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Tetrodotoxin – a review with special reference to its distribution in nature*

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Abstract: Tetrodotoxin is one of the most potent, naturally-occurring neurotoxins. Its distribution had been believed to be limited to the pufferfish. During the last decades, however, this toxin was also detected in some other vertebrates as well as a variety of invertebrates. These fiindings may afford a clue to elucidate the origin, biosynthetic pathway, and physiological significance of this toxin.

1. Introduction

In recent years, marine organisms have proved to produce various kinds of bioactives. Among these, tetrodotoxin (TTX) or pufferfish toxin, along with paralytic shellfish poison (PSP), has furnished a rich stock of topics to biologists, chemists and pharmacologists. TTX is one of the most potent neurotoxins. The lethal potency is comparable to that of saxitoxin, a representative member of PSP, and is about one-thousand times as high as that of sodium cyanide (MOSHER *et al.*, 1964).

It was TAHARA (1909) who first undertook chemical studies on TTX. The purity of his preparation was, however, as low as 0.2%. In 1950, YOKOO isolated and crystallized TTX from pufferfish ovaries. In 1964, the structure of TTX was elucidated simultaneously by three groups headed by TSUDA, WOODWARD and HIRATA (TSUDA *et al.*, 1964; WOODWARD, 1964; GOTO *et al.*, 1965). It was a highlighted topic at the International Symposium on the Chemistry of Natural Products, held in Kyoto in that year. Total synthesis of TTX was accomplished by GOTO, KISHI and co-workers in 1972 (KISHI *et al.*, 1972). The structure of TTX is characterized by the presence of one guanido and six hydroxy groups, along with a hemilactal functional group, which has never been found in any other natural products (Fig. 1). In the pharmacological aspect, TTX is known to inhibit the formation of action potential by blocking the sodium ion influx into excitable cells selectively. TTX has been appreciated as a useful pharmacological agent (KAO, 1966; NARAHASHI *et al.*, 1967; NARAHASHI, 1972, 1974).

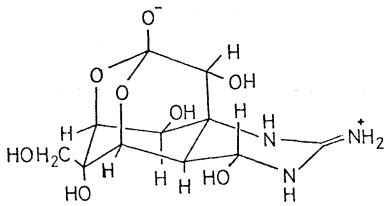

Fig. 1.

At the Kyoto Symposium, another important paper was presented: Tarichatoxin, a toxin isolated from the California newt *Taricha torosa*, was identified as TTX (MOSHER *et al.*, 1964). This finding negated the belief that TTX is distributed exclusively in the family *Tetraodontidae*. Since then, TTX has been detected also in various vertebrates and invertebrates as described below.

2. Distribution of tetrodotoxin in nature

As TTX-containing animals are known pufferfish, goby, newts, frogs, octopus, gastropod mollusks, starfish and crabs. There is no phylogenic relationship among these TTX-containing animals which makes, in turn, search of the biogenic origin of TTX extremely intriguing (Table 1).

2-1. Pufferfish

TTX has not been detected in any other fishes

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Table 1. Distribution of tetrodotoxin in nature.

Animals	Tissues containing tetrodotoxin
Vertebrates	
Fish	
Pufferfish (family *Tetraodontidae*)	viscera, skin, muscle, *etc.*
Goby (*Gobius criniger*)	viscera, skin, muscle, *etc.*
Amphibian	
Newts (genus *Taricha*)	viscera, skin, muscle, egg, *etc.*
Frog (genus *Atelopus*)	skin, ovary, egg, *etc.*
Invertebrates	
Mollusk	
Octopus (*Octopus maculosus*)	posterior salivary gland
Gastropods (*Charonia sauliae*, *Babylonia japonica*, *etc.*)	digestive gland
Echinoderm	
Starfish (genus *Astropecten*)	whole body
Crustacean	
Crabs (family *Xanthidae*)	whole body

than pufferfish except for a tropical and subtropical goby *Gobius criniger* (HASHIMOTO, 1979). More than ten people have been killed by ingesting pufferfish per year in Japan.

During the World War II, TANI (1945) surveyed toxicity of various tissues from 19 species of pufferfish caught from around the northern Kyushu Island. He reported that the pufferfishes were mostly toxic, and that the toxic potency widely differed even among specimens of the same species caught by a net.

His findings, however, seem not to be true for some pufferfish from other regions. In 1959, several persons were killed by ingestion of the flesh of a pufferfish caught off Vietnam. The fish was later identified as "dokusabafugu" *Lagocephalus lunaris lunaris*, which morphologically resembles non-toxic "sabafugu" *L. lunaris spadiceus* very well (HASHIMOTO, 1979). It has recently been found that the muscle of several pufferfishes inhabiting the Sanriku coasts is often toxified up to a level of several hundreds mouse units (MU)* per gram (KANOH *et al.*, 1984; KODAMA *et al.*, 1984), i. e. the flesh less than 100 g can kill a man. On the other hand, a high toxicity was detected in tissues of "shiroamifugu" *Tetraodon alboreticulatus* collected from Sagami Bay: e. g. 2,870 MU/g ovary and 31 MU/g liver (KANOH *et al.*, 1982). Under these situations, a new manual for utilization of pufferfish was issued by the Ministry of Health and Welfare in December 1983.

* One mouse unit (MU) is defined as an amount of TTX which kills a 20-g male mouse (ddY strain) in 30 min. The lethal dose of TTX in human is assumed to be about 10,000 MU (*ca.* 2 mg).

2-2. Newts

TTX has been identified in three groups of vertebrates other than pufferfish. It was observed about a half century ago that when the eye vesicles of the California newt *Taricha torosa* were transplanted into an embryo of the tiger salamander *Ambystoma tigrinum*, the host was paralyzed (MOSHER *et al.*, 1964). Causative agent isolated from the ovaries was named tarichatoxin (MOSHER *et al.*, 1964) which is identical with TTX. WAKELY *et al.* (1966) detected this toxin, TTX, also in the skin, muscle and blood of the California newt as well as other species than the genus *Taricha*.

2-3. Goby

People have never eaten some goby (*Gobius criniger*) in Ryukyu and Amami Islands because of the fear of poisoning. Toxic gobies were also reported in Philippines and Taiwan (HASHIMOTO, 1979). NOGUCHI and HASHIMOTO (1973) demonstrated that the toxin is TTX, and that TTX is distributed in all tissues of the goby. The toxicity is generally high in the skin, intermediate in the viscera and low in the

muscle. Subsequent research revealed that all specimens from Amami-Oshima Island, Ishigaki Island, Iriomote Island and Formosa were toxic. As is the case with puffer, the goby showed a clear regionality in toxicity (HASHIMOTO, 1979).

2-4. Frogs

Costa Rican had prepared an arrow poison from the frogs of the genus *Atelopus*. Toxicity was detected in the skin, ovary and egg, but not in the viscera, muscle and bone. KIM *et al.* (1975) demonstrated the presence of TTX in the skin of *A. varius varius* and *A. varius ambulatorius*. The toxin extracted from the skin of *A. chiriquiensis* proved to be a mixture of *ca.* 30% of TTX and a second major component designated chiriquitoxin (KIM *et al.*, 1975; PAVELKA *et al.*, 1977). From the Panamanian atelopid frog *A. zeteki*, on the other hand, the only toxin named zetekitoxin was detected from the skin extract but neither TTX nor chiriquitoxin was contained (SHILDERMAN and MOSHER, 1969; KIM *et al.*, 1975).

2-5. Octopus

The blue-ringed octopus *Octopus maculosus* is small-sized and often seen in shallow waters of Australia, especially in summer season. This octopus has a brown or yellow body with blue rings. When stimulated, the body color turns black, while blue gleaming spots appear as with a peacock feather. This phenomenon is so attractive as one might be unaware of bites by the octopus placed on his hand or arm. Paralysis or even death may occur. The toxin secreted from the posterior salivary glands was first named maculotoxin (FREEMAN and TURNER, 1970). The structure of maculotoxin had long been a matter of debate because of difficulty in collecting enough starting materials. SHEUMACK *et al.* (1978) isolated 1.8 mg of the toxin from *ca.* 500 posterior salivary glands and identified it as TTX. It was the first reporting the occurrence of TTX in invertebrates.

2-6. Gastropod Mollusks

In December 1979, a food poisoning occurred due to the ingestion of a trumpet shell, "boshubora" *Charonia sauliae* which was caught off Shimizu, Shizuoka Prefecture (NARITA *et al.*, 1981). The causative agent for this incidence was identified as TTX. In December 1982, the second poisoning associated with this shell occurred at Mihama, Wakayama Prefecture (MARUYAMA *et al.*, 1983). Toxicity was detected exclusively in the digestive gland in both cases. Most of the trumpet shell specimens collected from the adjacent waters of Shimizu were toxic, the highest toxicity score being 1,950 MU/g digestive gland. Toxicity survey of organisms inhabiting Suruga Bay revealed that the digestive gland of the following gastropod mollusks also showed a paralytic toxicity: "onarutobora" *Tutufa lissostoma*, "kakobora" *Monoplex echo*, "tengunishi" *Pugilina ternatana*, "hanamushirogai" *Zeuxis siquijorensis* and "araregai" *Niotha clathrata* (NARITA *et al.*, 1982). Among these, the toxins from *T. lissostoma* (NOGUCHI *et al.*, 1984) and *N. clathrata* (JEON *et al*, 1984) were identified as TTX, whereas that from *Z. siquijorensis* was not TTX (NARITA *et al.*, 1984a). The structure of the latter toxin is not clear, though it gives rise to C_9-base as does TTX. Recently, the same substance has been detected also in the trumpet shell and pufferfish at our laboratory. This suggests its probable involvement in the toxification of TTX-containing organisms.

On the other hand, a paralytic toxicity was detected in the Japanese ivory shell, "bai" *Babylonia japonica*, which was collected from Sakajiri Bay, Fukui Prefecture. This toxin was also identified as TTX (NOGUCHI *et al.*, 1981; YASUMOTO *et al.*, 1981).

2-7. Starfish

During our survey of benthic organisms in Suruga Bay, two starfishes of the genus *Astropecten*, "togemomijigai" *A. polyacanthus* (NOGUCHI *et al.*, 1982) and "momijigai" *A. scoparius* were found to contain TTX at significant levels at our laboratory. Since these starfishes are often detected in the digestive canal of the trumpet shell *C. sauliae*, the latter is supposed to be toxified by the food chain including those starfishes. TTX was also found in the starfish, "hiramomijigai" *A. latespinosus* from Sakajiri Bay (MARUYAMA *et al.*, 1984). It is not clear, however, as to whether the toxification of the Japanese ivory shell *B. japonica* there is associated with the starfish *A. latespinosus*, because of the feeding habit of the former.

2-8. Crabs

In the tropical and subtropical areas, food

poisonings sporadically occur by ingesting the crabs of a family *Xanthidae* (HASHIMOTO *et al.*, 1967). They contain PSP in most cases (NOGUCHI *et al.*, 1969; KOYAMA *et al.*, 1981). Recently, one of those toxic crabs, "subesube-manjugani" *Atergatis floridus*, inhabiting Miura Peninsula near Tokyo, was found to possess TTX as the major toxin, along with some PSP as the minor (NOGUCHI *et al.*, 1983). Detailed analysis of the toxin from Okinawan xanthid crabs has revealed that *A. floridus* contains TTX or its associated substance as the minor toxin. A small amount of TTX was detected in Okinawan specimens of another xanthid crab, "umore-ogigani" *Zosimus aeneus*, whose main toxin is PSP, as has already been demonstrated (KOYAMA *et al.*, 1981). YASUMOTO, T. and his group suggested recently that Philippine speciemens of this crab also contains TTX as the major toxin.

3. Conclusion

As described above, TTX is distributed in a variety of aquatic organisms which are not closely related phylogenically. In spite of many efforts, none can answer as yet to the question: "What does TTX originate in?"

Recently, SHIMIZU and KOBAYASHI (1983) studied on biosynthesis of TTX using the toxic newts, *T. torosa* and *T. granulosa*. Radioactive compounds such as acetate, arginine and glucose were administrated orally, externally or subcutaneously, to see if the *de novo* synthesis of TTX took place in the newts. However, no incorporation of them into TTX was observed, in contrast to a significant incorporation into amino acids, sterols, *etc.* No evidence has been presented to support the biosynthesis of TTX in pufferfish, either.

The blue-ringed octopus *O. maculosus* is considered to be the only TTX-containing organism in which physiological significance of this toxin can reasonably be accounted for: The TTX-containing venom is secreted from the salivary glands to immobilize and feed small crabs (FREEMAN and TURNER, 1970). In the TTX-containing gastropod mollusks, TTX is supposed to come from the toxic starfishes they feed, as described above and demonstrated recently by model experiments (NOGUCHI *et al.*, 1982, 1984; NARITA *et al.*, 1984b; SHIOMI *et al.*, 1984).

The authentic origin and biosynthetic pathway of TTX, along with physiological significance in most TTX-containing animals, still remain to be challenged by many scientists.

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