.04 mg toxin/mL), including NBAV that was raised from the SNTX-rich Taiwanese cobra venom. In a challenge-rescue experiment, envenomed mice were prevented from death by a maximal dose of VPAV (intravenous 200 μL) but the recovery from paralysis was slow, indicating the need for higher or repeated doses of VPAV. Our results suggest that optimal neutralization for Pakistani N. naja venom may be achieved by improving the formulation of antivenom production to enhance antivenom immunoreactivity against long and SNTXs.

INTRODUCTION

Bites by venomous snakes can result in envenomation, a life-threatening disease that is prevalent in many tropical and subtropical countries. The exact epidemiology and the global burden measure of snakebite envenomation, however, remains elusive due to the lack of reliable information on incidence, morbidity, and mortality.1,2 Most figures available were obtained from fragmentary surveys conducted in limited regions, representing only a small fraction of the true epidemiology.3 Persistent underreporting of the mortality and morbidity of snakebite envenomation earned it the most neglected status among the World Health Organization listed tropical neglected diseases.4 The problem is particularly aggravated in South Asia, where human populations are heavily engaged in agricultural activities.5 In Pakistan, the annual mortality estimate following envenomation soars as high as 20,000.6 The hidden toll of suffering continues to affect the families of the deceased, and patients who survived with crippling deformity.

With today’s medical advancement, snakebite envenomation is supposed a preventable and treatable condition. Unfortunately over the years, various challenges remain unresolved, hindering the solution for envenomation in many countries.7 One of these challenges is pertaining to the production and distribution of an effective antivenom tailored to the region that requires it. Countries that do not have local antivenom manufacturing plant such as Sri Lanka, and those with limited local antivenom production such as Pakistan, resort to importing antivenom from India.8,9 The Indian antivenoms assume the “Big Four” formulation using venoms of the common cobra (Naja naja), common krait (Bungarus caeruleus), Russell’s viper (Daboia russelii), and saw-scaled viper (Echis carinatus), sourced from a restricted area in southeastern India. These snakes although can be found in Pakistan, their venom profiles can vary geographically within the same species as demonstrated in several other venoms, attributed mainly to ecological factors.2,10,11 By the same token, the antigenicity of toxins can vary substantially too, thus limiting the efficacy of the imported antivenoms against local species.11,12 Here, a very pertinent concern arises when imported antivenoms are not rigorously evaluated against the venoms of local species. This contributes to uncertainty on the indication and dosing of the foreign antivenom, thereby exposing the patients to high doses of antivenom (and the risk of anaphylaxis) in which itself is probably ineffective to begin with.

In Pakistan, the landscapes differ greatly from fertile plains to deserts, forests, mountains, plateaus, and coastal lines. The extremely diverse bioclimatic and topographic profiles create multifarious habitats that cultivate unique fauna and flora with an exotic blend of Palearctic, Indo-Malayan, and Ethiopian forms.13 These include many venomous snakes of great medical importance, some of which are shared with the Indian subcontinent (the Big Four aforementioned), while many more are unique endemic species or those overlap with the middle eastern and Himalayan species.14 As with most developing countries, snakebite envenomation in Pakistan occurs following increased human contact with snakes during agricultural activities.1 This is particularly obvious with anthropometrically adapted snakes such as cobras (Elapidae: Naja).2 At least two cobra species distribute in Pakistan: N. naja, the black spectacled cobra that distributes across the southern and eastern Pakistan including Punjab, Baluchistan, and Sind provinces, and Naja oxiana, the brown ox cobra restricted to northern Pakistan at areas of higher elevations.15 A previously known subspecies in the southern Pakistan, Naja naja karachiensis, has been synonymized with the species N. naja under the current systematics.16 Cobra envenomation is characterized...