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The Processing of Pufferfish and Usage of Tetrodotoxin in the Pharmaceutical Industry

Nermin Berik[1], Ekrem Cem Çankırılıgil[2]

[1]Department of Fisheries and Processing Technology, Faculty of Marine Sciences and Technology, Canakkale Onsekiz Mart University, Çanakkale - Turkey
[2]Central Fisheries Research Institute, Yomra, Trabzon - Turkey

Abstract: The pufferfish is mostly distributed in tropical to temperate seas and they introduced several seas as an invasive species. The meat of the pufferfish is consumes mostly as sashimi especially in the Fareast countries. However, if the pufferfish is not processed properly, it poses a threat to consumers due to tetrodotoxin they contain. For this reason, several researchers were studied on developing toxin determination methods. With the development of studies in this field, it has paved the way to get benefits from tetrodotoxin. Nowadays, scientists has started to get benefits from this severe toxin with using as medicine for treatment of several diseases. In this review, studies related to usages of tetrodotoxin in pharmaceutical industry were examined. Besides, we evaluated to future of the tetrodotoxin in pharmaceutical industry as an alternative medicine.

Keywords: Pufferfish, tetrodotoxin, MSFD, medicine, invasive species, analgesic.

Introduction

There are 120 species of puffer fish belongs to tetraodontidae family exist in the world and most of them live in tropical seas (Aydın 2011). Several exotic species like puffer fishes in the Mediterranean Sea introduced from both the Red Sea and the Atlantic Ocean (Farrag et al. 2016) with the opening of Suez Channel since 1869 (Katikou et al. 2009). Among these Lessepsian migrant species, six puffer fish species such as Suez puffer fish (*Lagocephalus sceleratus*), silver-cheeked toadfish (*Lagocephalus suezensis*), blunthead puffer (*Sphoeroides pachygaster*), half-smooth golden pufferfish (*Lagocephalus spadiceus*), yellowspotted puffer (*Torquigener flavimaculosus*) and spiny blaasop (*Tylerius spinosissimus*) are found in coasts of Turkish seas (Çinar et al. 2005; Bilecenoğlu et al. 2014). Especially, *Lagocephalus sceleratus*, *L. suezensis* and *L. spadiceus* are widely distributed along the Turkish and eastern Mediterranean coasts (Acar et al. 2017) and several Lessepsian species like puffer fish cause serious harmful effects on both humans with intoxications and fishing gears with net damages (Turan 2010). Puffer fish species originated from Indo-Pacific, are distributed in Turkish seas recently. The development of opportunities to use tetrodotoxin content of the species is another key role in processing and utilizing of these invasive species. Within the review, present and further areas of utilization of tetrodotoxin was examined and summarized.

The pufferfish species that found on coast of Turkish Seas: *Lagocephalus sceleratus* (a), *Lagocephalus suezensis* (b), *Sphoeroides pachygaster* (c), *Lagocephalus spadiceus* (d), *Torquigener flavimaculosus* (e) and *Tylerius spinosissimus* (f). Photos were taken from Fishbase (Froese and Pauly 2017).

Consumption of Pufferfish

Seafood is important food source having high protein, long chained mono and poly unsaturated fatty acids and poor in terms of cholesterol and saturated fatty acids (Diaz and Hu 2009). In the world, several fish, shellfish and other aquatic organisms both obtained from fisheries and aquaculture are consume by the people increasingly (FAO 2017a, b). Also, 60% of European consumers like to try new fish products and species (European Commission 2017). Along with various fish species, the meat of puffer fish species are consume as grilled, boiled and fried especially in Fareast countries by processing avoiding intoxications (Hajeb et al. 2012). Besides, puffer fish meat can consume as sashimi which is called fugu particularly in Japan in spite of severe danger caused by tetrodotoxin (Tanaka 2008; Kheifets et al. 2012; Panão et al. 2016). Although the consumer' desires, the consumption of all species

especially species of tetraodontidae is not safe in every time. Puffer fish species may be dangerous to humans due to tetrodotoxin they contain if it is not processed properly (Kasapidis et al. 2007). According to Kosker et al. (2016) it is dangerous that consume puffer fish meat especially the female ones caught from Mediterranean Sea due to high tetrodotoxin levels in gonads, liver, intestine and skin. Thus, this high amount of tetrodotoxin can be used in pharmaceutical industry by processing.

Tetrodotoxin (TTX)

Tetrodotoxin is natural toxin which is toxic to mammals with LD_{50} (Moczydlowski 2013) found various organisms such as frogs, puffer fish, gobies, crab and gastropods produced by several bacteria in these organisms (Chau et al. 2011). But, studies carried out of tetrodotoxin occurrence associated with pufferfish species, especially (Kohane et al. 1998). Pufferfish species are contain this extremely dangerous neurotoxin in the liver and gonad tissues usually (Itoi et al. 2014). Pufferfish use tetrodotoxin especially for defensive purpose that protects prey species from predation (Toledo et al. 2016), communication between species about the location of mates or eggs (Williams 2010) and protection of pufferfish larvae (Itoi et al. 2014). For several years, researchers focused on human intoxications or potential danger of this severe toxin to humans (Buchwald et al. 1964; Kao 1966; Bane et al. 2014) Nowadays, tetrodotoxin has been shown to be safe with small doses in humans (Salas et al. 2015). Tetrodotoxin is powerful sodium channel blocker for several scientists (Kohane et al. 1998; Zhou and Zhao 2000; Marcil et al. 2006; Neuman-Lee et al. 2016; Toledo et al. 2016). It is known fact that, sodium channel blockers are effective for reducing pain in humans (Bagal et al. 2014; Dib-Hajj et al. 2017; Jensen 2017) and with that feature tetrodotoxin has become important for pharmaceutical industry.

Tetrodotoxin Use in the Field of Medicine

Tetrodotoxin has started to use for pain killer in recent years especially for terminal cancer and migraine patients. Also, tetrodotoxin is currently being used in cancer patients with the features of both anti-tumor activity and reducing side effects in chemotherapy. Besides, it can be used in the regulation of cardiac arrhythmias, treatment of some drug addictions, as an alternative anaesthetic and analgesic for several scientists. According to studies;

– Lihua et al. (1999) studied on the analgesic effect of tetrodotoxin on chemical and thermal stimuli in mice. They suggested that, compared to control group, tetrodotoxin has obvious analgesic effect during 30 minutes to 2 hours. Salas et al. (2015) determined to analgesic effect of tetrodotoxin on thermal hyperalgesia and mechanical allodynia associated with burning injuries in rats. According to results, tetrodotoxin reduced both of them rapidly and it can be used for battlefield burning injuries as a rapid and effective analgesic. Tetrodotoxin is also used in migraine patients. According to Zakharov et al. (2015) Spontaneous and pain spikes were strongly suppressed with tetrodotoxin. Besides, Lyu et al. (2000) showed that the small doses of tetrodotoxin can reduces neuropathic pain.

– Several studies carried out with regards to usage of tetrodotoxin as anesthetics. Schwartz et al. (1998) applied tetrodotoxin to New Zealand white rabbit' eye and evaluated to anesthetic features by observing delays of blinking reflex. As a result, in a rabbit model, tetrodotoxin is a long-acting topical anesthetic retains

effectiveness without harming to epithelial healing. Kohane et al. (2003) used coencapsulated tetrodotoxin with controlled release devices containing bupivacaine and dexamethasone for prolonged nerve blocking. According to their results, this devices were prolonged duration of nerve blocks. Berde et al. (2011) studied that the effect of combination of tetrodotoxin and currently used local anesthetics like bupivacaine and epinephrine for prolonged local anesthesia in rats. As a result, they suggested that combinations include tetrodotoxin generate significant prolonged sciatic nerve blockade compared to non-include tetrodotoxin groups.

– Tetrodotoxin is also very important for the branch of oncology. Several clinical trial are carrying out related to tetrodotoxin in cancer pain management (Garud et al. 2017). Fouda (2005) used tetrodotoxin obtained from the Masked Puffer fish (*Arothron diadematus*) as an anti-tumor activity agent in Swiss albino mice. According to findings, the tetrodotoxin increased lifespan of mice up to 46 with decreasing the number of tumor cells. Besides, the negative effects of tumor cells to liver such as fat oxidation, decreasing of anti-oxidation agents, unsystematic release of malondialdehyde (MDA) and gamma-glutamyltransferase enzymes were diminished six days after injection. Hagen et al. (2011) carried out a tetrodotoxin using trial with patients having moderate to severe phase of cancer. They suggested that the toxicity of tetrodotoxin that used in moderate to severe cancer patients is mild and tolerated. The similar research carried out Hagen et al. (2017) and they found that tetrodotoxin has favorable benefit-risk for cancer-related pain and it may suggested for patients who have persistent cancer pain despite best analgesic care.

– Tetrodotoxin can use preventing or delaying serious effects of other severe toxins such as aconitine. Ono et al. (2013) conducted a trial with mice and evaluated cardiac toxicity effects of aconitine which is cardiotoxic chemical by opening membrane of sodium channels and tetrodotoxin as a sodium channel blocker. According to their findings, mice treated with mixture of aconitine-tetrodotoxin showed lower mortality rates and delayed arrhythmia compared to mice treated aconitine alone. As a result, tetrodotoxin may use for antagonist to aconitine as severe plant toxin.

– Also tetrodotoxin can be helpful to treatment of drug addictions. Shi et al. (2009) randomly divided 45 heroin addicts to three treatment group such as placebo, 5 μg TTX, or 10 μg TTX. Besides, all groups exposed heroin related or non-related video with the aim of determining changes on drug craving, anxiety, blood pressure, and heart rate parameters. According to results, tetrodotoxin has no effect on blood pressure and heart rate. But, it was reduced drug craving and anxiety caused drug addiction.

Conclusion

Within the scope of this review, it is explicitly acceptable that tetrodotoxin has particular significance in the field of medicine. However, doses of tetrodotoxin that used as therapeutic purposes should be well adjusted to avoid poisoning. These determination techniques must be improved as sensitive and precise considering to amendments of “Contaminants” and “Contaminants in Seafood” named task groups of Marine Strategy Framework Directive (Law et al. 2010; Swartenbroux et al. 2010) described by the European Commission. With applying the standardized methods, it should be possible to get benefits from tetrodotoxin with the most effective way avoiding such health risks.

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Contact:
Nermin Berik
Department of Fisheries and Processing Technology, Faculty of Marine Sciences and Technology, Canakkale Onsekiz Mart University, 17100 Çanakkale-Turkey
E-mail: nberik@yahoo.com

Ekrem Cem Çankırılıgil
Central Fisheries Research Institute, Vali Adil Yazar Cad., No:14, 61250 Yomra, Trabzon - Turkey
E-mail: ekremcem19@gmail.com