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Pacific ciguatoxin 1B-induced modulation of inflammatory mediators in a murine macrophage cell line

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ABSTRACT

Ciguatoxins, potent marine neurotoxins responsible for ciguatera, exert their numerous damaging effects through primary binding to the voltage-sensitive sodium channels of excitable cells. Using RAW 264.7 murine macrophages, we report the first experimental study presenting evidence that P-CTX-1B (the most potent congener from the Pacific) could modulate mRNA expression of pro- and anti-inflammatory cytokines as well as of inducible nitric oxide synthase (iNOS). P-CTX-1B, unlike other less potent marine polyether toxins, P-CTX-3C and PbTx-3, induced the overexpression of interleukin (IL)-1β, IL-6, IL-10, tumor necrosis factor-α and iNOS with different magnitude and kinetic profiles, as compared to bacterial lipopolysaccharide (LPS). Unlike LPS, P-CTX-1B did not modulate IL-11 expression. In this report, we provide new evidence of the P-CTX-1B iNOS- and cytokines-inducing ability and shed new light on host response to potent neurotoxins.

1. Introduction

Among the worldwide existing seafood poisonings, Ciguatera Fish Poisoning (CFP) is one of the most common forms of ichtyosarcotoxism in the tropical areas especially in the Pacific, Indian and Caribbean regions (Glaziou and Legrand, 1994; Lewis, 2001). Nevertheless, CFP is no longer restricted to these areas due to the development of international tourism (Crump et al., 1999; de Haro et al., 2003), the increase of worldwide exports of coral fishes (Wong et al., 2005) and the global warming favouring its extent (Chateau-Degat et al., 2005). As a consequence, its prevalence is nowadays estimated to exceed 50,000 cases per year (Van Dolah, 2000; Lehane and Lewis, 2000). Even if its mortality rate is low, CFP results in variable combinations of gastrointestinal, neurological and cardiovascular manifestations leading to an elevated morbidity and important social and economic impacts (Lehane and Lewis, 2000).

The marine neurotoxins named ciguatoxins (CTXs) are responsible for CFP and belong to a class of lipophilic thermostable polyether compounds produced by certain strains of benthic dinoflagellates of the genus *Gambierdiscus* (Bagnis et al., 1980). These toxins are transferred up the marine food chain from these microalgae to herbivorous and to carnivorous coral fishes, and undergo an oxidation process concomitant with an increase in toxicity up the chain (Lewis and Holmes, 1993). Among the Pacific CTXs, P-CTX-1B is the most potent congener extracted from carnivorous specimens (Murata et al., 1989) and causes CFP at level as low as 0.1 μg/kg of fish flesh (Lewis, 1994). On the other hand, P-CTX-3C, the only congener found throughout the trophic chain, i.e. from the dinoflagellate *Gambierdiscus* spp. to carnivorous fish (Legrand et al., 1992; Yasumoto et al., 2000) exerts less damaging effects (Dechraoui et al.,

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1999; Darius et al., 2007). All CTXs possess a ladder-shaped polyether framework reminiscent of the brevetoxins (PbTxs), another family of marine neurotoxins produced by the dinoflagellate *Karenia brevis* (formerly *Gymnodinium breve*).

Due to their high affinity binding to receptor site 5 of voltage-sensitive sodium channels (VSSC), both PbTxs and CTXs have been extensively used in the past as neuropharmacological tools (Bidard et al., 1984; Lombet et al., 1987). The consequent increase of intracellular Na^+ leads to several phenomena, most notably the enhancement of nerve membrane excitability and the cell swelling process which can be both inhibited by tetrodotoxin (Benoit et al., 1996; Mattei et al., 1999). In addition, CTXs induce the selective activation of L-type Ca^{2+} channels and blockade of voltage-gated K^+ channels (Sauviat et al., 2006; Nicholson and Lewis, 2006). However, the multifaceted pathophysiology of CFP cannot be solely explained by the blockade of VSSC or the indirect action of CTXs on ion channels.

Patients suffering from CFP display a complex combination of a few to more than 30 symptoms with gastrointestinal manifestations such as diarrhoea, abdominal pain, vomiting and nausea which develop within the few hours of ingestion of contaminated fishes and usually resolve within few days. Though rarely fatal, cardiovascular symptoms are observed in acute cases with severe bradycardia, hypotension, respiratory difficulties and arrhythmias (Bagnis et al., 1979; Gillespie et al., 1986). The neurological disturbances include paresthesia, ataxia, dysesthesia, arthralgia, myalgia and pruritus, which become prominent after the gastrointestinal disorders. Some of these manifestations, especially pruritus, paresthesia in the extremities and arthralgia, in addition to neuropsychiatric signs such as generalized fatigue, malaise, depression or irritability have been reported to last for weeks or months (Friedman et al., 2008). The chronicity of these symptoms evokes the chronic fatigue syndrome (CFS) which occurs as a secondary state of CFP (Gillespie et al., 1986; Racciatti et al., 2001; Chateau-Degat et al., 2007).

It is noteworthy that some reports have suggested that CFS results from immunologic abnormalities (Klimas et al., 1990; Natelson et al., 2002) and that this illness is mediated by overproduction of inflammatory messengers such as nitric oxide (NO), circulating cytokines, C-reactive protein and β-microglobulin (Buchwald et al., 1997; Gupta et al., 1997; Cannon et al., 1999). Also well documented is the phenomenon of sensitization, recalling an allergy which is observed in previously intoxicated persons who suffer a recurrence of typical CFP symptoms after eating fish which do not produce symptoms in other persons or after consumption of non CFP-associated food like alcohol or nuts (Gillespie et al., 1986; Ruff and Lewis, 1994; Lewis, 2001; Friedman et al., 2008). In parallel, few cases of polymyositis have also been reported after CTXs exposure (Stommel et al., 1991, 1993), demonstrating inflammatory demyelinating diseases. Further observations concern extremely severe cases of CFP in which physicians have suspected an inflammatory neuropathy called the Guillain–Barré syndrome (GBS) (Angibaud and Rambaud, 1998; Gatti et al., 2008).

Based on the inflammatory nature of CFP symptoms collated from the literature, we have previously demonstrated the implication of nitric oxide (NO) in CFP through the dose- and time-dependant modulation of inducible NO synthase (iNOS) mRNA expression in RAW 264.7 murine macrophages treated with P-CTX-1B (Kumar-Roiné et al., 2008). In the present report, we have further explored the involvement of the inflammatory system in CFP by studying the modulation of cytokines expression upon P-CTX-1B treatment in the RAW 264.7 cell line. mRNA expression levels of cytokines and iNOS were measured by quantitative Polymerase Chain Reaction (qPCR) and the effects of P-CTX-1B were compared to a well-known inflammatory inducer, the bacterial lipopolysaccharide (LPS). The effects of less potent toxins, P-CTX-3C and PbTx-3, on selected cytokines and iNOS expression were also assessed to provide a potential link between toxins potency and inflammatory response.

Although CFP is the most frequently reported seafood poisoning in the world, until now few studies have investigated its immunological aspects. In this report, we provide new evidence of the possible implication of the inflammatory system in its complex pathophysiology.

2. Materials and methods

2.1. Materials

The murine macrophage RAW 264.7 cell line (TIB-71) was generously provided by Dr. M. Adib-Conquy (Institut Pasteur, Paris, France). P-CTX-1B and P-CTX-3C were purified at the Institut Louis Malardé (Tahiti, French Polynesia) from *Gymnothorax javanicus* liver and from cultures of the dinoflagellate *Gambierdiscus polynesiensis*, respectively, as previously described by Legrand et al. (1989). PbTx-3 was obtained from Latoxan (Valence, France). LPS (*Escherichia coli*, 0111:B4), thiazolyl blue tetrazolium bromide (3-(4′,5′-dimethylthiazol-2′-yl)-2,5-diphenyltetrazolium bromide; MTT) and reagents used for cell culture were obtained from Sigma Aldrich (Lyon, France), while those used for qPCR were purchased from Roche Applied Science (Auckland, New Zealand), unless otherwise stated.

2.2. Cell cultures

RAW 264.7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 25 mM HEPES buffer, 4.5 g/l D-glucose, 0.2% sodium bicarbonate, 1 mM sodium pyruvate and 2 mM L-glutamine, and supplemented with 10% fetal bovine serum, 1% of antibiotic and antifungal solution (penicillin, 10,000 U; streptomycin, 10 μg/ml; amphotericin B, 25 μg/ml). Cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2. RAW 264.7 cells were seeded at 5.0×10^4 cells/well in 96-well plates and at 2.5×10^5 cells/well in 24-well plates, respectively, for cytotoxicity and mRNA quantification assays.

2.3. Cytotoxicity assay

The effect of LPS and P-CTX-1B on cell viability was determined by MTT assay according to previous methods