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Short communication

Use of monoclonal antibodies as an effective strategy for treatment of ciguatera poisoning

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ABSTRACT

Ciguatera is a global food poisoning caused by the consumption of fish that have accumulated sodium channel activator toxins, ciguatoxins. At present, most diagnosed cases of ciguatera are treated with symptomatic and supportive remedies, and no specific therapy has been devised. Here we report that ciguatoxin CTX3C can be effectively neutralized in vitro and in vivo by simultaneous use of two anti-ciguatoxin monoclonal antibodies, providing the first rational approach toward directly preventing and treating ciguatera.

Ciguatera seafood poisoning is the most significant form of fish toxicoses in terms of the number and severity of poisoning events. The poisoning occurs after eating intoxicated tropical and subtropical fish (Lewis, 2006; Nicholson and Lewis, 2006). Ciguatoxins [e.g. CTX3C (**1**) (Satake et al., 1993), Fig. 1], the principal causative agents, are biologically synthesized by an epiphytic dinoflagellate, *Gambierdiscus toxicus*, and transferred to fish of more than 100 species through the aquatic food chain, at the end of which humans are exposed. Ingestion of ciguatoxic fish leads to gastrointestinal, cardiovascular and neurological disorders. Gastrointestinal symptoms often last for several days, while the neurological symptoms may persist from several weeks to several years. In severe cases, death may be caused by respiratory failure due to paralysis of the respiratory musculature or complete cardiac failure. As high as 50,000 cases annually have been estimated as worldwide ciguatera incidence, with most cases reported from the region of the Pacific Ocean, Indian Ocean and the Caribbean. Alarmingly, it is fast becoming a global health issue as the advancement of rapid cold transport and shipping technologies has enabled the increased fish export to other regions, where ciguatera poisoning is under-recognized. In addition, global warming could further increase the frequency of poisoning events worldwide, since it extends the region favorable to *G. toxicus* proliferation.

Ciguatoxins bind to voltage-sensitive sodium channels in nerves, heart and muscle, and open the channels at resting membrane potential, resulting in neural hyperexcitability. The potent toxicity of ciguatoxins is attributable to their extremely high affinity toward the target proteins (**1**: $K_D = 81$ pM) (Lombet et al., 1987; Dechraoui et al., 1999; Strachan et al., 1999) and subsequent multimodal functional modulation (Yamaoka et al., 2004). Despite recent advances in understanding of the pharmacological properties of ciguatoxins, no direct treatment for the ciguatera poisoning has been devised based on its molecular

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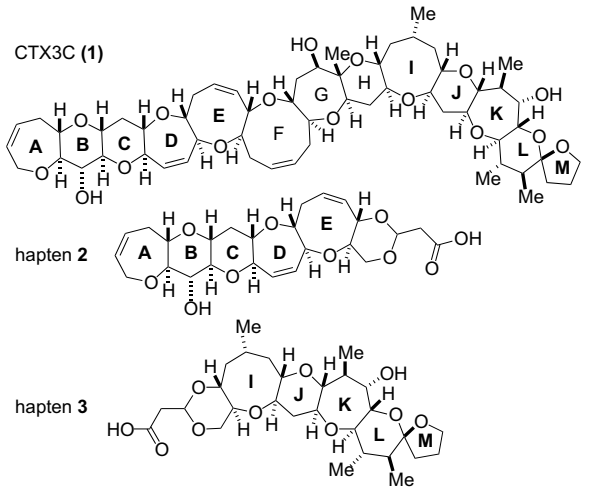

Fig. 1. Structures of ciguatoxin CTX3C (**1**) and synthetic haptens (**2**, **3**) used to elicit specific anti-CTX3C antibodies. The rings that corresponds to the left- and right-hand epitopes are indicated in bold face.

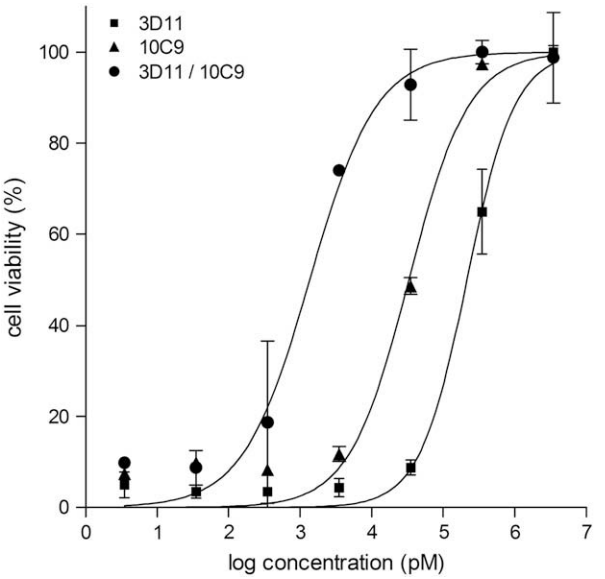

Fig. 2. In vitro toxin neutralization of Neuro-2A cells by the anti-CTX3C monoclonal antibodies. Antibodies were administered before challenge with CTX3C. After 24 h of CTX3C challenge, the survival of Neuro-2A was determined by an XTT assay. The results are expressed as percentages of viable cells, and the IC_{50}s of the antibodies was deduced from the graph.

mechanism of action. Treatment remains primarily symptomatic and supportive, and includes gastric lavage for removal of unabsorbed toxins, opiates for pain, oxygen and ventilatory assistance, cold showers and antihistamines for pruritus and antidiarrhoeal and antiemetic agents. The most widely used treatment is intravenous D-mannitol, following several reports indicating its ability to improve neurological status when administered early in the illness (Palafox et al., 1988). However, a recent double-blind randomized study revealed neither clinical nor biochemical benefits of D-mannitol compared with normal saline solution (Schnorf et al., 2002).

Direct targeting and scavenging of ciguatoxin in theory can be achieved by immunity of the toxin exposed individual, and could provide a new strategy toward preventing ciguatoxins-induced illness. Since vaccines require time to induce protective immunity and depend on the host's ability to mount an immune response, the only practical method of providing immediate immunity against ciguatoxins is passive immunotherapy, using neutralizing antibodies. Importantly, neutralizing antibodies can confer protection regardless of the immune status of the host.

We recently achieved the first total synthesis of ciguatoxin CTX3C (**1**) (Hirama et al., 2001; Inoue et al., 2004). The synthetic route uniquely allowed us to synthesize non-toxic haptens to prepare monoclonal antibodies (mAbs) specific to ciguatoxins (Oguri et al., 2003). We report here that ciguatoxin CTX3C can be potently neutralized in vitro and in vivo by combining two CTX3C-specific mAbs, paving the way to rational design of drugs for treating ciguatera.

As previously reported, compounds **2** and **3**, which represent the left and right half substructures of CTX3C (**1**) were used to prepare the specific anti-CTX3C mAbs, 10C9 and 3D11, respectively (Fig. 1). Accordingly, 10C9 binds to the right half of **1** with a dissociation constant (K_D) of 2.8 nM, while 3D11 has high affinity for the left half of **1** ($K_D = 122$ nM). It is important to note that these two antibodies are able to capture **1** simultaneously from both ends of the molecule, because of their non-overlapping epitopes.

In vitro neutralization of toxicity by the anti-CTX3C antibodies was evaluated using mouse neuroblastoma Neuro-2A cells, which express voltage-sensitive sodium channels on their membrane, with an XTT assay (Scudiero et al., 1988; Manger et al., 1993). CTX3C is highly cytotoxic against Neuro-2A, and the concentration of it yielding 50% cell viability (EC_{50}) was determined to be 28.1 pM, while both 10C9 and 3D11 were confirmed to be non-toxic up to 3.5 μM. When Neuro-2A was incubated with either 10C9 or 3D11 prior to addition of 200 pM CTX3C, which corresponds to 7-fold the EC_{50}, CTX3C-induced toxicity was neutralized in concentration-dependent fashion (Fig. 2). The 50% inhibitory concentrations (IC_{50}) of 10C9 and 3D11 were determined to be 33.2 nM and 215 nM, respectively. The higher neutralizing activity of 10C9 than of 3D11 appears to reflect its lower dissociation constant for CTX3C. Most importantly, significant synergy in toxin neutralization was observed, when an equimolar mixture of 10C9 and 3D11 was tested. The IC_{50} value of the 10C9/3D11 mixture was found to be 1.36 nM. The pair of mAbs was thus 24-fold and 158-fold more effective as an antitoxin than 10C9 and 3D11, respectively, alone and only 7 equivalents of the two mAbs to **1** were required for 50% neutralization of cytotoxicity.

Next, we applied the pair of mAbs to an in vivo mouse acute toxicity assay. The 50% lethal dose (LD_{50}) of **1** in mice (i.p.) was 1.3 μg/kg, with a mean survival time of 12 h. To evaluate in vivo neutralization of toxicity, various concentrations of the mixture of the two antibodies (3D11:10C9 = 1:1) were injected into six mice before injection of a dose of **1** 15 times the LD_{50} (15 LD_{50}s = 20 μg/kg). Ciguatoxic signs, time to death, and number of surviving mice were then determined over 24 h. When a dose equivalent to 15 LD_{50}s of CTX3C was administered