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Immune effects of the neurotoxins ciguatoxins and brevetoxins

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ABSTRACT

Ciguatoxins (CTXs) and brevetoxins (PbTxs) are phycotoxins that can accumulate along the marine food chain and thus cause seafood poisoning in humans, namely “ciguatera fish poisoning” (CFP) and “neurotoxic shellfish poisoning” (NSP), respectively. CFP is characterized by early gastrointestinal symptoms and typical sensory disorders (paraesthesia, pain, pruritus and cold dysaesthesia), which can persist several weeks and, in some cases, several months or years. NSP is considered a mild form of CFP with similar but less severe symptoms. After inhaled exposure, PbTxs can also cause respiratory tract irritation in healthy subjects and asthma exacerbations in predisposed subjects, whose respiratory functions may be disrupted for several days following PbTx inhalation. Mechanistically, it is well established that CTX- or PbTx-induced disturbances are primarily mainly due to voltage-gated sodium channel activation in sensory and motor peripheral nervous system. However, little is known about the pathophysiology or a potential individual susceptibility to long lasting effects of CFP/NSP. In addition to their action on the nervous system, PbTxs and CTXs were also shown to exert effects on the immune system. However, their role in the pathophysiology of syndromes induced by CTX or PbTx exposure is poorly documented. The aim of this review is to inventory the literature thus far on the inflammatory and immune effects of PbTxs and CTXs.

1. Ciguatoxins (CTXs) and brevetoxins (PbTxs): phycotoxins of potential human health concern through the activation of voltage-dependent sodium channels

CTXs and PbTxs are two classes of lipid-soluble cyclic polyether phycotoxins that are mainly produced by the dinoflagellates *Gambierdiscus toxicus* and *Karenia brevis*, respectively (Chinain et al., 1999; Lewis and Molgo, 2000; McFarren et al., 1965). Both toxins have analogous chemical structures and biological activities. Moreover, CTXs and PbTxs share the same major primary target, namely the voltage-dependent sodium channel (Na_v; Dechraoui et al., 1999). In humans, both toxins cause characteristic syndromes described below. The oral route is the main route of CTX exposure, whereas PbTx exposure can occur through both the oral and inhaled routes.

Ciguatera fish poisoning (CFP), with an estimated annual incidence of 10 000 to 50 000 cases worldwide, is the most frequently reported seafood-induced syndrome caused by contaminated fish consumption (Lehane and Lewis, 2000; Friedman et al., 2008). Endemic areas comprise tropical and subtropical regions of the Pacific and Indian Oceans and the Caribbean Sea; however, there is a growing number of cases in more temperate zones (Gingold et al., 2014; Lehane and Lewis, 2000). Typical ciguatera syndrome includes early gastrointestinal symptoms (diarrhoea, vomiting, nausea, and abdominal pain) and sensory disorders whose combination following a fish meal is pathognomonic: perioral and acral paraesthesia, painful sensations on contact with cold (cold dysaesthesia) and pruritus, possibly associated with pains including headaches, myalgia, dental pain and arthralgia. Autonomic (sweating, salivation and lacrimation), generalized (fatigue and weakness) and, in the worst cases, cardiovascular disturbances can also occur (Glaziou and Legrand, 1994; Lewis, 2001). Gastrointestinal and neurological symptoms generally appear within hours after the toxic meal, although the severity may vary depending on the ingested toxin dose but also on a prior exposure which leads to increase the severity (Chateau-Degat et al., 2007). Only rare cases of intoxication lead to death (Lehane and Lewis, 2000). Symptoms are usually alleviated after several weeks but may reoccur or last several months or years after the toxic meal. Persistent or relapsing

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symptoms consist especially of sensory disturbances including distal paraesthesia, cold dysaesthesia, pruritus, arthralgia and myalgia associated with generalized weakness (Bagnis and Legrand, 1987; Calvert et al., 1987; Chateau-Degat et al., 2007; Derouiche et al., 2000). Although symptom recurrence or worsening is clearly associated with the consumption of alcohol, peanut or non-contaminated fish meat (Lewis, 2001; Vucic and Kiernan, 2008; Friedman et al., 2008), little is known about the pathophysiology or a potential individual susceptibility to this phenomenon (Glaziou and Martin, 1993; Katz et al., 1993).

"Harmful algal blooms" (HABs) of the main producer of PbTxs, *Karenia brevis*, (red tides) occur with an increasing frequency and cause both public and environmental health issues, especially in New Zealand waters and in the Gulf of Mexico where the Texas coast and East coast of Florida are commonly affected (FAO, 2004; Glibert et al., 2005; Steidinger, 2009). Human oral exposure to PbTxs through contaminated bivalve shellfish can trigger « neurotoxic shellfish poisoning » (NSP). Clinically, NSP is considered to be a mild form of CFP with similar although less severe gastrointestinal, neurological and cardiovascular disturbances. Effective risk management of contaminated shellfish, by monitoring toxin levels in shellfish during HAB events, maintains a low NSP incidence (FAO, 2004). In contrast, inhaled exposure to aerosolized PbTxs is less effectively prevented and occurs in humans during recreational or occupational activities on coastal regions affected by red tides. Reported symptoms, including upper respiratory disorders (eye irritation, nasal congestion, non-productive cough and throat irritation), occur in healthy humans from an exposure to moderate levels (up to 36 ng PbTx/m^3 air) of aerosolized PbTxs for less than 1 h, with reported frequencies increasing with exposure duration (recreational versus occupational exposure; Backer et al., 2003, 2005). Rhinorrhoea, sneezing and lacrimation have also been reported (Asai et al., 1982). Anatomical localization of such symptoms suggests a deposition of inhaled PbTxs in the upper airways, which is in accordance with the predominant coarse particle size measured for red tide aerosols (Cheng et al., 2010). Lower respiratory (asthma-like) symptoms (wheeze, shortness of breath, and chest tightness) reported during exposure to higher levels may reflect the deposition of sufficient fine particles in the lower respiratory tract and subsequent irritant effects. In healthy subjects, these transient disturbances are rapidly reversed by leaving the beach area, and do not impair functional respiratory tests. However, in asthmatic subjects, these disturbances cause decreased respiratory function and exacerbations of asthma (wheeze, chest tightness, irritation, cough and shortness of breath) which may persist for several days (Asai et al., 1982; Fleming et al., 2005, 2007, 2009; Steensma, 2007; Kirkpatrick et al., 2011). At a mechanistic level, bronchoconstriction elicited by PbTxs in healthy subjects results from an indirect action on airway smooth muscle. Indeed, airway smooth muscle contraction has been shown to be mediated by acetylcholine that is released from PbTx-activated parasympathetic postganglionic nerve terminals (Asai et al., 1982; Baden et al., 1982; Shimoda et al., 1988; Richards et al., 1990; Richards and Bourgeois, 2010). However, in contrast to healthy tissue, airway smooth muscle cells from asthmatic subjects were directly depolarized by a PbTx extract and, surprisingly, that effect was not inhibited by tetrodotoxin, *i.e.*, did not depend on Na_v activation (Richards and Bourgeois, 2010). Taken together, these data demonstrate a substantial human health risk associated with inhaled PbTxs, especially in subjects with pre-existing airway disease, while pathophysiological mechanisms remain incompletely known. In addition, despite the regularity of *Karenia brevis* blooms along the coasts of Florida and Texas, little is known about the effects of chronic exposure to inhaled PbTxs.

In addition to humans, wildlife is commonly exposed to aerosolized and/or ingested PbTxs during Florida and Texas red tides. It represents therefore not only a good sentinel for human health but also a source of *in vivo* data for human risk assessment (FAO, 2004) and pathophysiological studies. Indeed, in contrast to human cases, exposed wildlife allows extensive tissue examination of afflicted or dead animals. Thus, reports from manatees (Bossart et al., 1998), cormorants (Kreuder et al., 2002), turtles (Perrault et al., 2014, 2016; Walsh et al., 2010), dolphins (Schwacke et al., 2010) and coyotes (Castle et al., 2013) during HABs of *Karenia brevis* are informative data for studying pathophysiology of PbTx effects. In addition, data from rats (Benson et al., 2004a, 2004b, 2005) and sheep (Abraham et al., 2005a, 2005b; Zaias et al., 2011) experimentally exposed to inhaled PbTxs are available. Among those data, significant immune effects were reported.

PbTxs and CTXs bind to a common site located within the S6 transmembrane segment in domain I and the S5 segment in domain IV of the Na_v α-subunit (commonly named neurotoxin binding site 5; see for details Deuis et al., 2017), albeit with variable affinity (Dechraoui et al., 1999; Lombet et al., 1987). The major CTX isolated from Pacific fish P-CTX-1 (or CTX-1B), which binds with the highest affinity to this site, is the most potent CTX both *in vivo* and *in vitro*. Among the PbTxs, PbTx-1 is the most potent *in vivo* and *in vitro* and binds with the highest affinity to this site. Potency comparison between toxins (see Table 1 for potency parameters) indicates that P-CTX-1 is approximately 1000 times more potent than PbTx-1, which is slightly more potent than PbTx-2 and PbTx-3, two of the major congeners produced by *Karenia brevis*. Interestingly, both PbTx and CTX parameters that quantify relative affinities for Na_v, as well as Na^+-induced cytotoxicity and mouse intraperitoneal toxicity potencies are not perfectly correlated, suggesting possible additional cellular and *in vivo* toxicity pathways besides Na_v activation (Dechraoui et al., 1999).

Mechanistically, it is well established that human symptoms induced by exposure to PbTxs or CTXs are primarily mainly due to the activation of Na_v on sensory and autonomic nerves (Ramsdell,

Table 1
Potency parameters for P-CTX-1 (CTX-1B), PbTx-1, PbTx-2, and PbTx-3. From Dechraoui et al., 1999 (1); Inserra et al., 2017 (2); Lewis et al., 1991 (3); Gawley et al., 1995 (4); Baden and Mende, 1982 (5).

	Affinity to Na_v "site 5" [K_i or IC_{50} in competitive $^{[3H]}$PbTx-3 (1 nM or 1.8 nM, respectively) radioligand binding assay in rat brain synaptosomes]	Sodium-dependent cytotoxicity (ED_{50} in mouse neuroblastoma cell Neuro-2A assay)	Acute toxicity in mice (LD_{50} expressed in μg/kg after intraperitoneal injection)
P-CTX-1	$K_i = 41$ pM (1) with functional potency in Na_v subtype 1.8 > 1.3 > 1.1 > 1.2 > 1.5 > 1.7 > 1.6 > 1.4 (2)	$ED_{50} = 11.3$ pM (1)	$LD_{50} = 0.25–0.33$ (1; 3)
PbTx-1	$K_i = 2$ nM (1); $IC_{50} = 4.4$ nM (4)	$ED_{50} = 5.8$ nM (1)	$LD_{50} > 100$ (1)
PbTx-2	$IC_{50} = 15.02$ nM (4)		$LD_{50} = 200$ (5)
PbTx-3	$K_i = 2.24$ nM (1); $IC_{50} = 8.48$ nM (4)	$ED_{50} = 15$ nM (1)	$LD_{50} > 200$ (1); $LD_{50} = 170$ (5)

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