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SMALL INTESTINAL INJURIES IN MICE CAUSED BY A NEW TOXIN, AZASPIRACID, ISOLATED FROM IRISH MUSSELS

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ABSTRACT

Pathological changes of the small intestine caused by a new toxin, azaspiracid, from Irish mussels were studied. Human poisoning cases included both diarrhetic shellfish and paralitic shellfish poisoning symptoms. The present paper focused on the former. Injuries were observed in the upper part of the small intestine, where lamina propria in the villi became atrophied at the initial stage, followed by desquamation of epithelial cells and shortening of villi. The injuries were different from the DSP toxin okadaic acid; 1) they developed very slowly after a lag time of about 3 hr, 2) recovery was very late, 3) initial target and process were different.

INTRODUCTION

Occurrence of a new type of food poisoning resulting from ingestion of the mussel *Mytilus edulis* was first reported in the Netherlands in 1995. These mussels were cultivated in Killary Harbour in Ireland [1; 2]. The mussel toxicity was again observed in 1997 at Arranmore Island region in Ireland [3]. Symptoms observed in patients were nausea, vomitting, diarrhea and stomach cramps, and thus resembled those of diarrhetic shellfish poisoning (DSP). However, in bioassay tests with mice, other than DSP symptoms, neurological symptoms were also observed. In the mussel farming area, there were no DSP or PSP (paralytic shelfish poisoning) producing plankton. The new toxin, azaspiracid (Fig. 1) was isolated from the mussels [4].

Pathological changes in orally administered mice were fatty changes in the liver, decreased number of lymphocytes, and small intestinal erosion. The injuries of the villi in the small intestine by azaspiracid were different from those by the DSP toxin okadaic acid, that is, the former had a time lag of about 3~4 hr before destruction and recovery took a long time, whereas the latter had transient changes [5].

In the present report, the changes in the small intestine according to dose and time-course by orally administered azaspiracid were studied in *in vivo* experiments in mice.

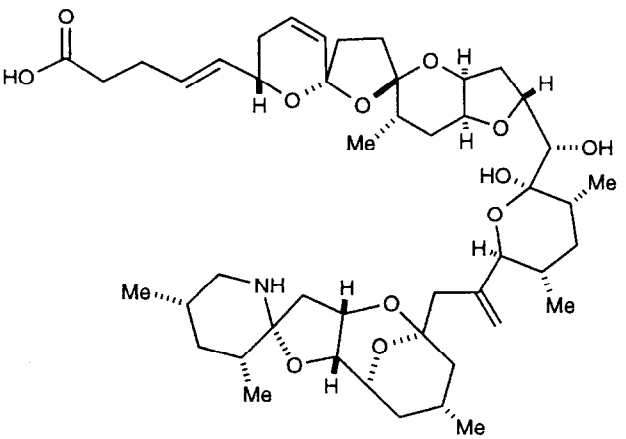

Fig.1 Structure of azaspiracid

MATERIALS AND METHODS

Azaspiracid was extracted from mussels *Mytilus edulis* collected at Killary Harbor in Ireland in 1996 and purified as described previously [4]. Stock solution of azaspiracid was prepared by dissolving 100μg of the toxin in 1 ml of aqueous 50% ethanol (w/v). ICR male mice were used for the experiments. For administration by gastric intubation, aliquots of the stock solution were diluted in 0.2 ml of saline. After administration at 300μg/kg, the mice were killed at 30, 60 min, 2, 3, 4, 8, 16 and 24 hr. With lethal doses of 500 and 600 μg/kg, mice were killed at 16 and 24 hr, and mice given 700 and 900 μg/kg were killed at very weakened stages. Internal organs from all mice were fixed in 20% neutral formalin for light microscopy and in Karnovsky's solution (4% paraformaldehyde, 5% glutaraldehyde in 0.2M cacodylate buffer, pH 7.4) for scanning electron microscopy. For observation, the specimens were treated as described previously [6].

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RESULTS

General observation

With the lethal dose of 500 μg/kg, mice showed neither diarrhea nor body weight loss within 24 hr. Even at the higher dose of 900 μg/kg, mice did not show any behavioral changes during 4 hr before sudden death. At autopsy, characteristic changes were swelling and fatty changes of the liver and accumulation of fluid in the small intestine.

Changes in the small intestine

Tissue changes caused by azaspiracid were observed in the upper part of the small intestine, and moderate volume of fluid was seen; these were similar to those caused by okadaic acid.

With 600 μg/kg, epithelial cells were eroded, followed by exposure of the lamina propria in the lumen and intestinal glands of Lieberkühn after 8 hr (Fig. 2a). With the higher dose of 900 μg/kg, desquamation of epithelial cells occurred, resulting in exposure of the lamina propria and empty crypts of Lieberkühn after 4 hr (Fig. 2b). Thus, in fatal cases, azaspiracid injured epithelial cells in a short period.

Fig. 2a

Fig. 2 Small intestinal injuries of mice treated p.o. at higher doses.
a After 8 hr with azaspiracid at 600 μg/kg, epithelial cells of villi became desquamated. LP: lamina propria; arrows: intestinal glands of Lieberkühn. SEM.

Fig. 2b. At 4hr with azaspiracid at 900 μg/kg. Epithelial cells of villi desquamated together with crypt cells. LP: lamina propria; arrow: hole of crypt of Lieberkühn. SEM

On the contrary, with a lower dose the injury started in the lamina propria. With 300 μg/kg, the onset of sporadic necrosis caused by nuclear pyknosis in the lamina propria of the villi was confirmed after 3 hr (Fig.3a). The atrophied lamina propria was spatially separated from epithelial cells, and vacuolization of epithelial cells was prominent after 4hr (Fig. 3b). Then villi became shorter by losing their upper parts after 8 hr, and the degenerating cells were still separating from the top (Fig. 3C), but neither the lamina propria nor crypts of Lieberkühn were exposed.

After 24 hr, epithelial cells showed signs of recovery but lamina propria lagged in recovery. Some villi were still empty inside or contained necrotized lamina propria (Fig. 4a), but on the surface, villi did not reflect the inside condition and looked as if they were undergoing a normal recovery process (Fig. 4b).

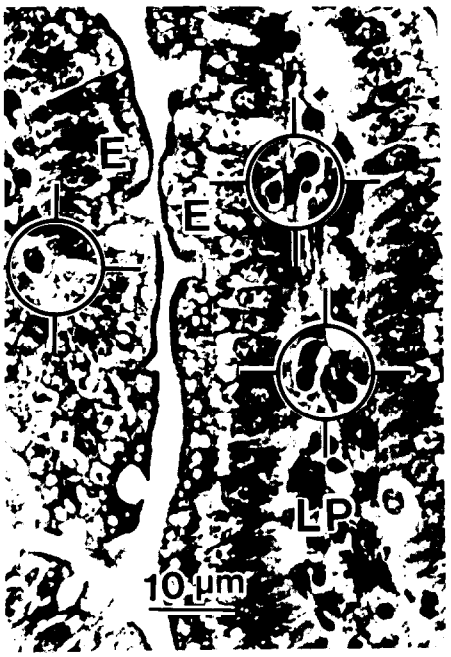
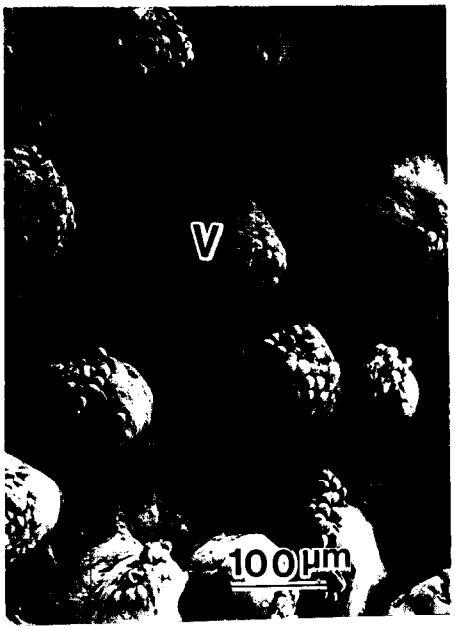


Fig. 3a

Fig. 3 The progression of small intestinal injuries.

a. Changes initiated at the lamina propria after 3 hr with 300 μg/kg, and sporadic necrosis with nuclear pyknosis is seen.
E: epithelial cells of the villi; LP: lamina propria circles: necrosis with nuclear pyknosis.LM.

3b. After 4 hr with 300 μg/kg. Atrophyc lamina propria was spacially separated from epithelial cells and the inside of villi became almost empty LP: lamina propria; arrows: vacuolar degeneration of epithelial cells (E). LM.

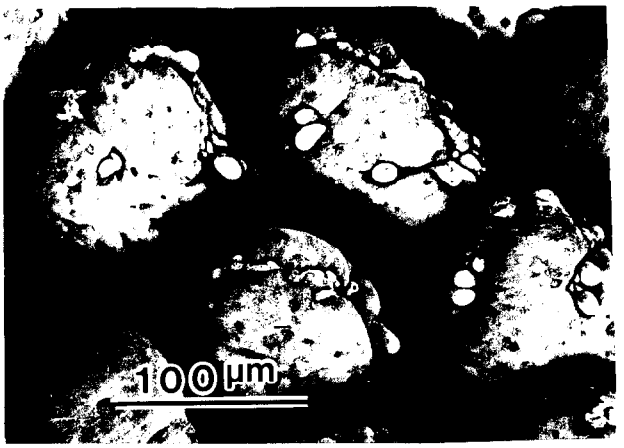


3c. After 8 hr, villi (V) became shorter, losing the upper parts. Necrotizing cells still cover the tops. SEM.

Fig. 4a

Fig. 4 Recovery process 24hr after administration of 300 μg/kg.

4a. Lamina propria lagged in recovery, and some villi were still empty inside (*) or contained necrotized lamina propria. LP: lamina propria; E:epithelial cells. LM.



4b In the lumen, the surface arrangement of villi was irregular, but normal recovery seemed underway. V: villi. SEM.

DISCUSSION

In the small intestine, severe changes by azaspiracid at 600~900 μg/kg caused desquamation of the epithelial cells during 8 hr. With a sublethal dose of 300 μg/kg, the onset of the degeneration of villi was characterized
by necrotic atrophy of the lamina propria by nuclear pyknosis within 3 hr.

There are some types of pathological changes of villi; 1) hypersecretion of mucus from goblet cells, such as cholera toxin and ciguatoxin [7], 2) edema of epithelial cells resulting in desquamation, such as okadaic acid [8], 3) degeneration of blood vessels (a rise in permeability or breaking) such as palytoxin [9], lyngbyatoxin [unpublished] and aplysiatoxin [6; 10], 4) atrophy of lamina propria, and 5) others.

With sublethal doses of okadaic acid or azaspiracid, although the appearance of villi looked to have similar changes at the developed stage, the representative DSP of okadaic acid was type 2) and its injuries were not so severe and the recovery was rapid, meaning that the process was transitional [5; 8]. On the other hand azaspiracid was type 4), because the target in the villi was the base of its structure, and therefore the recovery was extremely late. The partially purified toxin fraction KT3 caused almost the same injuries in each organ as azaspiracid, such as fatty changes in the liver and necrosis in lymphoid tissues, but it caused much more fluid accumulation in the small intestine [11] than this pure toxin. Thus the azaspiracid fraction should be explored in the future to explain the differences in severity of diarrhea.

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