Response of Mice to *Gambierdiscus toxicus* Toxin

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ABSTRACT—Response to toxins extracted from cultured Gambierdiscus toxicus *was evaluated in mice. Toxin preparations administered intraperitoneally or intravenously gave similar dose-response curves, whereas oral administration elicited no response. Time-to-death determination was dose dependent and was quantitated to the dose-response based on lethality. The 48-hour lethality dose curves for the dinoflagellate toxin were comparable to those previously published for ciguatoxin extracted from fish, whereas the time-to-death curves showed a strong difference. The LD_{50} response of scheduled multiple injections suggested a 4- to 8-hour half-life for toxin activity in the mouse model. Sex and strain of the mouse did not affect susceptibility.*

Introduction

Ciguatera seafood poisoning is a serious human illness brought on by ingesting certain coral reef-associated fish in tropical and subtropical regions. The toxin carried by these fish was first isolated by Scheuer et al. (1967). The presence of the toxin has been confirmed in red snapper, *Lutjanus bohor*, moray eel, *Gymnothorax javanicus*, amberjack, *Seriola aureovittata*, and others. Toxic fish probably acquire ciguatoxin through their diet, and that one source of toxin is probably the benthic dinoflagellate, *Gambierdiscus toxicus*, found in association with certain macroalgae of coral reefs (Yasumoto et al., 1977). Extracts of laboratory grown cultures of *G. toxicus*, however, have yielded a more polar toxin similar to maitotoxin described from surgeonfish, *Acanthurus* sp. (Yasumoto et al., 1979). The relationship of this dinoflagellate toxin to ciguatoxin is unknown, though one possible explanation is that the dinoflagellate toxin becomes chemically converted as it is metabolized during transfer through the marine food web.

Toxin(s) extracted from cultured *G. toxicus* injected intraperitoneally (i.p.) into laboratory mice is reported to evoke gross symptoms indistinguishable from those reported for partially purified fish extracts containing ciguatoxin (Hoffman et al., 1983; Sawyer et al., 1984). Both toxins exhibit similar dose-response curves and both elicit in mice a striking hypothermia, which is reversed by increasing ambient temperature (Sawyer et al., 1984). It is important, therefore, that the biological and chemical relationship between these toxins be clarified. In this investigation, we have utilized the mouse bioassay to examine further the biological activities of the dinoflagellate toxin for comparison with ciguatoxin and to gain an estimation of the biological half-life of *G. toxicus* toxin in mice.

Materials and Methods

Cells of *G. toxicus*, Adachi and Fukuya, were supplied by Rick York of the Hawaii Institute of Marine Biology. The isolate, clone T-39, was cultured in 100-liter vats in F/2 medium supplemented with a seaweed extract. Harvested dinoflagellate cells were shipped to South Carolina in aqueous methanol, and upon arrival, cells were extracted for 7 days at room temperature in methanol:water (80:20). The suspension was clarified by centrifugation, the supernatant dried, and the resulting solids weighed and dissolved in absolute methanol. This suspension was filtered, designated as crude dinoflagellate extract, and stored at 4°C.

Assays for toxicity were conducted on ICR female, ICR male, and C57BL/6 female mice all weighing approximately 20 g each. Animals were maintained on Wayne Laboratory Animal diets (Lab-Blox)[1] and water, ad libitum. A known quantity of the crude dinoflagellate extract was resuspended in phosphate-buffered saline (PBS) containing 5 percent Tween-80 and administered (in 0.2 ml aliquots unless otherwise stated) to mice intraperitoneally, intravenously (i.v.), and by gavage. Control animals received an equal volume of the solvent vehicle. Lethality was recorded at 48 hours for some studies, and time-to-death after injection was recorded for other studies. One mouse unit (MU) of toxicity is defined as that quantity of crude extract capable of eliciting a fatal dose in half of the test animals by 48 hours. Body temperatures were recorded using a YSI rectal probe (Model 43TA, Yellow Springs Instrument Co., Yellow Springs, OH) within 2 hours after administration to obtain early indications of toxicity.

For the retention time study, a stock solution of dinoflagellate extract (13.3 MU/ml) was prepared in PBS containing 5 percent Tween-80 (67 μg extract/ml). Each ICR female mouse was administered 0.15 ml (10 μg) i.p. of the stock solution or 0.15 ml of a 1:3 dilution; a reduced volume was used due to administration of multiple injections. One control group received 10 μg of tox-

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[1]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

ic extract as a single injection, and another control group received 3.3 μg in a single injection. Each test animal in the eight remaining groups received three equally spaced injections over a time interval ranging from 0 to 30 hours between individual injections. Lethality was recorded after 48 hours of the last injection.

Results and Discussion

Sex and Strain Differences

Determination of the dose-response relationship to the crude dinoflagellate extract administered i.p. to ICR female, ICR male, and C57BL/6 female mice suggested that sex and strain of the mouse did not markedly influence toxicity (Fig. 1). The LD_{50}'s for ICR female, ICR male, and C57BL/6 female mice were estimated to be 260 μg/kg, 393 μg/kg, and 192 μg/kg, respectively. ICR females were used in later tests.

The i.p. injection of ciguatoxin extracts into laboratory mice has been a reliable bioassay method for ciguatera-associated toxins and has been widely adopted by investigators of this field. The variability that may be present in the assay due to the sex and strain of mice used has not been studied. The results of this limited study indicated that no apparent difference should be expected in dose-response with respect to sex and strain of the test animal.

Route of Administration

To determine the effect of the route of administration on the toxicity of *G. toxicus* extract, animals received the toxin in three ways: Intraperitoneally, intravenously, and orally (p.o.). Response of mice to 10 MU of toxin administered by i.p. and i.v. routes was uniformly fatal (Table 1). Average time-to-death, however, was much shorter following i.v. injections. Administration by gavage of approximately 20 MU resulted in no test mice fatalities.

The potency of the dinoflagellate toxin, as defined by percent fatalities of the treated mouse populations at 48 hours, was not significantly different between mice receiving the dinoflagellate extract i.p. or i.v. However, mice administered the toxin i.v. responded earlier (time-to-death) than mice injected i.p. The toxicokinetics of the dinoflagellate and fish toxins are not known. It is interesting that the time to develop overt symptoms, such as hypothermia in mice (Sawyer et al., 1984) or clinical symptoms in man (Bagnis et al., 1979), occurs over a period of hours. In view of the high lipophilicity of these toxins, which should enhance their equilibration across membranes and promote their accessibility to the site(s) of action, a much more rapid response, perhaps within minutes, would be expected.

The lack of any response, including temperature depression in mice administered the dinoflagellate extract by gavage, raises interesting questions regarding the toxin's rate of absorption, physical or biochemical inactivation, etc. Preliminary studies (not reported), in which the toxicity of the dinoflagellate extract was not lost when incubated under acidic conditions or with various mouse tissue extracts, suggest that simple physical or enzymatic inactivation was not responsible for abrogation of dinoflagellate toxicity when administered orally. The observation that the route of administration influenced the dose-dependence of the toxicity was first made by Baden[2] and is in contrast to the animal response with fish toxin (putative ciguatoxin) described by Banner et al. (1960), in which an equivalent response was obtained whether mice were injected with toxic fish extracts or fed the extract equivalent to twice the injected dose.

Determination of Mean Death Time

A standardized mouse bioassay has been universally accepted to determine the toxicity of saxitoxin, a marine toxin of dinoflagellate origin and the causitive agent of paralytic shellfish poisoning. The method described by Schantz et al. (1957) involved determination of median death time for a given dilution of a suspect extract injected i.p. relative to a saxitoxin standard. For ciguatoxin, Tachibana (1980) reported the relationship between the dose of toxin and time-to-death in the mouse. For comparison with these published reports, we observed the lethality of the dinoflagellate toxin as judged by time-to-dealth response in relation to the 48-hour LD_{50} response (Fig. 2). Groups of test mice were injected i.p. with dinoflagellate extract equivalent to 2-33 MU. At these doses, death occurred between 2 and 16 hours, and the mean time-to-death was

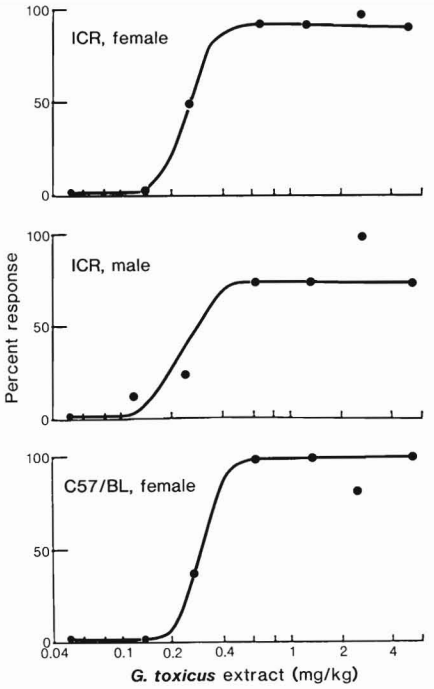

Figure 1.—Percent response (death) in 48 hours displayed by three mouse strains to i.p. administration of *G. toxicus* extract diluted in PBS. Data points represent a minimum of 8 animals.

Table 1.—The effect of administration route on lethality of *G. toxicus* extract.

Administration route	Amount	No. of animals[1]	Percent response[2]	Time to death (h)
I.p.	50 μg	6	100	4 to 22
I.v.	50 μg	6	100	1 to 3
Gavage	100 μg	4	0	

[1]Female ICR mice.
[2]Percentage of fatalities after 48 hours.

[2]Baden, D. Department of Biochemistry, University of Miami School of Medicine, Miami, FL 33101. Personal commun., 1982.

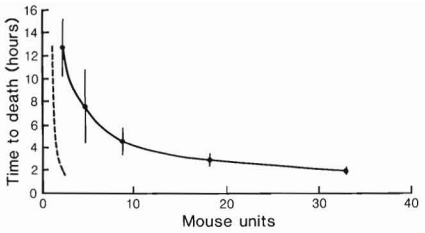

Figure 2.—Relationship between *G. toxicus* extract dose and time-to-death of ICR female mice (solid line). Mice received doses expressed in equivalents of mouse units (1 MU = 5.2 μg extract). Error bars indicate one standard error of the mean (n = 8). Curve defined by the dotted line represents the administration of fish ciguatoxin as reported by Tachibana (1980).

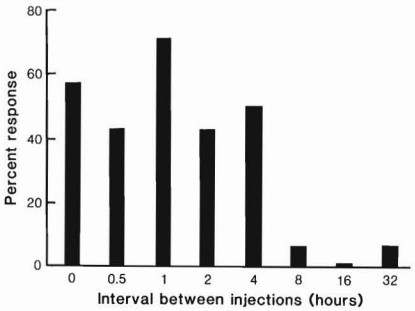

Figure 3.—Percent response (death) within 48 hours following three equally spaced i.p. injections of *G. toxicus* extract.

dose dependent. A nonlinear equation estimated the relationship between the independent variable (dose) and the dependent variable (time-to-death). Using the Simplex method for determining the values of the parameters by least squares, the following equation gave a reasonable approximation of the curve in Figure 2:

$$\text{Dose (MU)} = 0.8\ (\text{time-to-death in hours})^{-1}.$$

Coupled with differences in sensitivity to orally administered toxins, the marked difference in time-to-death response to ciguatoxin reported by Tachibana (1980) and that obtained in our study with *G. toxicus* extracts further illustrates that the two toxins may not be equivalent biologically. The dashed line in Figure 2, superimposed on the dinoflagellate response curve, represents a curve based on the formula reported by Tachibana (1980) for ciguatoxin:

$$\text{Log dose (MU)} = 2 \log (1 + \text{time-to-death in hours})^{-1}.$$

The differences between the fish-toxin-derived curve and the dinoflagellate-derived-curve are evident from this figure.

Estimation of Toxin Retention Time

Our knowledge regarding the ability of the animal to clear, sequester, or otherwise inactivate ciguatera-associated toxins is vitally important to our understanding of the human illness, ciguatera. Unfortunately, direct methods of determining the biological fate of these toxins is not readily available. To gain insight as to the "half-life" of the dinoflagellate toxin, portions of an otherwise lethal dose were administered over several time intervals in order to extrapolate the time point in which the effect of individual doses were no longer additive (Fig. 3). A control group which received a single 10 μg standard dose of the toxin extract elicited the expected 75 percent lethality response. Another control group received a single 3.3 μg dose and exhibited no fatalities. Groups that received three injections of 3.3 μg each at time intervals ranging from 0 to 4 hours between injection gave between 40 and 60 percent response, while those animals in groups injected at intervals between 8 and 30 hours gave less than 10 percent response. From these results, it appeared that an estimation of biological half-life for this toxin in the mouse model lies somewhere between 4 and 8 hours, and that this toxin may not accumulate in the body.

The persistence for months of the neurological symptoms associated with ciguatera poisoning in humans certainly suggests that either ciguatoxin is retained and remains active for long periods of time or that the damage caused by ciguatoxin is not quickly repaired. As yet, a similar "half-life" study using crude extracts of ciguatera fish has not been performed. It will be of interest to know if ciguatoxin, unlike the dinoflagellate toxin, is retained in the mouse model.

Biological studies of the toxins associated with ciguatera have been minimal. However, many of the biological properties described can be very useful in defining the various toxins that have been isolated. As a clear understanding of the biological activities of these ciguatera-associated toxins is obtained, their relationship to each other and a more rational approach to minimizing the impact of ciguatera may become evident.

Acknowledgments

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