Venomics of Naja sputatrix, the Javan spitting cobra: A short neurotoxin-driven venom needing improved antivenom neutralization

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ABSTRACT
The venom proteome of Naja sputatrix (Javan spitting cobra) was elucidated through reverse-phase HPLC, nanoESI-MS/MS, and data mining. A total of 97 distinct protein toxins belonging to 14 families were identified. The most abundant toxins are the three-finger toxins (3FTx, 50.22%) and phospholipase A_2 (PLA_2, 31.46%), followed by serine proteases (1.63%) and a few antitoxins of lower abundance (<1%) including a variety of venom enzymes. At subprime venom, the 3FTx is dominated by ophistoxin (40.49%), while short neurotoxins (7.36%) predominate over the long neurotoxins (31.46%) among other neurotoxins of lesser toxicity (neutral toxins-like proteins, 5.51% and weak neurotoxins, 2.28%). The major SNTX, CTX and PLA_2 toxins were isolated with intravenous median lethal doses determined as 0.13, 1.06 and 0.39 μg/g in mice, respectively. SABU, the Indonesian manufactory homologous tri-specific antivenom could neutralise the CTX and PLA_2 fraction with moderate potency (potency = 0.14-0.16 mg toxin per ml antivenom). The SNTX, however, was very poorly neutralised with a potency level of 0.034 mg/ml, indicating SNTX as the main limiting factor in antivenom neutralization. The findings help elucidate the inferior efficacy of SABU reported in neutralizing N. sputatrix venom, and supports the call for antivenom improvement.

Biological significance: The Javan spitting cobra, Naja sputatrix is by itself a unique species and should not be confused as the Indian and the Indo-Chinese spitting cobras. The distinction among the spitting cobras was however unclear prior to the recent addition of snake systemsatics in the mid-90s, and results of some earlier studies are now questionable as to which species was implicated back then. The current study successfully profiled the venom proteome of authenticated N. sputatrix, and showed that the venom is made up of approximately 54% three-finger toxins (including neurotoxins and ophistoxins) and 36% phospholipase A_2 by total venom protein. The findings verified that the paralyzing components in the venom i.e., neurotoxins are predominantly the short-chain subtypes (SNTX), for encoding the long-chain subtype (LNTX) which is more abundant in the venom of monocled cobras and Indian common cobras. The neurotoxicity of N. sputatrix is hence almost exclusively NTX-driven, and effective neutralization of the SNTX is the key to early reversal of paralysis. Unfortunately, as shown through a toxin-specific assay, the neurotoxicological neutralization of the SNTX using the Indonesian antivenom (SABU) is extremely weak, implying that SABU has limited therapeutic efficacy in treating N. sputatrix envenomation clinically. From the practical viewpoint, actions need to be taken at all levels from laboratory to production and policy making to ensure that the shortcoming is overcome.

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1. Introduction
Snakebite envenomation is a well-recognized neglected tropical disease, but also a new environmental, occupational and climatic health hazard predominantly in developing and less developed countries [1]. The global figure of snakebite envenomation has been estimated to be >1.8 million with approximately 94,000 deaths occur annually [2]. The associated health burdens in South Asia have been revealed through several recent large community-based studies [3–6], however, the true scale of this problem in South Asia remains unclear due to continuing inadequacies in the reporting system of snakebite mortality and morbidity. The problem is in particular prevalent in countries such as Indonesia where early clinical practice is common while venemous snakes can be found throughout 14,000 islands of the country. One of the highly venomous snake species, classified under Category 1 of medically important snakes in Indonesia, is the Javan spitting cobra,