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Procedures for detoxification of the brevetoxins PbTx-2 and PbTx-3 were investigated using the Japanese madaka (Oryzias latipes) as the assay model. PbTx-2 was lethal to these fish at concentrations above 70 ng/ml in the aquarium water, while PbTx-3 was approximately twofold less potent. Treatment with 0.5 ml 0.1 N NaOH for 10 min detoxified a lethal dose of either PbTx-2 or PbTx-3 to below the detection limits of this assay (10 ng/ml). Decreasing NaOH concentration required correspondingly longer incubation times. Potency of both toxins was also destroyed by incubation at 500°C for 10-15 min. Steam autoclaving at 122°C (30 min, 18 psi) was not sufficient to detoxify brevetoxins. These results show that washing or soaking in a dilute NaOH solution will decontaminate laboratory glassware and equipment and render it safe for normal handling. Disposable waste can be either soaked in a NaOH solution prior to disposal or burned in an incinerator with a combustion chamber of at least 500°C. Steam autoclaving, however, is not a viable method of decontamination.

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Procedures for Detoxification of Brevetoxins

PbTx-2 and PbTx-3

(Neurotoxins from the Florida Red Tide

Dinoflagellate Ptychodiscus brevis)

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ABSTRACT

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Procedures for detoxification of the brevetoxins PbTx-2 and PbTx-3 were investigated using the Japanese medaka (Oryzias latipes) as the assay model. PbTx-2 was 100% lethal to these fish at concentrations above 70 ng/ml in the aquarium water, while PbTx-3 was less potent. Treatment with 0.5 ml 0.1 N NaOH for 10 min detoxified a lethal dose of either PbTx-2 or PbTx-3 to below the detection limits of this assay (<10 ng/ml). Decreasing NaOH concentration required correspondingly longer incubation times. Potency of both toxins was also destroyed by incubation at 500°C for 10-15 min. Steam autoclaving at 122°C (30 min, 18 psi) was not sufficient to detoxify brevetoxins. These results show that washing or soaking in a dilute NaOH solution will decontaminate laboratory glassware and equipment and render it safe for normal handling. Disposable waste can be either soaked in a NaOH solution prior to disposal or burned in an incinerator with a combustion chamber of at least 500°C. Steam autoclaving, however, is not a viable method of decontamination. ←

INTRODUCTION

Brevetoxins are a novel class of cyclic polyether neurotoxins produced by the Florida red tide dinoflagellate Ptychodiscus brevis. These compounds first came under scientific scrutiny as a result of the public health implications associated with blooms of P. brevis ("red tides") in the Gulf of Mexico. These blooms resulted in massive fish kills, human respiratory irritation, and human intoxication known as neurotoxic shellfish poisoning (1,2). Investigation of potent fractions isolated from cell culture extracts (3-8) indicated diverse physiological effects in vivo (4,8-14) and in a variety of nervous and muscle tissue preparations (15-25). On the molecular level, brevetoxins recently have been shown to bind to a unique receptor site on the voltage-dependent sodium channel in mammalian nervous tissue (26,27), where they cause persistent channel activation, increased Na⁺ flux, and subsequent depolarization at resting membrane potentials (25). They are considered new neurological probes for investigation of the structure and function of the voltage-dependent sodium channel.

Because of their potential value as tools for the study of neurological function, brevetoxins are attracting a great deal of research interest. As a result, the decontamination of laboratory equipment and surfaces, the detoxification of waste prior to disposal, and the containment and control of accidental

spills have become necessary for the safety and protection of research personnel. The purpose of this work was to evaluate methods and establish protocols for general laboratory decontamination and safe treatment and disposal of contaminated wastes.

EXPERIMENTAL

Toxins

The brevetoxins PbTx-2 and PbTx-3 were supplied by Dr. D.G. Baden (University of Miami, Florida) as HPLC-purified fractions (single peak of absorbance at 215 nm) extracted from P. brevis laboratory cultures. Toxins were kept at -10°C in acetone at a stock concentration of 1 mg/ml. Dilutions of stock solution were made as required and used immediately.

Fish

Japanese madakas (Oryzias latipes, 2-3 cm) of undetermined sex were obtained from Dr. William Van der Schalie (U.S. Army Medical Bioengineering Research and Development Laboratories, Frederick, Maryland), kept in a glass aquarium containing original water from breeding tanks, and fed once per day with a standard commercial fish food preparation.

Bioassay

Individual madakas were placed in 100-mL beakers with 30 mL aquarium water and allowed to acclimate 30-60 min in a quiet room. Toxins (PbTx-2 and PbTx-3), in 30 μ L acetone (experimental) or 30 μ L acetone alone (controls), was added; the beakers were gently swirled once to mix, and the fish were observed for 24 h. Signs of nonlethal intoxication included lethargy, impaired balance, and tremors. Severe intoxication resulted in partial or complete loss of righting reflex, hyperactivity, and a spiral swimming motion progressing to seizure, paralysis, and death. Fish were considered dead when all body movement ceased and no further opercular pumping motion was discernible. Dose-response curves were constructed from duplicate experiments with four fish per concentration, and LD₅₀ values were approximated from these curves.

Detoxification with NaOH

Lethal doses of PbTx-2 (3 μ g) or PbTx-3 (12 μ g) in acetone were dried in test tubes under a stream of nitrogen. The desired concentration of NaOH was added in 0.5 mL of distilled water and allowed to incubate for the indicated time. The solutions were neutralized with 0.5 mL of HCl, added to beakers containing 29 mL aquarium water, and the pH adjusted to that of the controls (7.9 - 8.0). One fish was then added to each beaker and observed for 24 h. Control tubes contained toxin incubated in 0.5 mL of aquarium water and "neutralized" with an additional 0.5 mL at the end of the incubation period. All experiments were performed with four fish per NaOH concentration group.

Detoxification via elevated temperature

PbTx-2 (3 µg) or PbTx-3 (12 µg) was dried in a glass boat and incubated at 500°C for the desired time in a Thermolyne Type 10500 oven. The boats were then removed and allowed to cool for 10 min. Each boat was washed four times with 50 µL acetone and the pooled acetone rinses dried in test tubes under a stream of nitrogen. The boats were then rinsed again four times with 250 µL water. The water rinses were combined with the acetone rinse residues, mixed, and added to beakers containing 29 mL aquarium water. One fish was added to each beaker and observed for 24 h. Controls were incubated at room temperature and then treated as above. All assays were performed with four fish per experimental group. In experiments in which an autoclave was used as the heat source, the toxins were treated for 30 min at 18 psi in a Castle steam sterilizer and treated as above.

RESULTS and DISCUSSION

Because of the potency of the brevetoxins as ichthyotoxins, fish have often been used as assay models to assess toxicity of P. brevis cultures (28,29) or to screen for the presence of isolated toxins (5,6,30,33). The Japanese madaka (Oryzias latipes) was chosen as the assay organism in this study for several reasons. It is small, easily handled, hardy, and, when kept for only a few days, requires little care in captivity other

than periodic feeding. Symptoms of intoxication are clear and easily seen. Finally, as the following experiments indicate, it was very susceptible to the neurotoxic effects of the brevetoxins.

The toxicity of PbTx-2 to the madaka is demonstrated in Figure 1. Under these conditions, the LC_{50} was approximately 15-25 ng/ml. One death occurred at 10 ng/ml. In three separate experiments, 70 ng/ml resulted in 100% mortality. By comparison, PbTx-3 was less potent, with an approximate LC_{50} in the range of 50-100 ng/ml (Figure 2). In two separate experiments, mortality was complete at concentrations in excess of 167 ng/ml. These potencies are slightly lower than those reported for Gambusia affinis (31) and Brachydanio rerio (33).

The brevetoxins are a novel class of compounds consisting of at least eight different derivatives of two parent polyether backbone structures (27). PbTx-2 and PbTx-3 share a common backbone and differ only at the C42 position, where the alcohol function of PbTx-3 imparts greater solubility in water than the aldehyde function of PbTx-2 (Figure 3). This solubility difference, and the resulting difference in partitioning between the aqueous phase and the lipid environment of the gill membranes, may be the basis for the difference in potency observed in O. latipes, as well as that noted by Baden et al. (31) and Baden and Mende (9) with G. affinis.

Incubation with NaOH inactivated brevetoxins. Table 1 shows the results of experiments in which a lethal concentration of PbTx-2 (100 ng/ml) was incubated with several concentrations of

NaOH for varying lengths of time. As little as a 10-min exposure to 0.1 N NaOH at room temperature was sufficient to detoxify PbTx-2 to levels below the detection limit of this assay (<10 ng/ml). Concentrations of 0.05 and 0.01 N NaOH were also adequate for detoxification, but required extended incubation (4 h and 16 h, respectively). In a similar experiment, 400 ng/ml PbTx-3 incubated for 10 min with 0.1 N NaOH elicited no signs in a group of madakas, even after 72-h exposure (data not shown).

Elevated temperatures also destroyed brevetoxin potency. Fifteen min at 500°C rendered 100 ng/ml PbTx-2 nontoxic to madakas. Again, in a similar experiment, 10 min at 500°C detoxified 400 ng/ml PbTx-3 to below assay detection limits. Steam autoclaving, however, was not an effective method of detoxification. No decrease in potency was observed in PbTx-2 autoclaved for 30 min at 122°C (18 psi) (data not shown).

In conclusion, these results indicate laboratory equipment and surfaces can be easily decontaminated and brevetoxin-contaminated waste can be safely discarded when properly handled. For general glassware and equipment decontamination, soaking in a solution of 0.1 N NaOH for a minimum of 10 min will destroy toxin activity and render the item safe for normal handling. Bench-tops, floors, and other flat surfaces can be cleaned by sponging with NaOH solution, waiting at least 10 min, and then sponging again with clean water. Disposable waste can be either soaked in a NaOH bath before discarding or burned in an incinerator with a combustion chamber temperature of at least 500°C. Steam autoclaving, however, is not a viable method of decontamination.

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Figure 1. Toxicity of PbTx-2 in Japanese madaka (Oryzias latipes). Concentration refers to final aqueous toxin concentration to which the fish were exposed for 24 h. The curve represents the mean of two experiments, each consisting of four fish per concentration group. Vertical bars span the range of measurements between experiments.

Figure 2. Toxicity of PbTx-3 in Japanese madaka (Oryzias latipes). Concentration refers to the final aqueous toxin concentration to which the fish were exposed for 24 h. The curve represents the mean of two experiments, each consisting of four fish per concentration group. Vertical bars span the range of measurements between experiments.

Figure 3. Structures of the brevetoxins PbTx-2 (a) and PbTx-3 (b) isolated from the marine dinoflagellate Ptychodiscus brevis.

Table 1. Detoxification of PbTx-2 After Incubation With NaOH

NaOH Concentration	Incubation Time			
	10 min	1 h	4 h	16 h
0.50 N	---	---	---	---
0.10 N	---	---	---	---
0.05 N	ND	+(s)	---	---
0.01 N	ND	+(d)	+(s)	---

(-) no visible intoxication during 24 h of exposure

(+) visible intoxication of at least one fish

(s) = signs, (d) = death

(ND) not determined





