CIGUATERA IN FRENCH POLYNESIA

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Résumé : *la ciguatéra est une intoxication alimentaire par la consommation de poissons de récif coralliens. Chaque année, en Polynésie française, environ 800 cas d'intoxication font l'objet de déclarations médicales. Le terme "ciguatéra" est réservé aux empoisonnements par poisson exception faite des intoxications de type histaminique et des intoxications par tétrodotoxine ou par palytoxine. La ciguatéra est considérée comme un problème de santé publique indéniable. Il est aussi et surtout préoccupant à cause de ses répercussions économiques. En effet, l'impact le plus important est lié à son incidence sur le développement de la pêche.*

L'origine du phénomène a été associée à la prolifération dans certaines conditions écologiques d'une algue microscopique, le dinoflagellé toxique Gambierdiscus toxicus, *Adachi et Fukuyo, et à la transmission de la toxicité aux poissons herbivores et à leurs prédateurs carnivores ichtyophages.*

Deux familles de toxines sont impliquées, l'une liposoluble composée de la ciguatoxine et des toxines qui lui sont apparentées, l'autre hydrosoluble composée de la maitotoxine et de ses analogues éventuels. Une vingtaine de toxines a été isolée à l'état pur, deux seulement ont été complètement caractérisées par leur structure moléculaire. Les données pharmacologiques indiquent que les ciguatoxines font partie des toxines marines les plus puissantes. Elles agissent de façon spécifique sur les canaux sodiques des membranes excitables.

Abstract : *ciguatera is a seafood poisoning provoqued by ingestion of tropical reef fish. Every year, about 800 clinically documented cases of ciguatera fish poisoning are registered in French Polynesia. The name "ciguatera" is used to qualify the kind of seafood poisoning distinct from histaminic poisonings, tetrodotoxin poisonings or palytoxin poisonings. It is considered as an appreciable public health problem and, most of all, as having a high economical incidence. The most important impact is connected with the effects on fishing resource development for local consumption and for export.*

The origin of the phenomena has been associated with the toxic dinoflagellate Gambierdiscus toxicus, *Adachi and Fukuyo, and the transmission of the toxins through a marin food chain.*

Two groups of toxic compounds have been isolated, one is liposoluble including ciguatoxin and related compounds, the other one is watersoluble including maitotoxin. More than twenty pure toxic fractions have been identified, but molecular structure was determined only for two of them. Pharmacological studies indicate that ciguatoxins are very potent compounds acting on the sodium channels of excitable membranes. The question of the chemical or immunochemical dosage of the ciguatoxins in fish muscle is discussed. The difficulty is dependent on the very low concentration (a few nanograms per one hundred grams) of so potent molecules.

Introduction

Every year about 800 clinically documented cases of ciguatera fish poisoning caused by consumption of certain marine reef fishes are registered in French Polynesia. The name "ciguatera" is used to qualify the kind of seafood poisoning distinct from histaminic poisonings, tetrodotoxin poisonings (associated with the pufferfish) or palytoxin poisonings (associated with Balistidae). Even through ciguatera fish poisoning is a notifiable disease, morbidity statistics are unreliable due to the tendency of many individuals not to take medical advice when not severely affected. Actually, annual incidence value may be scaled up at least by a

factor of 2. Although rarely fatal and not always severe, ciguatera fish poisoning is an extremely debilitating disease including gastrointestinal, neurological and eventually cardiovascular distress. It is considered as an appreciable public health problem in French Polynesia and, most of all, as having a high economical incidence. The most important impact of ciguatera is connected with its effects on fishing resource development for local consumption and for export.

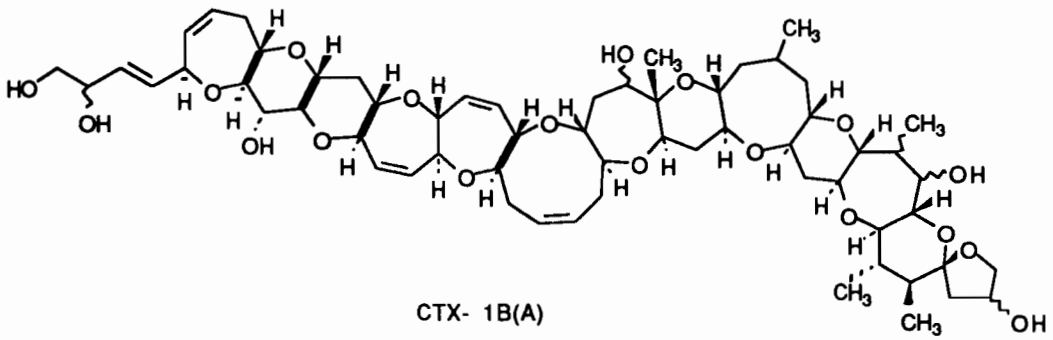
The cause of ciguatera poisoning is a group of lipid-soluble, heat-resistant toxins, the ciguatoxins. A tentative screening of these toxins is in process in our laboratory using various fish and algal toxic materials collected in the endemic areas.

The origin of the toxicity and the food chain theory

Despite the long history of ciguatera, the origin of the toxins involved in the fish poisonings was long time unknown. First, many theories implicated fish disease or pollution. Then the food chain theory was proposed by Randall (1958). The toxins were presumed to be produced by a benthic organism first ingested by herbivorous fish and then transmitted to the carnivorous fish. This theory proved to ensure a great reliability to the field observations. The breakthrough occurred when samples of biodetritus collected on the surface of damaged coral beds in the Gambier Islands (French Polynesia), were found to be highly toxic at a time when most of the reef fish were also toxic in this area (Yasumoto *et al.*, 1977 ; Bagnis *et al.*, 1977 ; Chanteau, 1978). The high toxicity of these samples was correlated to the great abundance of a dinoflagellate which was also found abundant in the gut contents of the herbivorous fish. This dinoflagellate was identified as belonging to a new genus and named *Gambierdiscus toxicus* (Adachi and Fukuyo, 1979). Lipid-soluble and water-soluble extracts of this wild dinoflagellate yielded two kinds of toxicity, one closely related to ciguatoxin as characterized from carnivorous fish by Scheuer *et al.* (1967) and the other resembling maitotoxin as characterized from surgeonfish gut contents by Yasumoto *et al.* (1976). These data fixed *G. toxicus* as the benthic micro-organism elaborator of the ciguatera toxins. Since then, cultures of *G. toxicus* collected in the Gambier Islands have been developed (Chungue *et al.*, 1979 ; Hurtel *et al.*, 1979 ; Bagnis *et al.*, 1980 ; Lechat *et al.*, 1985 ; Durand-Clément, 1986) in a tentative to obtain *in vitro* production of ciguatoxin or its analogs. In standard culturing conditions, *G. toxicus* yields mostly maitotoxin-like toxicity and only traces of ciguatoxin-like toxicity. Important clonal and sub-clonal variability in growth and toxin potency of *G. toxicus in vitro* poses a question regarding the precise origin and mechanism of synthesis of the involved toxins. The possible role of bacteria closely associated to the dinoflagellate cell surfaces and extracellular matrices during culture growth has been evoked (Hurtel *et al.*, 1979 ; Tosteson *et al.*, 1989). On another hand, genetic variability in toxin potencies among several clones of *G. toxicus* collected in various areas has been suggested (Bomber *et al.*, 1989). Further determinant experiments are needed to solve the question.

Chemical data on ciguatoxins

The main toxin involved in ciguatera fish poisoning, named ciguatoxin, was first extracted from red snapper (Scheuer *et al.*, 1967) and purified to cristals by Scheuer's group (Tachibana, 1980 ; Nukina, 1984 ; Tachibana *et al.*, 1987). At that time, even though unsuccessfully analyzed by x-ray and NMR methods, its partial characterization as a polyether compound with a molecular weight of 1111 has been made possible. Later on, another tentative using very performant NMR equipment and 0.35 mg of a pure sample isolated in Tahiti from moray eel livers (Table 1) collected from the Tuamotu and Marquesas Islands led to the complete structure elucidation of the toxin. Furthermore, the structural characterization of an analog isolated from wild *G. toxicus* (Table 2) collected in the Gambier Islands was also achieved (Murata *et al.*, 1989 ; Legrand *et al.*, 1989 ; Murata *et al.*, 1990). The typical structure of those toxins was disclosed to consist of a succession of thirteen contiguous trans-fused ether rings and to resemble the structure of the brevetoxins and yessotoxin (Fig. 1).



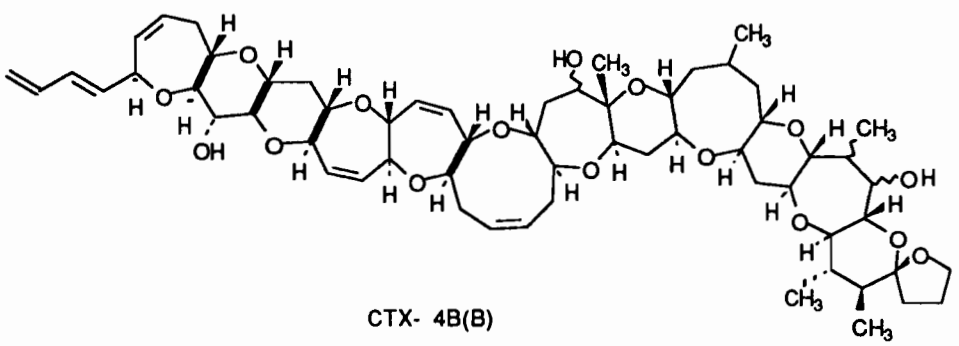


Figure 1

The isolation work conducted with careful examination of all the toxic components present either in carnivorous fish (the moray eel *Gymnothorax javanicus*, the groupers *Lutjanus bohar* and *Plectropomus leopardus*), herbivorous fish (the parrotfish *Scarus gibbus*) and wild *G. toxicus* has allowed the detection and characterization by high performance liquid chromatography and mass spectra methods of more than 20 lipid-soluble toxins. These toxins were listed into four distinctive groups considering their elution rank from a reversed phase column (Legrand *et al.*, 1991). Ciguatoxin (coded CTX-1B) was the major toxin in carnivores but was insignificant or absent in parrotfish and *G. toxicus*. A less polar toxin tentatively coded toxin-3B appears to be the major component isolated up to now from herbivorous fish (Table 3). Its structure still remains unknown due to the very small amount of the sample available. The algal material i.e. the wild *G. toxicus* yielded the less polar toxins. Polarity differences noticed among the *G. toxicus* and fish toxins suggests that oxidative modifications occur along the food chain.

Although a structural relationship has been established between toxins of wild *G. toxicus* and those of carnivorous fish and despite eager attempts for production of ciguatoxins by cultured *G. toxicus*, the latter remains very limited. Actually, there were numerous reports on lipid-soluble extracts from cultures that kill mice, but the high potency of maitotoxin and the likelihood that quantities of it remain in the lipid fraction any time purification happens to be insufficient, make some studies difficult to interpret. Correct evaluation of the ciguatoxins produced by cultured *G. toxicus* requires a very careful testing via mouse bioassay and must be confirmed with any specific *in vitro* method such as the inhibition of 3H-PbTx-3 binding to rat brain membranes reported by Holmes *et al.* (1991). Furthermore, chemical evidence for the production of CTX precursors by *G. toxicus* in cultures still needs to be established. For that purpose, several strains of that dinoflagellate collected from various areas in french Polynesia are on study.

Toxicology and pharmacology of the characterized ciguatoxins

The pharmacological studies undertaken on ciguatoxins mainly concerned the reference toxin coded CTX-1B. Most of them were performed using partially purified samples. Our isolation work in order to supply pure toxins for structural analysis lead us to carefully evaluate the acute toxicity in mice by intraperitoneal injection of CTX-1B (Legrand *et al.*, 1989) and two of the less polar toxins isolated in pure form from wild *G. toxicus*, CTX-4B and -4C (Legrand *et al.*, 1991). The methodology followed was as described previously (Legrand *et al.*, 1988). The lethal doses (LD50) are presented in Table 4 in comparison with the LD50 of some other well-known marine toxins. The data confirmed the very high potency of ciguatoxins.

Ciguatoxin as characterized by Scheuer was from long time presumed to exert its toxic effects via the sodium channels of the excitable membranes (Rayner, 1972 ; Ohizumi and Yasumoto, 1983 ; Bidard *et al.*, 1984 ; Lewis and Endean, 1986 ; Legrand *et al.*, 1987).

Evidences for an action on sodium channels was provided by voltage-clamp experiments on frog myelinated nerve fiber (Benoit et al., 1986), patch-clamp experiments on cardiac myocytes (SEINO *et al.*, 1988) and binding studies on rat brain membranes (Lombet *et al.*, 1987). The maintained inward Na current induced by partially purified ciguatoxin during long lasting depolarizations of frog nerve fiber (Benoit *et al.*, 1986) was later on confirmed using pure CTX-1B (2.10^{-12}M) and recently noted in the same manner for CTX-4B (8.10^{-11}M) (Benoit et Legrand, unpublished data). These results constitute the first tentative approach for a structure-activity analysis of the ciguatoxins.

Detection of the ciguatoxicity

The public health problem induced ciguatera toxins is related to the very high toxicity of these natural substances occuring at very low concentration in seafood i.e. a few nanograms per one hundred grams.

The water-soluble ciguatera toxin, maitotoxin, produced by *G. toxicus in natura* and *in vitro* is very potent. First isolated from the gut content of the surgeonfish *Ctenochaetus striatus* also named maito in French Polynesia (Yasumoto *et al.*, 1976), then chemically characterized as a polyether (Yokoyama *et al.*, 1988), it was defined as a unique pharmacological tool for research on calcium-dependent mechanisms (Gusovsky et Daly, 1990). Nevertheless, maitotoxin was not proven to accumulate in fish tissues. It seems to play a minor role in ciguatera fish poisonings when herbivorous fish with flesh contaminated by the maitotoxic gut content during cooking are consumed.

The most important toxins to detect in order to prevent the ciguatera fish poisonings are the ciguatoxins. Mouse and mosquito bioassays (Bagnis *et al.*, 1987 ; Legrand *et al.*, 1988) are mainly used up to now but they are time and toxin consuming and moreover not totally reliable. Correct detection of the hazardous reef fish requires a chemical or immunochemical dosage of the ciguatoxins. These two approaches are in process, in collaboration with Yasumoto's group and Avramea's group (Immunocytochemistry Unit, Pasteur Institute in Paris) respectively. Recently in Hawaii Hokama's work has allowed the setting up of an immunobead assay (Hokama, 1990) likely to be available soon. Its routinely use by a few control laboratories in the endemic areas is necessary to evaluate the sensitivity and local adaptability of this method.

Conclusion

In conclusion, research on ciguatera fish poisoning has expanded over the last 15 years mainly thanks to the discovery of the benthic causative dinoflagellate followed more recently by the chemical characterization of the ciguatoxins. Nevertheless, progress in order to solve the many remaining questions is still hampered by the lack of routine methods for reliable dosage of the toxins.

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Carnivorous fish (liver, viscera or flesh)

↓ Extracted with Acetone
↓ Partitioned Et_2O/H_2O
↓ Defatted with n-C_6H_{14}

Toxic extract

↓ $SiO_2/CHCl_3$-MeOH 97:3 ; 9:1

$CHCl_3$-MeOH 9:1

↓ Florisil/EtOAc ; EtOAc-MeOH 9:1 ; 3:1

EtOAc-MeOH 9:1

↓ Sephadex LH-20/$CHCl_3$-MeOH 6:4

Toxic fraction

↓ ODS cartridge/MeOH-H_2O 5:5 ; 6:4 ; 7:3 ; 8:2

MeOH-H_2O 8:2

↓ LiChrosorb RP-18 /MeOH-H_2O 8:2 twice
↓ /MeCN-H_2O 65:35 twice

CTX-1B

Table 1 : Purification procedure of ciguatoxins from carnivorous fishes (moray-eel *Gymnothorax javanicus*, *Lutjanus bohar* or *Plectropomus leopardus*)

Dinoflagellate cells

↓ Extracted with methanol
↓ Partitioned CH_2Cl_2/aqueous MeOH

CH_2Cl_2 fraction

↓ Florisil

Acetone/MeOH (9:1)

↓ Develosil Lop-ODS

90 % and 100 % MeOH eluate

↓ Sephadex LH_{20}/MeOH and 85% MeOH

Toxic fraction

↓ Develosil ODS-7
↓ MeCN 85% —> 100%

CTX-4B (GT-4B) and CTX-4C

Table 2 : Purification procedure of ciguatoxins from wild *Gambierdiscus toxicus*

Parrotfish flesh
↓ Extracted with Acetone
↓ Partitioned Et_2O/H_2O
↓ Partition/aqueous MeOH/n-C_6H_{14}
Methanol layer
↓ $SiO_2/CHCl_3$-MeOH 97:3, 9:1
$CHCl_3$-MeOH (9:1)
↓ Sephadex $LH_{20}/CHCl_3$-MeOH (2:1)
Toxic fraction
↓ Lichrosorb RP-2/MeOH-H_2O (4:1)
Toxic fraction
↓ Develosil ODS-7/MeCN 85% —> 100%
CTX-3B

Table 3 : Purification procedure of ciguatoxins from herbivorous fish

Marine Toxins		LD_{50}, i.p., in mice (µg/kg)
Okadaic acid		192
Brevetoxins	PbTx-2 type	200
	PbTx-3 type	100
Saxitoxin		10
Tetrodotoxin		10
Ciguatoxins	CTX-4B	10
	CTX-4C	1
	CTX-1B	0.33
Maitotoxin		0.15

i.p. = intraperitoneally
LD_{50} = lethal dose 50 %

Table 4 : Acute toxicity in mice of certain marine toxins.

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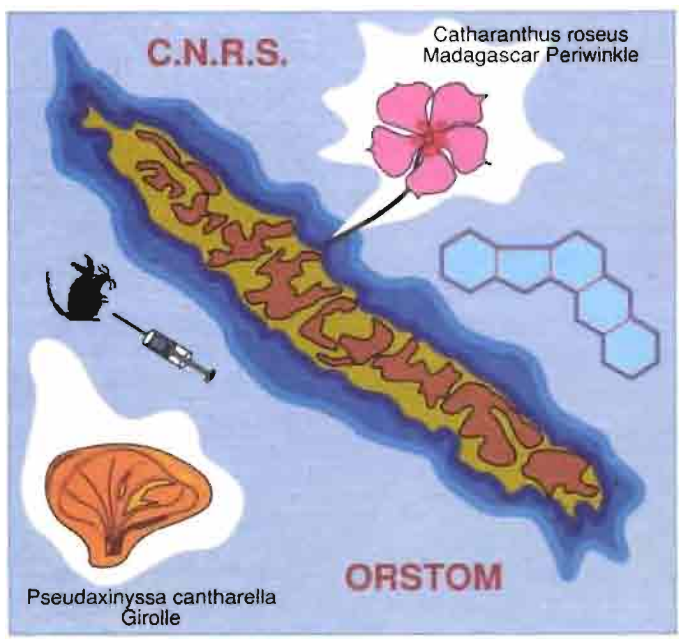

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