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Acute toxicity of karlotoxins to mice

Allen R. Place [a, *], R. Munday [b], J.S. Munday [c]

[a] Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Sciences, 701 East Pratt Street, Baltimore, MD 21202, USA
[b] AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand
[c] Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand

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ABSTRACT

Karlotoxins, polyketide derivatives produced by the dinoflagellate *Karlodinium veneficum*, are associated with fish kills in temperate estuaries world-wide. In this study, the acute effects of 3 pure karlotoxin analogs (KmTx 1, KmTx 3 and KmTx 2) have been examined in mice. Transient lethargy and increased respiratory rates were observed soon after dosing with the karlotoxins by intraperitoneal injection, but no deaths were recorded in animals dosed with KmTx 2 at up to 500 μg/kg or with KmTx 1 or KmTx 3 at up to 4000 μg/kg. Animals dosed intraperitoneally with KmTx 1 and KmTx 3 at 4000 μg/kg showed a pronounced decrease in food and water intake, lasting 3–4 days after dosing, accompanied by a significant decrease in body weight. After this time, the lost body weight was regained and the behavior and appearance of the mice remained normal throughout the following 10-day observation period. No effects were seen in mice dosed orally with KmTx 1 or KmTx 3 at a dose of 4000 μg/kg. It is concluded that contamination of seafood if it were to occur with these karlotoxins is unlikely to pose a major risk of acute intoxication in consumers.

1. Introduction

The athecate dinoflagellate *Karlodinium veneficum* produces a number of polyketide toxins, collectively known as karlotoxins (Peng et al., 2010; Place et al., 2012; Van Wagoner et al., 2008, 2010). These toxins have a surprising structural similarity to amphidinols produced by dinoflagellates of the *Amphidinium* genus which are characterized by long carbon chains with multiple hydroxyl groups and polyolefins (Satake et al., 1991). The karlotoxins have been reported to display a variety of interesting effects on biological systems including cellular lysis (Deeds et al., 2002; Kempton et al., 2002), damage of fish gills (Deeds et al., 2006; Nielsen, 1993), and immobilization of prey organisms (Van Wagoner et al., 2010). The cytolytic activity of the karlotoxins is modulated by membrane sterol composition, which has been proposed as a mechanism for *K. veneficum* avoiding autotoxicity (Deeds and Place, 2006; Place et al., 2006, 2009). The ichthyotoxicity of karlotoxins can be traced to its targeting the gills of fish and especially the chloride cells responsible for osmoregulation in these animals (Deeds et al., 2006).

Originally two families of karlotoxins were described as belonging to the KmTx 1 & KmTx 3 and KmTx 2 groups, which differ from one another in UV absorbance maxima, potency, and geographic distribution (Bachvaroff et al., 2009; Deeds et al., 2004). Although the reports of toxic compounds from *K. veneficum* (originally *Gymnodinium veneficum*) date back to the 1950s (Abbott and Ballantine, 1957), it has only been in recent years that structures were reported for KmTx 1 & KmTx 3 (Van Wagoner et al., 2008, 2010) and KmTx 2 (Peng et al., 2010) including the

Abbreviations: KmTx, Karlotoxins; CCMP, Culture collection of marine phytoplankton.

* Corresponding author. Tel.: +1 301 410 234 8828; fax: +1 3410 234 8896.
E-mail address: place@umces.edu (A.R. Place).

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absolute configuration for the latter compound. With the structures now reported, the difference between the compounds in carbon chain structure is known to be localized to the length of the lipophilic side chain. In KmTx 1 the side chain is 18 carbons in length (C48–C65), whereas in KmTx 2 it is two carbons shorter (C48–C63). KmTx 3 differs from KmTx 1 in having one less methylene group in the saturated portion of its lipophilic arm (Van Wagoner et al., 2010). The karlotoxins, like the amphidinols, have a hairpin-like structure with three distinct regions: a polyol arm that exhibits variable hydroxylation and methylation, a hinge region containing two ether rings, and a lipophilic arm that often includes conjugated trienes in amphidinols but in karlotoxins contain a terminal diene, which gives these compounds their distinctive UV spectra. While these substances are known to be highly toxic to fish (Deeds et al., 2006) and mammalian cells (Deeds et al., 2002), little information is available on their toxicity to mammals. In the present study, the acute effects of three karlotoxins KmTx 1, KmTx 2, and KmTx 3 have been investigated in mice through both intraperitoneal injection and oral administration.

2. Material and methods

Samples of purified KmTx 2 were from a culture of CCMP 2064 (Bachvaroff et al., 2008) and samples of purified KmTx 1 and KmTx 3 were from a *K. veneficum* bloom in the Baltimore Inner Harbor in September of 2005 (Adolf et al., 2008). Based on integration of the MS spectra of the purified UV peak, KmTx 3 was a mixture of two congeners KmTx 3 (80% 1347.8 Na adduct) and KmTx 7 (20% 1331. 8 Na adduct). Similarly, KmTx 1 was a mixture of two congeners KmTx 1 (70% 1361.8 Na adduct) and 30% KmTx 6 (1345.9 Na Adduct). The purity of all three substances based on UV absorbance exceeded 95%. They were dissolved in absolute ethanol and stored at −20 °C before use. Their planar structures are shown in the Graphical Abstract. The KmTx 1 and KmTx 3 mixtures will be referred to as KmTx 1 and KmTx 3 throughout the manuscript.

2.1. Experiment 1. Acute toxicity of KmTx 2 by intraperitoneal injection

In an initial study, 10 female Balb/c mice, of initial body weight 20 g, were injected intraperitoneally with KmTx 2 at 50, 100, or 500 μg/kg. The ethanolic solutions were diluted in 1% Tween 60 in saline, and 1 ml of the diluted solution injected. After 24 h, 2 mice from each treatment group were killed by cervical dislocation and major abdominal organs taken for histological examination. After fixation, paraffin-embedded tissues were stained with hematoxylin and eosin (H&E), according to Clark (1981).

2.2. Experiment 2. Acute toxicity of KmTx 1 and KmTx 3 by intraperitoneal injection

Female Swiss mice, initial body weight between 18 and 22 g, were injected intraperitoneally with the test substances at 500, 1,000, 2000 and 4000 μg/kg. The ethanolic solutions were diluted in 1% Tween 60 in saline, and 1 ml of the diluted solution injected. The appearance and behavior of the mice were closely monitored for 14 h after dosing, and abnormalities were recorded.

2.3. Experiment 3. Effect of intraperitoneal injection of KmTx 1 and KmTx 3 on mice at a dose of 4000 μg/kg

Groups of 4 mice were injected with KmTx 1 and KmTx 3 at a dose of 4000 μg/kg. A further group of 4 mice served as control, being injected with vehicle alone. After 24 h, the mice were weighed and then killed by carbon dioxide inhalation. Blood samples were taken for biochemistry and hematology. At necropsy, the liver, kidneys, spleen, lungs and heart of each animal were weighed, and these tissues, together with the pancreas and sections of the jejunum and peritoneum, were preserved in 4% buffered formalin for subsequent histological examination. The weights of the stomach and the whole of the intestinal tract were also recorded at necropsy, and weighing was repeated after the contents had been rinsed away under running water. From these measurements, the weights of stomach and intestinal contents were calculated. Blood packed cell volumes and hemoglobin levels were measured, and plasma activities of creatine kinase (CK), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as indicators of liver damage and plasma levels of creatinine, sodium, potassium and chloride as indicators of kidney damage were determined (Gribbles Veterinary, Hamilton, NZ).

2.4. Experiment 4. Effect of oral administration of KmTx 1 and KmTx 3 on mice at a dose of 4000 μg/kg

Groups of 4 mice were dosed by gavage with KmTx 1 and KmTx 3 at 4000 μg/kg or with vehicle. The mice were killed and necropsied 24 h later. Organ weights were recorded, and hematological and biochemical parameters were assayed as in Experiment 3.

2.5. Experiment 5. Long-term effects of intraperitoneal injection of KmTx 1 and KmTx 3 on mice

Three groups of 4 mice were housed individually. Their body weights and food and water intakes were measured daily. After 5 days, the mice were injected intraperitoneally with vehicle or with KmTx 1 and KmTx 3 at a dose of 4000 μg/kg. Body weights, food intakes and water intakes were measured for a further 14 days, after which time the mice were killed and necropsied. Organ weights were measured, and hematological and biochemical parameters were assayed as in Experiment 3.

3. Histology

Fixed tissues were cut at 3 μm and stained with hematoxylin and eosin (Clark, 1981) for examination by light microscopy.

4. Statistical analysis

Statistical significance of the data was evaluated by analysis of variance followed by the Student–Newman–Keuls