

Evaluation of Snake Venoms among *Agkistrodon* Species in China[†]

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Abstract. -Venom toxicity and enzymatic activities were examined in six species of Chinese *Agkistrodon* and *Deinagkistrodon acutus*. The venom toxicity of *A. intermedius* is the strongest, ten times that of *Deinagkistrodon acutus*. The venoms of *A. blomhoffii brevicaudus*, *A. blomhoffii ussuriensis*, and *A. shedaoensis* are the next strongest. *Agkistrodon saxatilis* venom had a similar toxicity as *A. strauchii* venom and they are more similar to *Deinagkistrodon acutus* in toxicity.

Key words: Reptilia, Serpentes, Viperidae, *Agkistrodon*, *Deinagkistrodon*, China, venom, toxicity.

Introduction

Agkistrodon snakes are widespread and abundant in China. According to Zhao et al. (1981) and Chen et al. (1984) they should be classified as: (1) *Agkistrodon blomhoffii brevicaudus* Stejneger, (2) *A. b. ussuriensis* Emelianov, (3) *A. intermedius* (Strauch), (4) *A. saxatilis* Emelianov, (5) *A. shedaoensis* Zhao, (6) *A. strauchii* Bedriaga (Plate 1), (7) *A. monticola* Werner, and (8) *Deinagkistrodon acutus* (Gloyd, 1979). The later was formerly regarded as *Agkistrodon acutus*.

The components and properties of venoms from these species are strikingly different from each other (Zhao et al., 1981). From the standpoint of venoms the general designation of Chinese *Agkistrodon* as only one species, *A. halys* Pallas, should not be accepted. Recently in China, *Agkistrodon* venoms have been used to make medicines to cure thrombotic disease and cancers. SVATE (Snake Venom Anti-thrombotic Enzymes) was first prepared from the venom of *A. shedaoensis* from Snake Island in Dalian, and was effective in curing thrombosis. Since there was a shortage of *A. shedaoensis* venom, the venom of *A. b. brevicaudus* from Zhejiang in eastern China was also used. However the product of *A. shedaoensis* seemed

better than that from *A. b. brevicaudus*. Later, two products, one named Qin Suan Mei using the venom of *A. b. ussuriensis*, and the other named Defibrinogenase using the venom of *D. acutus* also appeared in clinical application, but their efficiency and side reactions differed from each other. 787 Snake Venom Capsules, made by Shanghai Xin-Le District Hospital using the crude venom of *A. b. brevicaudus* for treatment of cancers has a magically inhibitory effect on the growth of malignant tumour cells. These observations aroused our interest to understand the differences of *Agkistrodon* venom properties. In this paper we determined the toxicity (LD₅₀), and enzymatic activities of arginine esterase, proteolytic and fibrinolytic enzymes to evaluate the quality of selected pit-viper venoms.

Methods

Snake Venoms

Agkistrodon b. brevicaudus were purchased from the Shanghai Experimental Animal Supply Station. *Agkistrodon b. ussuriensis*, *A. shedaoensis*, and *A. strauchii* were kindly provided by Professor Ermi Zhao of the Chengdu Institute of Biology. *Agkistrodon intermedius* was kindly provided by Mr. Jinbao Yu from Xinjiang Institute of Chemistry. *Deinagkistrodon acutus* were purchased from Jindezeng Snake Institute, Jiangxi.

[†] This publication was previously published in Chinese by Chen et al. (1990).

Chemical Reagents

BAEE (N-benzoyl arginine ethyl ester hydrochloride), are products of the Dongfeng Factory of Biochemical Reagents. Human fibrinogen and thrombin are products of the Shanghai Institute of Biological Products and casein is a product of the Factory of Chemical Reagents, Shanghai. All other chemical reagents are analytical grade.

Toxicity (LD_{50})

Toxicity (LD_{50}) was assayed (Litchfield and Wilcoxon, 1949). We dissolved 3-5 mg of snake venom in physiological saline, and then diluted the venom solution to 1-5 concentrations. Swiss mice of 18-20 grams of body weight, were divided into five groups, with six individuals each. Mice were injected intra-abdominally with 0.4 ml of venom solution. After injection observations were made within 48 hours. The death rate (LD_{50}) was then calculated.

Enzymatic Activities

1. *Arginine esterase* (United States Pharmacopea, 1980).—BAEE solution (0.8 mm moles) was prepared by dissolving BAEE in 0.05 M pH 8.0 tris-HCl buffer solution. Three ml of BAEE solution was inserted into a cuvette and 0.1 ml of snake venom solution was added. Spectrophotometric measurements were made at 253 nm and 25°C. The unit of activity is calculated according to the formula, $U/mg = (A_1 - A_2) / (0.003 \times T \times W \times A_1)$. The last value is at the linear part of the curve, where A_2 is the initial value, T is the time tested, and W is the weight of venom in milligrams.

2. *Proteolytic enzyme* (Rick, 1963).—One gram of casein was dissolved in 100 ml of 0.05 M Tris-HCl pH 7.8 buffer in a boiling water bath and the undissolved materials were filtered. We transferred 2 ml of filtrate to a test tube and incubated it in a 37°C water bath. Then two ml of venom solution (2 mg/ml) was added. After 15

minutes we added 15 ml of 15% trichloroacetic acid and mixed it thoroughly, filtering after 30 min. The filtrate was measured at 280 nm. We calculated the tyrosine released from the standard curve of tyrosine. The unit of activity is denoted by μg of Tyr/15 x mg of venom.

3. *Fibrinolytic enzyme* (Deogny et al., 1975).—We dissolved 0.5 grams of human fibrinogen in 10 ml of pH 7.75 barbiturate buffer and transferred 5 ml of the solution into each of two petri dishes (8 cm diameter). We subsequently added 1 ml of thrombin (about 4 units) and mixed it thoroughly. Then we immediately added 5 ml of 2% agar solution (below 60°C) and mixed it again to get a uniform plate. Wells were punched in the plate into which was put 10 μl of venom solution; incubated at 37°C for 18 hours. One unit of activity is equal to 1 mm² of lysis on the plate. The activity is denoted by units = mm²/mg of venom.

Results

The venom toxicity of *A. intermedius* is the strongest, ten times that of *Deinagkistrodon acutus* (Table 1). The venoms of *A. b. brevicaudus* and *A. b. ussuriensis* are the next strongest. Neurotoxins were isolated from these venoms. A presynaptic neurotoxin, Agkistrodotoxin, has been purified from the venom of *A. b. brevicaudus* (Chen et al., 1981) and its amino acid sequence also has been determined (Kondo, 1989). Three presynaptic neurotoxins were purified from the venom of *A. intermedius*. Their LD_{50} are 38, 49 and 49 $\mu g/kg$ of mice respectively, higher than that of Agkistrodotoxin which has a LD_{50} of 55 $\mu g/kg$ (Zhang and Hsu, 1985a). A fraction from column chromatography of the venom of *A. b. ussuriensis* has been confirmed to be neurotoxic (Zhang and Hsu, 1985b). The venoms of *A. shedaoensis* and *D. acutus* are non-neurotoxic. Therefore the potency of toxicity appears to be related to the neurotoxin content.

TABLE 1. Comparison of toxicity and enzymatic activities of *Agkistrodon* and *Deinagkistrodon* venoms.

Snake venom	Toxicity (LD ₅₀)	Enzymatic Activities (Units/mg)		
		Arginine esterase	Proteolytic enzyme	Fibrinolytic enzyme
<i>A. b. brevicaudus</i>	0.525	180	29	1.0 x 10 ³
<i>A. b. ussuriensis</i>	0.70	160	27	1.0 x 10 ³
<i>A. shedaoensis</i>	0.735	190	16.5	0.82 x 10 ³
<i>A. saxatilis</i>	2.064	220	18	0.82 x 10 ³
<i>A. intermedius</i>	0.285	450	11.5	1.1 x 10 ³
<i>A. strauchii</i>	1.75	250	24.5	0.65 x 10 ³
<i>D. acutus</i>	2.94	78	62	1.1 x 10 ³

Discussion

There are many components in the venom of *Agkistrodon* which react with the blood circulation causing bleeding, such as; hemorrhagin, arginine esterase, proteolytic and fibrinolytic enzymes. Three hemorrhagins had been purified from the venom of *D. acutus* (Xu et al., 1981), which have proteolytic activity, reacting with the blood vessel wall causing the leakage of red cells. Arginine esterase containing three enzymes: thrombin-like, kallikrein and plasminogen activator, which cause the failure of coagulation of the blood, depression of blood pressure and activation of fibrinolytic system.

Enzymatic activity data shows no sharp differences in the venom of *A. shedaoensis* compared to other venoms studied. The fibrinolytic activities are very close to each other due to the inaccuracy of the diffusion method. We should point out that the quality of snake venoms are deeply affected by the conditions of milking venom, such as; seasons, temperature and lyophilizing equipment.

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