Ultrastructural alterations of cabrilla sea bass Serranus cabrilla liver related to experimental Gambierdiscus toxicus (dinoflagellate) ingestion

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ABSTRACT: Ultrastructural effects of dietary Gambierdiscus toxicus on the liver of cabrilla sea bass Serranus cabrilla were investigated. No fish treated with G. toxicus died during the experiment. A marked perisinusoidal and intercellular fibrosis occurred after algal feeding. Microvilli decreased in number and shortened at the sinusoidal and canalicular areas of the hepatocytes. Lipid accumulation was also apparent. A high number of binucleated cells, nucleolar disorganization and loss of condensed heterochromatin at the periphery of the nucleus were also found, as well as nuclear pyknosis, dilated rough endoplasmic reticulum and swollen mitochondria. This work shows that toxins produced by this dinoflagellate may be considered to be harmful to fish through diet.

KEY WORDS: Gambierdiscus toxicus · Serranus cabrilla · Ciguatera · Ecotoxicology · Liver ultrastructure

INTRODUCTION

Gambierdiscus toxicus is considered as the major etiological agent of the ichthyosarcotoxism known as Ciguatera (Anderson & Lobel 1987). G. toxicus toxins are bioaccumulated, mainly in the liver, by fish which feed on these dinoflagellates (Randall 1958, Helfrich & Banner 1963, Yasumoto et al. 1971, 1977). G. toxicus toxins are reported to be fairly detrimental to mammals but it is established that, in the wild, there are no external differences between toxic and non-toxic fish (Banner et al. 1960).

However, it is well known that experimental ingestion of Gambierdiscus toxicus induces some macroscopical effects such as colour modifications, behavioural changes [Thalassoma bifasciatum, Labridae (Davin et al. 1986) and Chromis chromis, Pomacentridae (Durand-Clement et al. 1987)] and decrease in the liver size associated with a loss of colour of this organ [Serranus cabrilla, Serranidae (Durand-Clement et al. 1987)]. Moreover, purified extracts of the toxins have been shown to induce changes in the muscular Na⁺ content in Chelon labrosus [Mugilidae (Quod 1983)], epithelial degeneration of the intestine and dilatation of the gill filaments in Pomacentrus wardi and Chromis nitida [Pomacentridae (Capra et al. 1988)] and mortality in Gambusia affinis [Cyprinodontidae (Lewis 1992)].

Preliminary data on the histological effects of Gambierdiscus toxicus ingestion on Serranus cabrilla showed non-specific alterations of the hepatic structure (Amade et al. 1990). No modification was found in other organs (spleen, kidney and digestive tract; Gonzalez 1992). Taking into account that S. cabrilla has been shown to be a suitable species for ecotoxicological testing (Gonzalez et al. 1993), the aim of this study was to describe the ultrastructural alterations of the liver of the cabrilla sea bass following experimental G. toxicus ingestion.

MATERIALS AND METHODS

Serranus cabrilla used in this experimental series were juveniles (total length = 13.52 ± 1.47 cm; total...
TEM observations showed hepatic lesions in the *Serranus cabrilla* intoxicated with the medium dose (191.5 ± 71.2 mg *Gambierdiscus toxicus* d⁻¹) during the 3 periods tested. The untreated group exhibited the same hepatic ultrastructure as described previously by Gonzalez et al. (1993). After 5 d of treatment, a marked perisinusoidal and interhepatocyte fibrosis occurred. Micrornielli decreased in number and shortened (Fig. 1). Although the energy reserves of the hepatocyte were mainly constituted by glycogen, lipid accumulation of moderate to high osmiophily was also apparent (Fig. 2). After 10 d of intoxication, the fatty change was more evident and lipid droplets were found even in the nucleus of the hepatocyte (Fig. 3). Some myeline figures were also observed in the cytoplasm or inside the mitochondria (Fig. 3). Nuclear lesions started 10 d after the beginning of intoxication and were more common in the 20 d treated fish. These showed a high number of binucleated cells, nucleolar disorganization and loss of condensed heterochromatin at the periphery of the nucleus (Fig. 4). Hepatocytes with nuclear pycnosis, dilated rough endoplasmic reticulum (rER) and swollen mitochondria were also found (Fig. 4).

No fish treated with the high dose (471.0 ± 165.6 mg *Gambierdiscus toxicus* d⁻¹) died during experimentation. The disorganization of the sinusoidal pole of the hepatocyte was apparent, with loss of microvilli, and rupture of the endothelium of the sinusoid (Fig. 5). Within the sinusoids, cellular debris and altered organelles such as mitochondria with dense matrix were found (Fig. 5). The canalicular pole of the hepatocyte was also affected, exhibiting loss of microvilli (Fig. 6). The accumulation of lipids was more apparent at this high dose (Fig. 7), but no intranuclear lipid droplets were detected. The most common nuclear malformation was a loss of the spherical regular shape (Figs. 7 & 8). Extensive areas of hepatocytes with nuclear alterations, highly dilated rER and swollen mitochondria, as well as interhepatocyte fibrosis (Fig. 8) were found.

The low dose (59.4 ± 15.6 mg *Gambierdiscus toxicus* d⁻¹) induced minor changes in the liver of *Serranus cabrilla* after 20 d of intoxication. The sinusoids and the microvilli of both sinusoidal and canalicular poles were not affected. The energy stores were still constituted by glycogen, even though some lipid droplets were observable. Some hepatocytes exhibited a dilation of the rER and swollen mitochondria as well as some lipofuscin granules in the cytoplasm (Fig. 9).

**RESULTS**

The present observations showed that the experimental ingestion of *Gambierdiscus toxicus* induces ultrastructural alterations of the liver of *Serranus cabrilla*. No mortality was detected during experimentation in this fish species, even following the highest dose treatment (471.0 ± 165.6 mg *G. toxicus* d⁻¹). Mortality induced by *G. toxicus* ingestion has been reported by Durand-Clement et al. (1987) in *Chromis chromis* (Pomacentridae) fed for 3 d with 500 mg of algae. Our data show that *S. cabrilla* is less sensitive to *G. toxicus* toxins than other fish species such as *C. chromis*. However, the severity of the hepatic lesions...
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Figs. 1 to 4. Serranus cabrilla after ingesting medium dose of Gambierdiscus toxicus. Fig. 1. Liver after medium dose for 5 d, TEM, 6900 X. Note the perisinusoidal and intercellular fibrosis (arrowheads) in hepatocytes with few microvilli and some lipid droplets (star). Fig. 2. Liver after medium dose for 10 d, TEM, 9100 X. Two hepatocytes showing lipid droplets (star) of moderate osmio-philicity. Fig. 3. Liver after medium dose for 10 d, TEM, 13600 X. Lipids (star) inside the nucleus and a mitochondrion exhibiting a myelin figure (arrowhead). Fig. 4. Liver after medium dose for 20 d, TEM, 6500 X. Binucleated hepatocyte with pyknotic nuclei (arrowhead) undergoing degeneration. Loss of heterochromatin and nucleolus in the nucleus of a neighbouring hepatocyte.

m: mitochondria; mv: microvilli; N: nucleus; S: sinusoid
Figs. 5 to 8. *Serranus cabrilla* after ingesting high dose of *Gambierdiscus toxicus*. Liver after 5 d. Figs. 5 & 6. TEM, 6250× and 10 400×. Sinusoidal pole (Fig. 5) and bile pole (Fig. 6) are disorganized with loss of microvilli. Note the presence of mitochondria and cellular debris (star) within the sinusoid. Fig. 7. TEM, 4600×. Numerous lipid droplets (star) filling the cytoplasm of cells which exhibit nuclei of abnormal shape. Fig. 8. TEM, 5950×. Most of the cytoplasm of degenerating cells is occupied by a dilated rough endoplasmic reticulum and swollen mitochondria. Some very osmiophilic lipid droplets (star) as well as collagen fibres (arrowheads) are seen in this area. bc: bile canaliculum; m: mitochondria; mv: microvilli; N: nucleus; S: sinusoid.
observed in the cabrilla sea bass after 5 d of intoxication with the high dose led us to believe that these toxins are not without effect on *S. cabrilla* liver.

The main ultrastructural lesions of hepatocyte due to *Gambierdiscus toxicus* treatment are disorganization of the sinusoidal and canalicular areas and perisinusoidal fibrosis with loss of the microvilli. Numerous microvilli within the space of Disse can be considered as a good indicator of healthy hepatocyte metabolism (Byczkowska 1968, Nopanitaya et al. 1979, Ferri & Sesso 1981, Hampton et al. 1988). Loss of microvilli of the hepatocytes and sinusoidal fibrosis have been reported in fish in association with pesticide (Biagianti 1990) or heavy metal (Gony 1990) intoxication. Our observations show that degenerating hepatocytes with dilated rER and swollen mitochondria have been found at the 3 time periods and doses tested. Degenerative processes have also been reported in the heart, adrenal glands, thymus, stomach (Terao et al. 1988, 1991), liver (Partridge & Bergman 1982) and intestine (Coombe et al. 1987) of mouse after intoxication with purified *G. toxicus* toxins. The nucleolar lesions observed after 20 d of intoxication with the medium dose could be related to disturbances in the protein synthesis, since the presence of well-developed nucleoli is commonly associated with intense cellular metabolism (Ishii & Yamamoto 1970, Byczkowska 1971).

The accumulation of lipid droplets appears to be related to the *Gambierdiscus toxicus* dose, being more important after the highest dose treatment and varying with time of intoxication. Fatty change is a common alteration induced by many experimental intoxications in fish (Apostol et al. 1980, Biagianti 1990) or by environmental pollution (Grawinski 1990), mainly related to liposoluble toxins. Ciguatoxins, which are liposoluble (Lewis & Sellin 1992), could accumulate also in the hepatocyte and could modify the lipidic metabolism (Köhler 1990, Mosconi-Bac 1991). The lipid droplets observed in our study exhibited moderate to high osmiophily, related, according to the literature, to a high degree of non-saturated fatty acids (Stoeknius & Mahr 1965). A decrease of the degree of lipid saturation was also reported by Lagraulet et al. (1975) in the surgeonfish *Ctenochaetus striatus* living in ciguatoxic areas.

We conclude that the ciguatoxins could induce ultrastructural hepatic disturbances in fish fed with *Gambierdiscus toxicus*. However, these modifications must be considered as non-specific lesions that can be induced in polluted areas by a great number of etiolog-
ciguatera areas (such as the Pacific and Indian Oceans and the Caribbean Sea), may help to elucidate the effects of ciguatoxins on fish.

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LITERATURE CITED


Mathiessen, P., Roberts, R. J. (1982). Histopathological changes in the liver and brain of fish exposed to endosulfan insecticide during tse tse fly control operations in Botswana. J. Fish Dis. 5: 153-159


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