Experimental Animals

Original

Drastic hypothermia after intraperitoneal injection of okadaic acid, a diarrhetic shellfish poisoning toxin, in mice

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Abstract: The mouse bioassay for diarrhetic shellfish poisoning (DSP) toxins had been used as the official method in Japan and also used in the world. In this study, hypothermia, one of the symptoms observed in mice after inoculation with DSP toxins, were characterized. Lethal and sublethal doses of okadaic acid (OA), a representative component of DSP toxins, were inoculated intraperitoneally into mice. Body-temperature changes over time were measured by an electronic thermometer or monitored by an infrared camera. Drastic hypothermia (<30°C in some mice) was observed in a few hours after administration of a lethal dose of OA. Dose-dependency was clearly seen between doses of OA inoculated and body-temperature decrease. Drastic hypothermia was also detected by using an infrared camera. These results suggest that hypothermia could be used as an index for the humane endpoint in experimental animal toxicological studies.

Key words: diarrhetic shellfish poisoning (DSP) toxin, hypothermia, mouse, okadaic acid

Introduction

Diarrhetic shellfish poisoning (DSP) is a food poisoning disease caused by the ingestion of shellfish contaminated with algal toxins produced by marine dinoflagellates [1]. The main symptoms are diarrhea, nausea, and vomiting. DSP has been recognized as a worldwide public health problem since the first case reported in Japan [2].

The mouse bioassay (MBA) was first determined as the official method for DSP toxin detection in Japan in 1981 [3]. Since then, the MBA (also known as Yasumoto’s method) had been widely used in many countries of the world [4], including the European Union [5], with some minor modifications. The MBA was a useful and reliable screening method and no human cases due to contaminated bivalves with DSP toxins distributed in commercial markets have been reported for more than 30 years in Japan. However, the MBA was time-consuming and criticized on ethical grounds. No humane endpoints were provided and mouse death was the only index for levels of DSP toxins above the regulation limit.

The European Commission authorized the use of the MBA until December 31, 2014 [5], and the Japanese Government has also decided to replace the MBA with LC-MS/MS for okadaic acid (OA) group toxins since April 1, 2017 [6]. However, the MBA has been still used in some countries in Africa and Asia [7].

In our previous study [8–11], the mice injected with a lethal dose of OA, a representative component of DSP toxins, showed asthenia and hypothermia within a few hours after inoculation. And we preliminarily reported that the decreases of body temperature were observed in mice intraperitoneally injected with lethal dose of OA.
by a thermometer and an infrared camera [12].

In the present study, we aimed to ascertain if hypothermia was correlated with the doses of toxin injected and/or whether hypothermia could be used as a humane index of the MBA for the detection of DSP toxins.

Materials and Methods

Specific-pathogen-free male ICR mice (4 weeks) were purchased from Japan SLC Inc. (Shizuoka, Japan). Mice were allowed to acclimatize in our animal facility for 1 day and used at 18–20g body weight. The room lighting was 12 h light (09:00–21:00) − 12 h dark (21:00–09:00) cycle. Mice were housed in plastic cages with woodchip bedding. They had access to commercial pellets (CRF-1; Charles River Japan Inc., Kanagawa, Japan) and tap water ad libitum. All animal experiments were conducted with the approval of the Animal Care and Use Committee of the National Institute of Health Sciences, Japan, and all experiments were performed in accordance with the Ethical Guidelines for Researchers of the National Institute of Health Sciences, Japan.

Chemicals

OA was purchased from LC Laboratories (purity >98%, Woburn, MA, USA). OA inoculum was prepared as described previously [8–10]. Briefly, OA was first dissolved in acetone and mixed with soybean oil. After removing acetone by evaporation, 1% Tween-60 saline was added to the residue and sonicated. The final inoculum contained 10% soybean oil.

Experimental design

Changes in colorectal temperature after administration of a lethal dose of OA: A lethal dose of OA (4 µg/mouse) [13] was injected intraperitoneally (i.p.) into each mouse. Only vehicle was injected i.p. into control mice. Experimental and control groups each comprised 4 or 5 mice. The colorectal temperature of mice was measured before and every 1 h (up to 12 h) and 24 h after inoculation using an electronic thermometer (CTM-303; Terumo Corp., Tokyo, Japan). Each mouse was removed from the cage and held manually in order to insert a probe (lubricated with olive oil before each insertion) 2 cm into the colon. The colorectal temperature was read 30 s after insertion. This experiment was repeated twice.

Changes in colorectal temperature after administration of sublethal doses of OA: Sublethal doses of OA (1 and 2 µg/mouse) were injected i.p. into each mouse. Only vehicle was injected i.p. into the control mice. Experimental and control groups each comprised 5 mice. Colorectal temperature was measured before and every 1 h (up to 8–10 h) and 24 h after inoculation using an electronic thermometer. The procedure for measurement of colorectal temperature was the same as that described above.

Changes in body surface temperature by thermography after administration of lethal and sublethal doses of OA: Lethal and sublethal doses of OA (1, 2, and 4 µg/mouse) were injected i.p. into each mouse. Only vehicle was injected i.p. into control mice. Experimental and control groups each comprised 5 mice. The body surface temperature of mice was monitored before and every 1 h (up to 10 h) and 24 h after inoculation using an infrared camera (FLIR i5, FLIR Systems, Inc., Wilsonville, OR, USA). In the first experiment, only lethal-dose OA and control groups were compared. In the second experiment, lethal- and sublethal-OA groups, together with the control group, were compared.

Results

Changes in colorectal temperature after administration of a lethal dose of OA

In the lethal-dose OA group, the colorectal temperature of all mice fell to <35°C at 1 h after inoculation, and decreased to <30°C at 2 h and/or 3 h after inoculation in some mice (Figs. 1 and 2). In the first experiment (Fig. 1), 2 out of 5 mice died and 3 mice survived. At 24 h after inoculation, the colorectal temperatures of the 3 surviving mice almost recovered to those recorded before inoculation. In the second experiment (Fig. 2), 4 out of 5 mice died and 1 mouse survived. However, the colorectal temperature of the surviving mouse did not recover, and the mouse died a few hours after the end of the observation period (≥24 h after inoculation). In the control group, the colorectal temperature of mice showed variations but not such drastic decrease as seen in the OA-inoculated group.

Changes in colorectal temperature after administration of sublethal doses of OA

In the 2 µg OA group, the colorectal temperature of most mice decreased to <35°C at 2 h and/or 3 h after inoculation, but then recovered (Fig. 3). In the 1 µg OA-inoculated group, the colorectal temperature of mice decreased by a few°C at 2 h and/or 3 h after inoculation, but then recovered (Fig. 4). No deaths were observed in either experiment.

The colorectal temperature of mice at 2 h after inoculation and dose of OA administrated are shown in Fig. 5. The temperature at 2 h after inoculation was chosen because one of the mice that died after being inoculated a lethal dose of OA died before 3 h after inoculation. The
Changes in body surface temperature by thermography after administration of lethal and sublethal doses of OA: In the first experiment, a lethal dose (4 µg) of OA group and control group were compared using an infrared camera. Thermograms of mice in the lethal-dose OA group changed from red to yellow at 1 h after inoculation, and to green / blue at 2 h after inoculation or later (Fig. 6), suggesting drastic decrease in body surface temperature. In the lethal-dose OA group, 4 out of 5 mice died within 24 h after inoculation. The thermogram of the surviving mouse was blue, which meant that body temperature had not recovered, and the mouse died a few hours after the end of the observation period (≥24 h after inoculation). The thermograms of mice in the control group did not show such changes.

In the second experiment, lethal- (4 µg) and sublethal (2 and 1 µg)-dose OA groups and the control group were compared using an infrared camera. At 1 h and 2 h after inoculation, thermograms of 4 out of 5 mice in the 4-µg OA group changed from red to yellow, and to green / blue at 4 h after inoculation or later (Fig. 7), suggesting drastic decrease in body surface temperature. Thermograms of one mouse, however,
changed slightly to yellow at 6 h after inoculation but returned to red at 8 h after inoculation or later. In the 4-µg OA group, 3 out of 5 mice died within 24 h after inoculation. The thermogram of the one surviving mouse was green and that of the other mouse was red. The former died a few hours after the end of the observation period but the latter survived. In the 1-µg and 2-µg OA groups, thermograms changed slightly to yellow at 1–4 h after inoculation (Fig. 7), suggesting slight decrease in body surface temperature, but returned to red (normal) later. No deaths were observed in 1-µg and 2-µg OA groups. Thermograms of mice in the control group did not show such changes. The highest temperature of thermograms of each mouse area at 4 h after inoculation and dose of OA administrated in the second experiment are shown in Fig. 8 The body surface temperature of mice at 4 h after inoculation and dose of OA inoculated showed a moderate relationship with a relatively high coefficient of determination (R^2=0.7227).

**Discussion**

Hypothermia, one of symptoms observed after OA inoculation in mice, was examined in the present study. The lethal dose of OA inoculation induced very rapid and drastic decrease in colorectal temperature in mice.
Fig. 5. Dose-colorectal temperature relationship at 2 h after inoculation.

![Graph showing colorectal temperature vs dose with equation and R^2 value.

Fig. 6. Thermograms after lethal dose of OA inoculation.

![Thermograms of OA inoculated group and control group with deaths noted.

Fig. 7. Thermograms after lethal and sublethal doses of OA inoculation.

![Thermograms showing effects of different doses and group comparisons.

Fig. 8. Dose-body surface temperature relationship at 4 h after inoculation.

![Graph showing body surface temperature vs dose with equation and R^2 value.
That is, the colorectal temperature fell to <35°C in all mice at 1 h and decreased to <30°C in some mice at 2 h and/or 3 h after inoculation. Sublethal doses of OA also induced relative decrease in colorectal temperature in mice, and the decrease in colorectal temperature showed clear dose-dependency with OA doses. Such decrease in body temperature measured by insertion of a probe could also be detected using an infrared camera, but the temperature was less accurate and dose-dependency less clear.

Lethality of mice injected with a lethal dose of OA was 40–80% at 24 h after inoculation, but rapid and drastic decrease in body temperature was observed within a few hours after inoculation in all mice. In the former official method, the lethality of mice must be observed at 24 h after inoculation. On the other hand, rapid and drastic decrease in body temperature was observed within a few hours after inoculation. In the present study, the extent of body-temperature decrease and fate (death or survival even later than 24 h after inoculation) of mice were thought to be related. These data suggest that decrease in body temperature might be used as a rapid and humane index of the MBA for DSP toxin detection. In particular, by using an infrared camera, stress-free monitoring of body temperature is possible. Though the temperature obtained is not accurate compared with colorectal temperature, it is sufficiently accurate to estimate the outcome. Using an infrared camera for monitoring the body temperature of mice is stress-free for both investigators and animals, because holding animals is quite stressful not only for the animals but also for the investigators.

The European Commission has announced that the biological test for the detection of DSP toxins in bivalve molluscs such as mussels and scallops was replaced by a liquid chromatography–mass spectrometric (LC-MS/MS) method and that the MBA might still be used until 31 December 2014 [5]. The Japanese Government also replaced the MBA with LC-MS/MS for OA group toxins since April 1, 2017 [6]. In other countries or area, however, whether the MBA will be replaced or continued to be used for the detection of DSP toxins is not clear.

There have been reported that the reduced body temperature of the mouse might be a good marker for determining the humane endpoint in the several study, including infectious diseases [14–16], aging [17], and shock [18]. In this experiment, for instance, lower than 30°C in colorectal temperature of the mouse at any points of the experiment might be able to set as a humane endpoint (and also be able to estimate the test sample might contain DSP toxins more than regulation limit (0.05 mouse unit/g)), although the mechanisms for the hypothermia after OA inoculation should be elucidated. This study would be not only for the MBA for DSP toxins, but also for presenting a good example to use body temperature as an index for the humane endpoint in experimental animal toxicological studies, especially for the toxic substances which induced hypothermia followed by death.

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Conflict of Interest

The author declares that there are not conflict of interest.

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