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SPIEZ LABORATORY

FACT SHEET

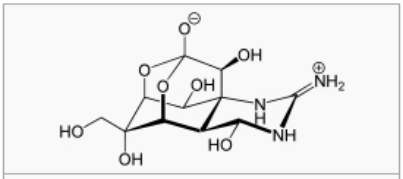
Tetrodotoxin (TTX)

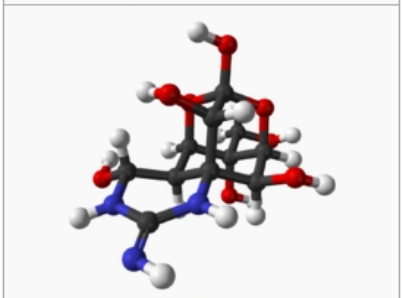
1. General information

Tetrodotoxin and its analogues (TTXs) are neutrotoxins widely common among marine and terrestrial organisms and best-known as the causative agent of pufferfish (Tetraodontidae) poisoning.
TTX is specified on the NATO-list of dangerous toxins. The Australian group includes TTX in one of the 19 toxins on their checklist. TTX is resistant to cooking and will not be destroyed by proteases in the gastrointestinal tract.
TTX poisonings are always life-threatening. Tetrodotoxin blocks the voltage-gated sodium channels. In neurological research Tetrodotoxin is often used as an inhibitor to selectively block sodium channels.

2. Chemical structure and properties

In 1964, the chemical structure of TTX was elucidated and the racemate was successfully synthesised for the first time in 1972. TTX and its derivatives are aminoperhydroxyquinazoline derivatives that exist as dipolar ions with a polarity comparable to Saxitoxin. However, TTX consists of only one positively charged guanidinium group and the cation is stabilised by resonance effect.
The TTX has a unique heterocyclic structure. According to SIMS et al. (1986) the molecule's structure is partly similar to morphine. The toxin is scarcely soluble in water but well soluble in diluted acids, e.g. 0.03 M acetic acid.





Names	
IUPAC name	(4*R*,4a*R*,5*R*,6*S*,7*S*,8*S*,8a*R*,10*S*,12*S*)-2-azaniumylidene-4,6,8,12-tetrahydroxy-6-(hydroxymethyl)-2,3,4,4a,5,6,7,8-octahydro-1*H*-8a,10-methano-5,7-(epoxymethanooxy)quinazolin-10-olate
Other names	anhydrotetrodotoxin, 4-epitetrodotoxin, tetrodonic acid, TTX
Identifiers	
CAS Number	4368-28-9 ✓
ChEBI	CHEBI:9506 ✗
ChEMBL	ChEMBL507974 ✗
ChemSpider	9349691 ✗
IUPHAR/BPS	2616
Jmol 3D image	Interactive graph
KEGG	C11692 ✗
PubChem	11174599
InChI	[show]
SMILES	[show]
Properties	
Chemical formula	$C_{11}H_{17}N_3O_8$
Molar mass	319.268

Fig. 1: Properties of TTX (Source: Wikipedia)

3. Toxicology

Tetrodotoxin inhibits the voltage-sensitive sodium channels of myelinated neurons. The blockage already occurs with low concentrations of 10-9 to 10-8 mol/l. The binding to the sodium channel receptor is reversible, as the TTX with its guanidine group imitates the sodium ion in its hydration shell.
As a consequence of TTX blockage the propagation of action potential ceases, and the neuronal and muscular information processing is impaired, resulting in sensorimotor paralysis.
The TTX's mechanism of action is comparable to that of Saxitoxin. The toxicity of the various TTX analogues varies significantly. 5-deoxyTTX, trideoxyTTX and anhydroTTX are non-toxic analogues, whereas TTX and its epi-forms are extremely toxic; LD50: 8-20 µg/kg when administered orally, and 8 µg/kg when administered intravenously. The toxin can be taken up via oral, inhalation or dermal routes. Tetrodotoxin is one of the most potent non-protein toxins and exceeded in toxicity by only few toxins, e.g. Maitotoxin.

Different sources list 0.5 – 1.5 mg as the lethal dose of TTX for an adult.

5. Analytics

The high toxicity of the TTX requires very low detection limits in analytical methods. Clear identification and precise quantification of TTX and TTXs are very important for food control and in clinical specimen in case of poisoning. Pharmaceutical studies involving these substances require sensitive and selective detection methods as well.

As for many other marine toxins, the mouse-bioassay is used as standard procedure to determine the toxicity in TTX. With this method, however, it is impossible to distinguish between PSP-toxin and TTX poisoning, as the effects of these groups of neurotoxins are similar. Both the lack of specificity and ethical concerns with respect to animal experiments have led to the development of chemical analytical methods.

Today, HPLC methods coupled with MS are therefore used for the detection of TTX and its analogues, as the combination of high separation performance and low detection limits allows clear quantification of TTXs. The analyses are mostly carried out on ZIC-HILIC® HPLC columns. The SPIEZ Laboratory has established the LC-MS method as a non-accredited procedure.

5. Literature / information

[1] Wikipedia; the free encyclopedia (http://en.wikipedia.org/wiki/Tetrodotoxin)

[2] M. Diener, Dissertation - Entwicklung von chromatographischen Analyseverfahren zur Bestimmung von Neurotoxinen aus Lebensmitteln mariner Herkunft; Friedrich- Schiller-Universität Jena, 2008.

[3] Daunderer - Klinische Toxikologie - 105. Ere.-Lfg. 4/96.

[4] E. Teuscher, U. Lindequist: Biogene Gifte; Wissenschaftliche Verlagsgesellschaft Stuttgart; 2010.

[5] L. M. Botana: Seafood and freshwater toxins; CRC Press, Taylor & Francis Group, 2014.

[6] Chung-Him Yu: Rapid screening of Tetrodotoxin in urine and plasma of patients with puffer fish poisoning by HPLC with creatinine correction; Food Additives and Contami-nants; Vol. 27, N0. 1, January 2010, 89-96.

[7] Universität Jena: LC-MS-Bestimmung von Tetrodotoxin (TTX) aus Fischen; Versuchsanleitung; Hydrophilic Interaction Chromatography (HILIC) sowie MS-Bestimmung von Tetrodotoxin und seiner Analoga. (https://www.uni-jena.de/unijenamedia/-p-36938.pdf?rewrite_engine=id).

[8] Bonnie Mei-Wah Fonga et al.: Development and validation of a high-throughput double solid phase extraction–liquid chromatography–tandem mass spectrometry method for the determination of tetrodotoxin in human urine and plasma; Talanta 83 (2011) 1030-1036.

[9] Margaret A. O'Leary et al.: Use of high performance liquid chromatography to measure Tetrodotoxin in serum and urine of poisoned patients; Toxicon 44 (2004) 549-553.

[10] M. Schär: Untersuchungen zur Trennung von polaren CWÜ-relevanten Verbindungen mit Hydrophilic Interaction Chromatography (HILIC) und dem LC/MS System Agilent 1200-AB/MDS Sciex 3200QTrap, LABOR SPIEZ, Laborbericht LS 2012-05.

[11] I.Putzier, S. Frings: Tiergifte in der biomedizinischen Forschung; Vom Jagdgift zur neuen Schmerztherapie; Biologie in unserer Zeit, 32. Jahrgang 2002, Nr. 3.

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