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Editorial

Chemistry, Toxicology and Etiology of Marine Biotoxins

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Marine biotoxins refer to bioactive natural products primarily produced by microalgae and bacteria and may affect aquatic organisms and human health [1]. The blooming of certain species of dinoflagellate or diatom causes plankton-feeding bivalve mollusks to become poisonous, and consuming these bivalves prompts human intoxication, inducing paralytic, diarrheic, neurologic, and amnesic shellfish poisonings. Having elucidated these microalgae's appearance patterns and principal toxins' chemical properties, the risk of shellfish poisoning has been avoided by monitoring plankton and toxins. This Special Issue aims to present new findings on the chemical, toxicological, and etiological aspects of marine biotoxins, as well as observations and evidence in health risk assessment, analysis, and management, which might contribute to the research areas mentioned above. In this Special Issue, eleven manuscripts (two reviews and nine original articles) contributed and covered various marine biotoxins, including tetramine, palytoxins, ciguatoxins, tetrodotoxins, and okadaic acids.

Tetramine, tetramethylammonium ion, is the smallest marine biotoxin, $(CH_3)_4N^+$, and a major component of the salivary gland of marine snails belonging to the genus *Neptunea* (Buccinidae) [2]. Most marine biotoxins are produced by microorganisms such as dinoflagellate, cyanobacteria, and bacteria and are transmitted to other animals through the food chain. However, tetramine is one of the few exceptions of endogenous origin. Shiomi reviewed tetramine in marine snails (contribution 1) for the first time in over 30 years since the previous review in 1989 [3]. This review covered widespread aspects of tetramine, implicating marine snails, and incidents of human poisonings. The review also included an anatomical description of salivary glands, a method for analyzing tetramine, the distribution of tetramine in marine snails, the pharmacological properties of tetramine, and the occurrence and prevention of intoxication.

Palytoxin is one of the most potently toxic and giant structural organic compounds [4]. It was first isolated and structurally elucidated from a tropical marine cnidarian, *Palythoa toxica* [5,6]. Its analogs, ostreocins and ovatoxins, have been isolated from the marine dinoflagellate of the genus *Ostreopsis* [7,8]. Palytoxin analogs have been involved in clupeotoxism, a kind of food poisoning acquired by consuming tropical Clupeidae fish that has significant symptoms and a high lethality rate [9,10]. They were responsible for respiratory disorders and dermatitis in people exposed to marine aerosols during *Ostreopsis* cf. *ovata* blooms in the Mediterranean Sea [8]. Furthermore, there have been several case reports of these symptoms and keratitis following exposure to aerosols from domestic aquaria-housed *Palythoa* [11]. Carlin et al. review the recent development of biological and biochemical methods of detecting palytoxin analogs (contribution 2). The methods include immunological assays using specific anti-palytoxin antibodies and functional cell-based assays. They are usually sensitive, cost- and time-effective, and do not require highly specialized operators.

Tetrodotoxin (TTX) is a voltage-gated sodium channel blocker and is one of the best-known and well-studied marine natural toxins [12]. The representative vector of TTX

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are pufferfishes (globefishes), and there have been many cases of human intoxication (some fatal), especially in East and Southeast Asia [13,14]. TTX has been found in various marine animals, including goby, blue-ringed octopus, and xanthid crabs. In addition, it also has been found in terrestrial animals such as amphibians (e.g., newts and frogs). Four original articles related to TTX were collected in this Special Issue. Previously, Sato et al. produced a specific polyclonal antibody against TTX and developed an enzyme-linked immunosorbent assay kit (ELISA) to detect tetrodotoxin and its analogs TTXs [15]. They determined the distribution of the TTXs in various tissues of toxin-bearing pufferfishes (*Canthigaster revulata* and *Takifugu flavipterus*) and newts (*Cynops pyrrhogaster*) using both the ELISA and high-performance liquid chromatography with fluorescence detection (HPLC-FLD). The feeding experiment on the newts showed that a TTX analog, 5,6,11-trideoxyTTX, and a saxitoxin (STX) analog, decarbamoylSTX (dcSTX), were not metabolized into TTX or STX, respectively. The anatomical localization of TTXs was demonstrated by these methods and an immunohistochemical study using the antibody mentioned above. They also demonstrated TTXs-immunoreactive staining on various tissues. They suggested that TTXs absorbed from the environment are distributed to various organs or tissues in a species-specific manner, regardless of whether or not these are metabolized in the bodies of toxin-bearing animals (contribution 3). Tatsuno et al. demonstrated that TTX levels were reduced in wild specimens of toxic goby, *Yongeichthys criniger*, raised in laboratory aquaria, and fed a TTX-free diet for 60 days. Liquid chromatography–mass spectrometry (LC-MS) and liquid chromatography–tandem mass spectrometry (LC-MS/MS) were employed to analyze TTX; the decreasing tendency of TTX differed by tissue and was remarkable in the skin. They concluded that *Y. criniger* has low TTX retention ability (contribution 4). Nagashima et al. isolated the TTX-binding protein, HSTBP (Hemigrapsus sanguineus TTX-binding protein) from the hemolymph of nontoxic marine shore crab, *Hemigrapsus sanguineus*, and elucidated its primary structure. It comprised three subunits of 88 kDa (subunit 1), 65 kDa (subunit 2), and 26 kDa (subunit 3) arranged in tandem in the following order: subunit 3, subunit 1, and subunit 2. HSTBP showed a weak similarity (29–40%) to clotting proteins of crustaceans and the conserved vWF type D domain in subunit 2. The recombinant HSTBP subunit 2 was bound to TTX at a molecular ratio of 1:1. They concluded that the HSTBP may neutralize TTX to prevent the lethal toxicity of TTX, and could be applicable to possible antidotes for TTX intoxication (contribution 5). Oshiro et al. reported TTX levels in Japanese specimens of *Takifugu flavipterus*, formerly known as *Takifugu poecilonotus*, collected from the Seto Inland Sea. The flesh of this pufferfish is accepted for human consumption in Japan. However, unacceptable levels of TTX were detected in some individuals. They demonstrated TTX in flesh that had migrated from the skin. The TTX levels in the flesh were remarkable in individuals with poor freshness or extremely high TTX content in the skin. In addition, regionality rather than seasonality influenced TTX levels in the skin (contribution 6).

Okadaic acid (OA) was first isolated from a marine sponge, *Halichondria okadai*, and its structure was determined in 1981 [16]. Soon after, its analog, dinophysistoxin-1 (DTX1), and OA were determined as the principal toxins of a newly recognized form of shellfish poisoning, diarrhetic shellfish poisoning [17,18]. The Codex Alimentarius claims that an acceptable level of OA group toxins in bivalve mollusks is 0.16 μg OA equivalent/kg. Toxic equivalency factors (TEFs) are applied to the actual levels of each analog needed to convert to an OA equivalent. Ikehara et al. described the bioactivities of OA, DTX1, and DTX2 and their impact on protein phosphatase 2A (PP2A) inhibition and cytotoxicity. The relative activities were similar in both assays, and matched better with oral toxicity data than intraperitoneal toxicity in mice. Their result suggested a higher OA equivalent for DTX1 than that currently used (contribution 7).

Ciguatera poisoning is the most common seafood poisoning caused by marine biotoxin. Tropical fishes are the primary cause of human CP, frequently occurring in the tropical and sub-tropical Indo-Pacific Ocean and the Caribbean Sea. Several CP cases have recently been reported in Macaronesia in the northeastern Atlantic, which is recognized as a non-CP

endemic area, leading to consideration of the expansion of the biogeographic distribution of ciguateric fish. The principal marine biotoxins are ciguatoxins (CTXs), which are categorized into four groups: CTX4A, CTX3C, C-CTX (Caribbean ciguatoxin), and I-CTX (Indian Ocean ciguatoxin) derivatives based on their skeletal structures and regions [19]. In this Special Issue, four original articles contributed, one on the Atlantic and three on the Pacific. Castro et al. presented a detailed protocol for preparing purified C-CTX reference materials from the flesh and liver of ciguatoxic fish specimens obtained from Macaronesia. They isolated C-CTX based on a liquid chromatographic technique guided by CTX-like toxicity evaluated using a specific cell-based N2a assay. The purified products were ultimately profiled and quantified by comparison with authentic C-CTX1 using LC-MS/MS. These reference materials enable further research and monitoring of CP as a public health hazard (contribution 8). Oshiro and co-workers provided CTX profiles in the flesh of the fish collected from the tropical West Pacific and Japan using the LC–MS/MS technique. They analyzed 24 specimens of snappers, groupers, Spanish mackerel, and moray eel purchased from Fiji, the Philippines, Thailand, and Taiwan. Only the fish captured from Fijian coastal waters contained detectable amounts of CTXs. The toxin levels in the fish species found along the coastal regions and the toxin profiles differed from those in other coastal areas (contribution 9). They also reported CTX levels and profiles in the flesh of Japanese specimens of two grouper species, *Variola louti* (154 individuals) and *Variola albimarginata* (133 individuals), collected from the Ryukyu Islands (Okinawa and Amami Islands) and Okinawa Island, respectively. The former is the most frequently implicated fish, and the latter is regarded as safe for consumption in Okinawa. In the flesh of *V. louti* and *V. albimarginata*, the rates of individuals were 64% and 21%, 43% and 8%, and 3% and <1%, respectively; these values are CTXs detected (>LOD), above the FDA guidance level (0.01 μg/kg CTX1B equivalent) and the unacceptable level for human consumption in Japan (0.175 μg/kg CTX1B equivalent). These observations indicated the CP risk of *V louti* is higher than that of *V. albimarginata*. Considering the above observations, the frequency of the CP caused by these two species, and estimated fish catches, the authors stated that implementing risk management based on levels recommended in Japan effectively protects public health and enables appropriate exploitation of fisheries' resources (contribution 10 and 11).

While important progress has recently been made in the identification of the main toxins associated with ciguatera and pufferfish poisoning, further investigations that identify and structurally elucidate new analogs and transmission and accumulation modes are required. In addition, the isolation and structural elucidation of Indian Ocean ciguatoxin, charcheatoxin, and the substances implicated in Haff disease and similar types of intoxication are issues of interest in this field. The guest editors believe that articles collected in this Special Issue will play important roles in further scientific activities and measures to protect human health.

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