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SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM
(Rangiroa, French Polynesia, 16th - 22nd August, 1968)

OCCURRENCE OF TOXIC CRABS IN THE RYUKYU AND AMAMI ISLANDS AND SIMILARITY OF THE CRAB TOXIN TO SAXITOXIN

by Shoji Konosu and Yoshiro Hashimoto *

In a field investigation of ciguatera in the Ryukyu and Amami Islands, we found a widespread belief that toxic crabs were the primary source of the toxin causing fishes to become ciguatoxic. We also found reports of sporadic outbreaks of intoxication in humans and domestic animals following the ingestion of toxic crabs. To understand the ciguatera problem more clearly, the belief seemed to be worth studying, and a survey of cases of crab poisonings and toxic species of crabs was carried out.

Three toxic species, Zosimus aeneus, Platypodia granulosa (Y. Hashimoto et al. : Toxicon, 5, 85, 1967) and Atergatis floridus (A. Inoue et al. : Toxicon, in press), were found to contain a potent neurotoxin, which is identical with or closely related to saxitoxin (paralytic shellfish poison), but apparently different from tetrodotoxin (puffer toxin) (S.Konosu et al. : Toxicon, in press).

The present report deals with these results.

Epidemiological investigation

Ten cases of intoxication, including some questionable ones, were traced in the Amami and nine in the Ryukyu Islands. Patients had been poisoned by ingestion of boiled meat or only soup. The signs, characterized by vomiting, paralysis, and rapid death, suggested that the toxin was of a paralytic type. A typical poisoning case is given below.

On the morning of 30th May, 1928, a man (Case A, aged 52), his wife (Case B, aged 49), a son (Case C, aged 9) and two relatives (Cases D and E) in Naze City were poisoned by a crab locally known as "Hamugan". Cases A and B both died.

At around 6.30 a.m. the crab in question, three lobsters and an edible crab, were boiled in miso soup and served for breakfast. Both Cases A and B took three bowls of soup, Case C, meat of the chelae and a small quantity of soup, and Cases D and E, only the abdomen of the lobster. Soon after the meal, Case A gradually began to feel ill and was aware that he had been poisoned when he saw that a pig vomited and died after being fed the remnants of the miso soup. He died at about 11 a.m. Case B, who had left home to peddle crabs after breakfast, had fallen down on the road. When she was found, she could neither speak nor move, and died at about 10 a.m.

Case C, who had paralysis of the feet and felt ill, was hospitalized with Cases D and E. He was forced by an injection to vomit all he had eaten. After several days he completely recovered. Cases D and E had slight numbness and aphasia but treatment in the hospital contributed to their rapid recovery.

Six domestic fowls which ingested the vomitus of Case A were later found dead.

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Screening Test

Several toxic species seemed to be present in association with coral reefs in these islands, but no reliable report on the biology of toxic species was obtained. A screening test for toxic crabs, therefore, was carried out on a total of about 1,000 specimens covering 56 species collected in the Ryukyu and Amami Islands. Most of them were tested in the following way.

Specimens were cut into halves symmetrically, and a half of the body was minced thoroughly in a mortar. One gram of minced material was extracted with 9 ml of water for 5 minutes in boiling water and then filtered when cooled. A 0.5 ml portion of the extract was injected intraperitoneally into mice weighing about 20 g. The toxic extracts killed mice usually within 20 minutes. When they survived, specimens were regarded as non-toxic.

Three species, Z. aeneus, P. granulosa, and A. floridus, were found to be toxic. The last species caught along the southern coast of the main land of Japan was also poisonous. Z. aeneus was confirmed to be the causative species in most of the poisoning cases in the Amami Islands by reinvestigation showing the specimen to patients.

Responses of test animals to the crab toxin.

The intraperitoneal injection of a lethal dose induced in mice restlessness, paralysis in the hind limbs, and wobbling gait followed by gasping, jumping, and death. On autopsy immediately after death, no marked change in the internal organs was found, and the heart was observed to continue beating for more than 30 minutes.

To determine the dose-death time curve, the stock solution of partially purified toxin from Z. aeneus (the minimum lethal dose for mice, 0.04 y/g body weight) was diluted with water to several concentrations. Each 0.5 ml portion was injected intreperitoneally into 7 mice weighing approximately 20 g at each dose level and the death time was measured. The curve similar to that for tetradotoxin or saxitoxin was obtained, suggesting that the crab toxin is determinable by the mouse assay method as these neurotoxins.

Cats died in a short time following both oral and subcutaneous administrations, and displayed vigorous vomiting and paralysis of the limbs.

Toxicity of three species of toxic crab

The specimens were divided into the appendages and cephalothorax or several portions. Each portion was minced and extracted with water as described above. By intraperitoneal injection of a 0.5 ml portion into mice weighing approximately 20 g, the dilution necessary to kill mice in 10 to 15 minutes was sought. From the average death time observed on two mice at this dilution and the dose-death time curve, the toxicity was determined. To indicate the amount of toxin, one mouse unit (M.U.) was defined as the minimum lethal dose which kills a 20 g mouse, following the method commonly adopted for saxitoxin.

A marked individual variation in toxicity was observed in each of three species. So far as examined on 94 individuals of Z. aeneus, 45 of A. floridus and 28 of P. granulosa, the toxicity and frequency of toxic specimen were found to be highest in the first species, followed by the second species. In the last species, only three specimens were toxic.

In general, the appendages were more toxic than the cephalothorax. It is also interesting that the exoskeleton of both the appendages and cephalothorax contained a considerable amount of toxin. Toxicity of the viscera, muscle and gill was lower than that of the appendages.

Some chemical properties of the crab toxin

Using the hot water extracts of Z. aeneus and the mouse bioassay, some chemical properties were examined.

After being adjusted to pH 3.0 or 10.0 with HCl or NaOH, the extracts were kept in a boiling water bath for 15 minutes. The toxicity remained unchanged at pH 3.0, but was reduced to a half at pH 10.0. When the syrupy residue obtained by evaporating the extracts was extracted with organic solvents, toxin was completely extractable with methanol, only slightly so with ethanol, and not at all with diethyl ether, petroleum ether, hexane, chloroform, and n-butanol. The toxin was not partitioned into diethyl ether, petroleum ether, and chloroform from the aqueous solutions adjusted to pH 2.0 with HCl or pH 10.0 with NaOH or NH_4OH. It was easily dialyzable through a cellophane membrane.

Purification of the crab toxin

The ion-exchange column chromatographic method devised by Schantz et al. for saxitoxin (J. Am. Chem. Soc., 79, 5230, 1957) was found to be very effective in purifying the crab toxin. An example of the results obtained in the purification of toxin from Z. aeneus is given in Fig. 1.

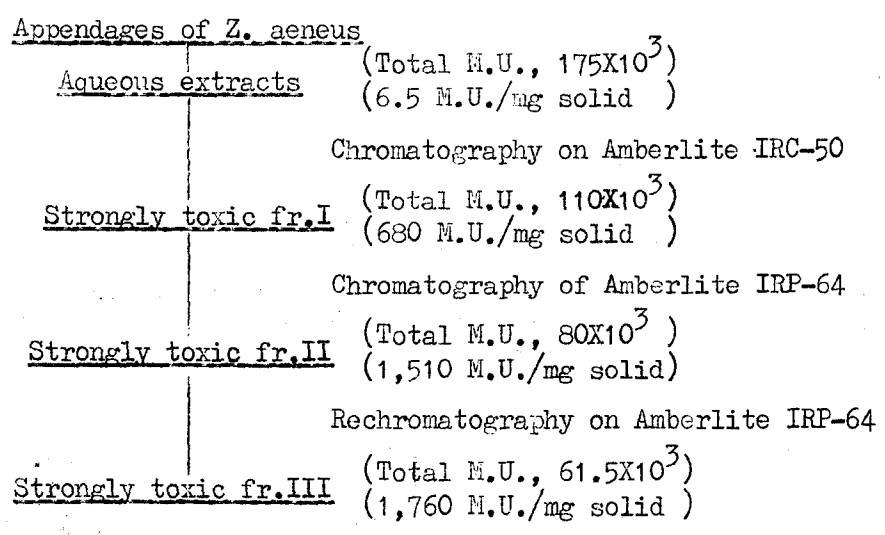

Fig. 1 Purification of crab toxin.

The behavior of crab toxin on the columns was almost identical with that of saxitoxin reported by Schantz et al., and the toxicity of the original extracts was improved about 270-fold by these treatments with a recovery of 35%.

Comparison of the crab toxin with saxitoxin and tetrodotoxin

In view of the close similarity of the crab toxin with saxitoxin and tetrodotoxin in chemical and pharmacological properties, a further comparison of these toxins was made, using a partially purified preparation of toxin from Z. aeneus (1,240 M.U./mg solid), a 97% pure specimen of saxitoxin supplied by Dr. J.E. Campbell and crystalline tetrodotoxin obtained from Sankyo Co. Ltd.,

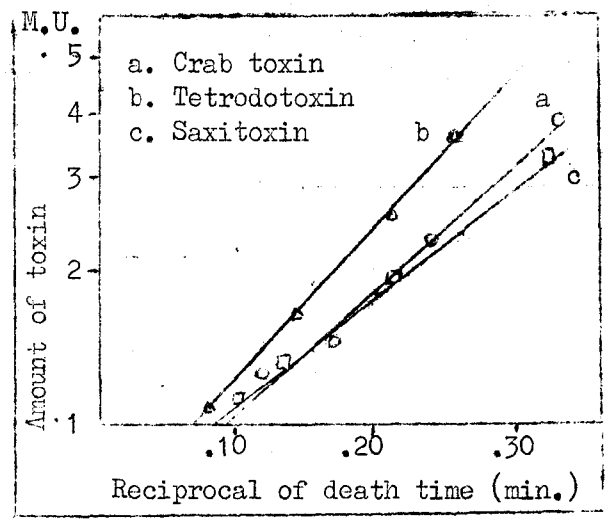

Fig. 2. The dose-death time relationship.

The crab toxin behaved quite similarly to saxitoxin both in paper and thin layer chromatography using several different solvent systems, while tetrodotoxin did apparently differently from the crab toxin.

The dose-death time curve for crab toxin was practically the same as that for saxitoxin and distinctly different from that for tetrodotoxin. The relations among these three toxins are clearly seen in Fig. 2, in which the reciprocal of death time is plotted against the logarithm of dosage.

Discussion

It is interesting that three toxin species found in this study all belong to the family Xanthidae, in which many non-toxin species are also involved. According to Banner and Randall (Atoll Research Bull., (13), ii + 62, 1952), a crab reported to be poisonous in the Gilbert Islands is presumably identical with one of our toxic species, Z. aeneus. This suggests the possibility that the species, known to be widespread in the Indo-Pacific, may be toxic throughout its range.

By the ion-exchange column chromatographic method, a preparation showing the minimum lethal dose for mice, 0.028 y/g, was obtained. This lead us to presume that the crab toxin, if purified to the utmost, may be almost comparable to saxitoxin (0.009 y/g) or tetrodotoxin (0.008 y/g) in toxicity. The crab toxin was not distinguishable from saxitoxin in all respects examined so far, while it was clearly discriminated from tetrodotoxin. It may be concluded that the crab toxin is identical with or closely related to saxitoxin.

There have been many poisoning cases reputedly due to toxin crustaceans (B.W. Halstead: Poisonous and Venomous Marine Animals of the World, Vol.1, 905, 1965), but no laboratory data is available regarding the toxicology, pharmacology, or chemistry of these toxins, except the paralytic one one found in the sand crab, Emerita analoga (H. Sommer et al.: Arch. Pathol., 24. 560, 1937). The sand crab was believed to become toxic from the ingestion of a toxic dinoflagellate, Gonyaulax sp., since toxicity was correlated with the occurrence of this toxic plankton and was limited to the digestive gland. In our study, the toxin was accumulated much more in the appendages than in the cephalothorax, and a considerable amount of toxin was found in the exoskeleton. In addition, there was no sign of a red tide, at least at the time of collections. These facts may suggest a quite different origin of the crab toxin, even if it is identical with saxitoxin.

The difference in pharmacological and chemical natures between the toxin in ciguatoxic fishes and that in our crabs is too great to allow us to accept the prevalent belief of the inhabitants who attribute the toxin in fish to these crabs.

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ABSTRACT

OCCURRENCE OF TOXIC CRABS IN THE RYUKYU AND AMAMI ISLANDS AND SIMILARITY OF THE CRAB TOXIN TO SAXITOXIN

by Shoji Konosu and Yoshiro Hashimoto *

Nineteen cases of illness associated with the ingestion of toxic crabs were traced in the Ryukyu and Amami Islands. The signs were characterized by vomiting, paralysis, and rapid death. In the screening test for toxic crabs, three species, Zosimus aeneus, Platypodia granulosa and Atergatis floridus, were found to contain a paralytic toxin, which killed mice in a few minutes. Specimens of A. floridus caught along the southern coast of the main land of Japan were also poisonous.

A marked individual variation of toxicity was recognized in each species. In general, the appendages were more toxic than the cephalothorax, and the exoskeleton also contained a considerable amount of toxin. The toxin was easily dialyzable, soluble in water and methanol and insoluble in most fat solvents.

For the purification of crab toxin, the ion-exchange column chromatographic method for saxitoxin was found to be applicable without any modification, and a partially purified preparation of toxin having a toxicity as high as 1,760 M.U./mg solid (the minimum lethal dose for mice, 0.028 y/g body weight) was obtained from the water extracts of Z. aeneus.

In paper and thin layer chromatographic behaviors and the dose-death time relationship in mice, the crab toxin was indistinguishable from saxitoxin and apparently different from tetrodotoxin. The crab toxin may be identical with or closely related to saxitoxin.

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