Rev. Agr. Acad., v.1, n.2, Jul/Ago (2018)

Revista Agrária Acadêmica

Agrarian Academic Journal

Volume 1 – Número 2 – Jul/Ago (2018)

Organisms that host Tetrodotoxin and researches related to its medicinal use

Organismos que possuem tetrodotoxina e pesquisas relacionadas ao seu uso medicinal

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Abstract

Tetrodotoxin (TTX) is a highly important animal toxin found in pufferfish, mollusks, arthropods, flatworms and amphibians. Although the toxin is related to several cases of food poisoning in humans, recent search demonstrated that TTX has a potent specific action in the blockage of voltage-gated sodium channels (VGSCs). The aims of current research is demonstrates that TTX is efficient in therapies related to the nervous, circulatory and motor systems, besides those against cancer. TTX-producing organisms, its mechanism and studies on the therapeutic use of the toxin in medicine are provided. Further studies may reveal other therapeutic uses of TTX related to voltage-gated sodium channels due to its efficiency and specificity in the blockage of these channels.

Keywords: Pain therapy, new medicine, sodium channels, tetrodotoxin, toxin.

Resumo

A tetrodotoxina (TTX) é uma importante toxina animal, a qual é comumente encontrada em peixes baiacus, moluscos, artrópodes, platelmintos e anfíbios. Esta toxina está relacionada com diversos casos de intoxicação alimentar em humanos. No entanto, diversas pesquisas têm mostrado que a TTX possui uma ação poderosa e específica no bloqueio de canais de Na+ voltagem-dependente. Esta pesquisa demonstra que a TTX têm revelado eficácia em terapias relacionadas ao sistema nervoso, sobretudo aquelas relacionadas a dores neuropáticas e crônicas, terapias relacionadas ao sistema muscular, cardíaco e motor, terapias contra cânceres, além de outras utilizações terapêuticas, relacionadas aos canais de Na+ voltagem-dependentes.

Palavras-chave: Dor, canais de sódio, nova medicina, tetrodotoxina, toxina.

Introduction

Animal venoms and poisons are a complex mixture of toxins used as a defense strategy and the capture of prey. These toxins are shown to have a relevant potential in pharmacological research and in the production of new therapeutic drugs (LEWIS, GARCIA, 2003). Tetrodotoxin (TTX) is an important poison found in animals, such as the pufferfish (NIETO *et al*., 2012; RAMIRES *et al*., 2012). Although TTX is related to human food poisoning (SANTANA-NETO *et al*., 2010; SILVA *et al*., 2010; ISLAM *et al*., 2011), it is highly efficient in therapies for the nervous system (ROSENBERG *et al*., 1999; MORALES *et al*., 2008), especially those featuring neuropathic and chronic pain (MARCIL e*t al*., 2006; HAGEN *et al*., 2007; KAYSER *et al*., 2010). It is also highly effective in therapies for the muscle, circulatory and motor systems (HIRN *et al*., 2008; ZIMMER, 2010) and in treatments against several types of cancer (MORALES *et al*., 2008; BRAGADEESWARAN *et al*., 2010; GOMES *et al*., 2011; NIETO *et al*., 2012). Current research demonstrates TTX-producing organisms, its mechanism and studies on the therapeutic use of the toxin in medicine.

Toxins in animals

Toxins are compounds, produced by vegetal, animal, fungus or bacterial organisms, with a great variety of molecular forms. They may be classified according to their physical state, structure, chemical stability, poisoning potential and other biochemical mechanisms. When discussing animal-derived toxins, a significant difference exists between toxic and poisonous animals. Poisonous animals have special body structures for the synthesis of toxins and poison-inoculating structures. Contrastingly, toxic animals do not synthesize their own toxins. Toxicity lies either in their body as a whole or in specific body tissues which may be caused by secondary metabolites or acquired within the food chain. In this case, intoxication occurs, as a rule, by ingesting the toxic organism (GOMES *et al*., 2011).

Some organisms are potently toxic, as is the case of fish of the Tetraodontidea family in which TTX abounds. In fact, it is the most potent marine toxin in food poisoning cases (LEE e*t al*., 2000; BRAGADEESWARAN *et al*., 2010; ISLAM *et al*., 2011; RAMIRES *et al*., 2012). Isolated in 1950, TTX was divided into groups in 1964, as a poison that inhibits the nervous and muscular systems due to the blockage of sodium channels (NOGUCHI, ARAKAWA, 2008). Tetrodotoxin is an aminohydroxyquinazoline compound of complex molecular structure (Fig 1).

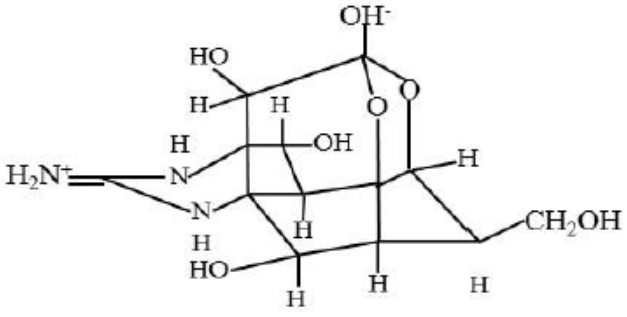

Fig. 1 - Molecular structure of tetrodotoxin(TTX). Adapted from Noguchi and Arakawa (2008).

TTX-producing organisms

Fish of the Tetraodontidae family, known as 'baiacu', 'fugu' or 'pufferfish', are perhaps the best known living beings in which TTX may be found. Some 120 species of pufferfish are known to exist worldwide, most of which are found in tropical and subtropical sea waters, including species living in rivers and lakes (SILVA *et al*., 2010). TTX may be found in the viscera, but mainly in the gonads, liver, spleen and intestines of the pufferfish which release the poison to defend themselves against predators (KRUMME *et al*., 2007; RAMIRES *et al*., 2012). TTX may also be detected in the skin (ISLAM *et al*., 2011), muscle and blood of some species (SILVA *et al*., 2010), with varying degrees of concentrations (SOBEL, PAINTER, 2005; NOGUCHI, ARAKAWA, 2008). However, it is in the Japanese and Chinese pufferfish of the genus Takifugu that TTX is found in great quantities (KHORA *et al*., 1991; NOGUCHI, ARAKAWA, 2008).

TTX is produced by bacteria that are part of the food chain of the organisms in which the toxin is found (SILVA *et al*., 2010). The bacteria *Vibrio alginolyticus*, *Shewanella alga*, *S. putrefaciens*, *Alteromonas tetraodonis* and others live in symbiosis or parasite several aquatic organisms (NOGUCHI, ARAKAWA, 2008). In other words, fish fed on non-toxic diet and without the producing bacteria do not demonstrate any toxicity (MATSUMOTO *et al*., 2010). Although TTX is largely found in fish of the Tetraodontidae family, the toxin may be found in other species such as arthropods, amphibians, mollusks, flatworms and others (PIRES JR *et al*., 2005; NOGUCHI, ARAKAWA, 2008; GOMES *et al*., 2011; ISLAM, *et al*., 2011). Some Species that host TTX are illustrated in the Fig 2 and listed in the table below.

Other forms of TTX

Other forms of the toxin, such as 4-epiTTX, 4.9-anhydroTTX (ISLAM *et al*., 2011) and 11-norTTX-(S)-ol from TTX degradation and metabolites, have also been identified. Tetrodonic acid found in the amphibian *Brachycephalus ephippium* with habitat in the Brazilian Atlantic Rainforest is also a related poison (PIRES *ET AL*., 2002). Further, *B. ephippium's* 11-oxoTTX is a toxin analogue which is four to five times more potent than TTX (PIRES *et al*., 2003). Toxins 6-epiTTX (YASUMOTO *et al*., 1988) and 11-norTTX-6(R)-ol have been isolated from tetraodotidiform fish (GOMES *et al*., 2011), whereas 11-oxotetrodotoxin is another tetrodotoxin analogue, albeit rare, detected in only two marine species, namely, the tetraodontiform *Arothron nigropunctatus*, and the crab *Atergatis floridus*. It has also been recently discovered in the salamander species *Notophthalmus viridescens* (GOMES *et al*., 2011).

Toxins 5-deoxiTTX and 11-deoxyTTX are approximately 1/20 and 1/100 times less toxic than TTX, respectively. On the other hand, 6-epiTTX is somewhat 10-20 times more potent than TTX (SHOJI *et al*., 2001; PIRES *et al*., 2003), whilst 5,6,11-trideoxyTTX, 4-anhydroTTX, 5-deoxyTTX, 4-ScysteinylTTX (4- CysTTX) and 1-hydroxy-5, 11-dideoxyTTX from the salamander *Taricha granulosa* are only slightly toxic (GOMES *et al*., 2011).

Table 1 - Species with TTX and the organ in which the toxin is found (MIYAZAWA *et al*., 1986; KHORA *et al*., 1991; HADDAD JR. 2003; SHIU *et al*., 2003; PIRES JR. *et al*., 2005; YOTSU-YAMASHITA *et al*., 2007; NOGUCHI, ARAKAWA, 2008; GOMES *et al*., 2011; ISLAM *et al*., 2011).

Animals	**Organs**
Fishes	
Pufferfish (Family *Tetraodontinae*)	ovaries, testes, liver, intestines and less frequently in the skin, muscle and/or blood
Pufferfish (Family *Diodontinae*)	ovaries, liver, intestines, skin and/or muscles
Pufferfish (Family *Ostracidae*)	ovaries, testes, liver, intestines, skin and/or muscles
Other living beings (species)	
Flatworms (*Planocera spp.*)	every body
Nemertinea (*Lineus fuscoviridis*, *Tubulanus punctatus* e *Cephalothrix linearis*)	every body
Mollusca Gastropoda (*Charonia sauliae*, *Babylonia japonica*, *Tutufa lissostoma*, *Zeuxis siquijorensis*, *Niotha clathrata*, *Natica lineata*, *Cymatium echo*, *Pugilina ternotoma*, *Polinices didyma* and other from genus *Conus*)	mainly in the digestive glands, and/or some species in whole body
Mollusca Cephalopoda (*Hapalochlaena maculosa*)	Mainly sallivary glands in adult
Annelida Polychaeta (*Pseudopolamilla occelata*)	Every body
Arthropoda (*Atergatis floridus*, *Zosimus aeneus* e *Carcinoscorpius Rotundicauda*)	Every body and eggs
Chaetognatha (*Parasagitta spp* e *Flaccisagitta spp.*)	Head
Echinodermata (*Astropecten spp.*)	Every body
Amphibia (*Taricha spp.*, *Notophthalmus spp.*, *Cynopsis spp.*, *Triturus spp.*, *Atelopus spp.*, *Colostethus sp.*, *Polypedates sp.*, *Brachycephalus ephippium*, *B. nodoterga* and *B. pernix*)	skin, ovaries, eggs, muscle, blood and/or liver

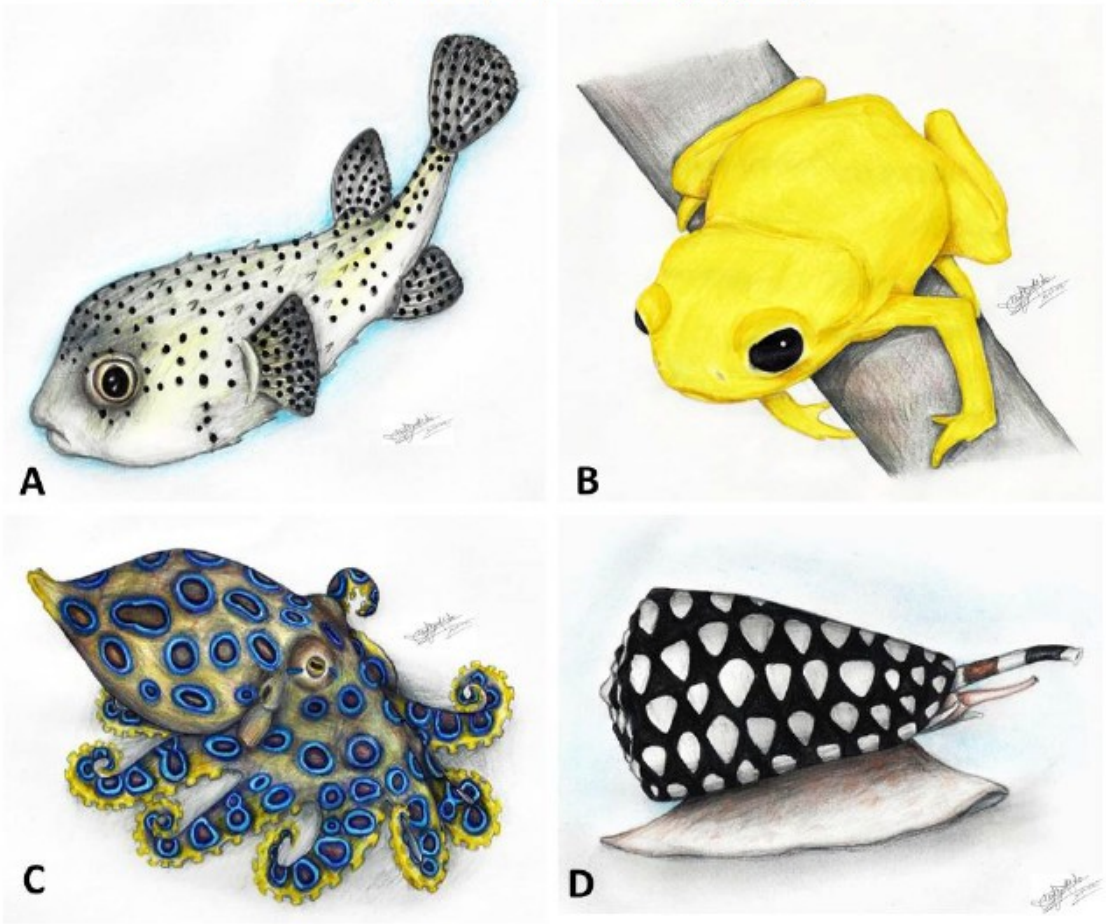

Fig. 2 - Illustrations from some species, which hosts TTX. A: *Chilomycterus reticulatus*. B: *Brachycephalus ephippium*. C: *Hapalochlaena maculosa*. D: *Conus marmoreus*.

TTX active mechanism

TTX works on voltage-gated sodium channels (VGSCs), which are proteins of the heteromeric membrane. They contain the main subunit α which doubles and forms selective poles of Na^+ through the plasmatic membrane. Subunit α is composed of six segments in α-helix structure repeated four times. The ion pathway is formed by a section between subunits 5 and 6 without any elliptical shape, called segment P. The fourth subunit comprises several groups of polar amino-acids directly linked to the voltage-sensitive channel, whereas groups in segment 6 are known for the metagenesis that contributes significantly towards local anesthesia linked to Na^+ channels (CATTERALL *et al*., 2007; NARAHASHI, 2008).

Alpha subunits are associated to unique transmembrane structures, very similar to the immunoglobulin called accessory subunit β. The subunit causes kinetics and modulation of the channel´s function. Besides being pore-forming factors, subunits α are link sites of anti-epileptic and anesthetic toxins bonded for channel blockage. In this case, TTX itself bonds at site 1 of the channel´s extracellular section and closes the pore (SOONG, VENKATESH, 2006; GOMES *et al*., 2011).

The discovery of a selective blocking action of TTX on VGSCs has been of immeasurable value for investigations of the properties and physiological roles of VGSCs, as well as their pharmacology (FARMER *et al*., 2008). TTX has potent and specific activity on the blockage of

VGSCs (GOMES *et al*., 2011) since it works selectively and without any effect on other systems of receptors and ion channels. However, the blocking activity is efficacious only when externally injected to the cell. It is unable to obstruct the channels when applied into the inside of the cell due to the guanidine group linked to the channel´s external aperture. Consequently, TTX´s mechanism is somewhat different from that of local anesthetics since these compounds block efficiently Na^+ and K^+ channels even when applied in the inside of the cell (NARAHASHI, 2008). Anesthetics are introduced within the cell in a neutral way and are converted into a cationic form within the cell´s interior (GOMES *et al*., 2011).

Voltage-gated sodium channels may be classified into TTX-resistant (TTX-R) and TTX-sensitive (TTX-S), according to their TTX sensitiveness. Currents in the former are slower than those in TTX-S, owing to their activation and de-activation kinetics (ULBRICHT, 2005; FARMER *et al*., 2008; NARAHASHI, 2008). If nociceptive primary afferent neurons contain significantly large quantities of TTX-R channels, there are channels which are sensitive to nanomolar TTX concentrations (KYLE, ILYIN, 2007; FARMER *et al*., 2008) or TTX-S channels. Consequently, within the context of TTX sensitiveness, in the brain, different VGSCs may be detected in damaged peripheral nerves, nociceptive neurons, thalamic neurons related to neuropathic pain, normal peripheral nerves, neuro-endocrine cells, skeleton muscles (HIRN *et al*., 2008) and heart (JO *et al*., 2004; GOMES *et al*., 2011).

Poisoning by TTX

TTX poisoning by ingesting fish of the Tetrodontidae family is a common type of intoxication in Japan, China, Bangladesh and other Asian countries (MAHMUD *et al*., 1999; NOGUCHI, ARAKAWA, 2008; ISLAM *et al*., 2011), even though other poisoning events have been reported in South Africa (NOGUCHI, EBESU, 2001), the United States (DEEDS *et al*., 2008) and Brazil (SANTANA-NETO *et al*., 2010; SILVA *et al*., 2010). Tiny amounts of TTX may cause a swift cruel death in animals and humans (BRAGADEESWARAN *et al*., 2010). Symptoms start with the paralysis of the mouth area (indicative of the accident), followed by muscle weakness, myalgia, dizziness, dysarthria, ataxia, difficulties in gait, visual disturbances and others. Worsening of neurological manifestations produces convulsions, breathlessness and heart-respiration stoppage which may occur within 24 hours. Gastro-intestine symptoms are characterized by nauseas, vomiting, bellyaches and diarrhea. Death occurs by muscular paralysis, respiratory depression and failure in the circulatory system (HADDAD JR *et al*., 2004; SANTANA-NETO *et al*., 2010; SILVA *et al*., 2010; ZIMMER, 2010; GOMES *et al*., 2011). Death by non-responsive bradycardia (total atrium-ventricular blockage) has also been reported in humans (HADDAD JR *et al*., 2004; SANTANA-NETO *et al*., 2010). The cause of death is practically always related to respiratory stoppage, although the heart is one of the organs that remain unchanged even in large toxin concentrations (ZIMMER, 2010).

Research related to the medicinal use of TTX

Nevertheless, TTX has proved to be useful in new therapies in different fields owing to its capacity of blocking voltage-gated Sodium channels. Recently the pharmacological activity of TTX is being extensively studied as a powerful therapeutic pharmacological agent (GOMES *et al*., 2011; NIETO *et al*., 2012). Studies on TTX application in research and in new techniques and its use as a toxin in therapies will be given below. Actually, TTX is highly effective in therapies against neuropathic and chronic pains and the toxin is applied in clinical studies with patients suffering from pains caused by several diseases (BHATTACHARYA *et al*., 2009). Besides being a painkiller and used in pain treatment, the toxin has been employed in reducing cancer metastasis. Since TTX blocks voltage-gated sodium channels, its effects have been investigated in therapies against pathologies involving the muscular and motor systems such as Duchenne muscular dystrophy, heart arrhythmia and arterial hypertension (GOMES *et al*., 2011).

In their analysis of TTX in heart and muscular system therapies, Jo *et al*. (2004) demonstrated its efficaciousness in the treatment of changes in the heart in the wake of ischemia and hypoxia which are characterized by a continuous electrical current caused by the opening of sodium channels of the muscle cells and which lead to arrhythmia and heart failure. The application of TTX as a hypertension agent has been highly successful through its direct action on the central nervous system, due to the drug´s capacity in crossing the hemato-encephalic barrier (ZIMMER, 2010; GOMES *et al*., 2011).

Moreover, the application of TTX to animal models in the wake of investigations on Duchenne muscular dystrophy with great concentrations of Ca^{2+} and Na^{+} and Na^{+}/K^{+}/ATPase in skeletal muscular fibers revealed itself beneficent in Na+ influx and avoided the death of cells caused by the dystrophy (HIRN *et al*., 2008).

TTX is effective in the treatment of mechanical or inflammatory lesions in therapies for the nervous system. The local administration of in vivo TTX prevents the loss of white matter in lesions of the spine marrow. TTX significantly decreases the loss of large diameter neurons and mitigates axoplasmatic pathology. In these cases, partially mediated by Na^{+}, the Ca^{2+} flows to the axon which starts a series of pathological events. The blockage of Na+ channels partially prevents lesions caused by Ca^{2+} influx (ROSENBERG *et al*., 1999).

Tam *et al*. (2002) demonstrated TTX efficiency in the blockage of axon ramification growth, or rather, the mechanism of motor loss compensation through an increase in the number of muscular fibers innervated by the same motor neuron caused by a decrease in innervations and skeletal muscular activity. The above characteristics may be found in poliomyelitis, Borne-poliomyelitis syndrome and amyotrophic lateral sclerosis in which the death of motor neurons is registered.

TTX may also be employed in cases of inflammatory, neuropathic and visceral pain in which voltage-dependent sodium channels are directly linked to the eruption of neuropathic pain (NIETO *et al*., 2012). However, highly relevant is the blockage of neuropathic pains in cases involving mechanical allodynia and thermal hyperalgesia (MARCIL *et al*., 2006). The changed expression of several TTX-S sodium channels occurs during pathological pain (inflammatory or neuropathic). Changes in gene regulation heads towards electro-physiological alterations which may have a leading role in the pathogenesis of pain (NIETO *et al*., 2012).

Even when associated with other drugs, Na+ blockers may introduce a new series of highly efficient treatments against several types of pain (KAYSER *et al*., 2010). Moreover, TTX is tolerant since some patients responded positively to minimum doses. On the other hand, response to the toxin was very efficient in patients suffering from somatic and visceral pain rather than from neuropathic pain (HAGEN *et al*., 2007; GOMES *et al*., 2011). Increase in the excitability of neurons is one of the mechanisms in the feeling of pain (CUMMINS *et al*., 2007). It is believed that TTX-R channels cause these impulses since they are expressed in the small neurons (LYU *et al*., 2000) with a lower caliber and transmit the large ones from the periphery to the central nervous system (CUMMINS *et al*., 2007; GOMES *et al*., 2011).

Several studies have also been undertaken with TTX in the therapy of cancer (GOMES *et al*., 2011) even though many authors underscore the need of further studies in this field (NIETO *et al*., 2012). TTX actually acts on the metastatic cells with more intensity and, as shown above, it is less aggressive against the other cells of the organism. Treatment with TTX becomes less invasive when compared to traditional methods such as chemotherapy (GOMES *et al*., 2011).

In several studies, TTX was applied at the root of the dorsal ganglia of rats and proved capable of decreasing mechanical allodynia (MARCIL *et al*., 2006) besides lessening the pain in patients with cancer (HAGEN *et al*., 2007). A study on the analgesic effect of TTX in patients with pain produced positive results in 50% of the patients during a long term treatment without any tolerance (HAGEN *et al*., 2011). Toxicity in these investigations is normally low and side effects are mainly sensorial and temporary, with dormancy or tingling around the mouth area (NIETO *et al*., 2012). The above authors suggest that further research is necessary on the use of TTX in mild and intense pain.

The ion channels are expressed in all cells and changes in their expression or activity which may be involved in several pathologies comprising heart arrhythmias, epilepsy, neuropathic pain and others (ONKAL, DJAMGOZ, 2009). A change in the VGSCs expression in cells has been reported in metastatic cancer cells. It is believed that the channels are closely related to the development of metastases by an increase in their aggressiveness (BENNET *et al*., 2004). The insufficiency of Na+ for cancer cells modifies their integrity and consequently the suppression of the cell´s invasion and spreading (BRAGADEESWARAN *et al*., 2010).

Conclusion

Current research shows that TTX, closely linked to food poisoning, is high efficient in therapies for the nervous system, especially those which feature chronic and neuropathic pain, in therapies related to the muscular, heart and motor systems, and in therapies against several types of cancer. Further studies may reveal other therapeutic employments of TTX related to voltage-dependent sodium channels due to their efficaciousness and specificity in the blocking of these channels.

ACKNOWLEDGMENTS

UEMASUL, UEMA, FAPEMA and FAPESP

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Recebido em 25/05/2018
Aceito em 26/06/2018