NOTE *Ethology*

Anti-anxiety Effect of Ovary Lipid Extracted from Skipjack Tuna (*Katsuwonus pelamis*) in Rats

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ABSTRACT. Using an elevated plus-maze test, we evaluated anxiety level in rats given ovary lipid extracted from Skipjack tuna (*Katsuwonus pelamis*; OLS). The percentage of open time was significantly higher in rats given OLS than in rats in the control group, but lower than in rats given diazepam (1.0 mg/kg body weight). Based on this fact and findings about other indicators, this study showed that OLS does not have as fast-acting and strong an anti-anxiety effect as diazepam but that continuous ingestion of OLS causes an anti-anxiety effect in animals.

KEY WORDS: anxiety, elevated plus-maze test, ovary lipid.

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Ovary lipid from Skipjack Tuna (*Katsuwonus pelamis*; OLS), extracted and purified using our patented method (JP Laid-Open No. 2004–2663), contains a large amount of docosahexaenoic acid (DHA) in the form of phospholipids such as phosphatidylcholine, phosphatidylethanolamine, and lysophosphatidylcholine. We hypothesized that if OLS ingestion relieves anxiety in companion animals, it could be useful in the treatment of problem behaviors. The elevated T-maze test is an excellent tool used for distinguishing an animal's underlying and intrinsic anxiety and/or fear from memorized behaviors [2, 5, 11, 13, 14]. Previously, we studied anxiety and/or fear using an elevated T-maze test with rats given OLS. We found that OLS shortened avoidance latency at the first trial, indicating that it has an effect against anxiety and/or fear [9]. In the present study, we evaluated the anti-anxiety effect of OLS using an elevated plus-maze test [1, 3, 4, 6–8, 12, 15], which is often used to determine anxiety level.

In the previous study using an elevated T-maze test, rats were given OLS mixed as a lipid component in feed. As OLS is rich in n-3 fatty acids, the control feed was prepared to give the same energy percentage as n-3 fatty acids and the same n-6/n-3 ratio [9]. In the present study, rats were also given OLS mixed as a lipid component in feed. As this study evaluated anxiety level, however, the control group was given AIN93 and the OLS group was given AIN93, in which the lipid content was partly replaced with OLS. The animal raising period for the previous elevated T-maze test was 42 days. OLS ingestion for a shorter period decreased the rodent stress hormone corticosterone under acute stress [10]. Thus, the raising period was set at 28 days in the present study. Diazepam was used as a positive control. Observations were recorded during the first 5 min [7] (0–5 min) and then up to 15 min [8] (5–15 min) thereafter.

The ovary was removed from the Skipjack tuna (*Katsuwonus pelamis*), washed with water, boiled, freeze-dried, powdered, and extracted with ethanol. The resultant extract was filtered and concentrated to obtain ovary lipid of Skipjack tuna (OLS).

Four-week-old male Wistar rats (Japan SLC, Inc.) were used in the experiments. They were raised in an animal room maintained at a temperature of 23 ± 1°C, humidity of 55%, and a light/dark cycle of 12 hr (lighting 7:00–19:00, automatically controlled) in Facility II at the Animal Experiment Center of the University of Shizuoka (Yada, Suruga-ku, Shizuoka-shi). The rats were divided into a control group (n=14), a diazepam group (n=11) and an OLS ingestion group (n=13); they were given test feeds for 28 days. The test feeds were prepared according to AIN93 and given to the animals *ad libitum*. The feed for the control and diazepam groups used corn oil as an oil/fat component (n-3 fatty acids: 0.2 energy %, n-6/n-3: 33.7) and the feed for the OLS group was prepared by partly replacing the corn oil that was used in the feed for the control and diazepam groups with OLS (the feed contained 0.9% OLS, n-3 fatty acids: 0.9 energy %, n-6/n-3: 5.7).

This study was conducted with the approval of the Animal Experiment Center Steering Committee at the University of Shizuoka, in compliance with the Guidelines for Animal Experiments at the University of Shizuoka.

The test was conducted between 19:00 and 0:15. Rats were transferred to a test cage 60 min before the start of the test. An intraperitoneally administered diazepam solution of 500 μg/m*l* (0.9% NaCl, 2% TWEEN 20) was administered to rats in the diazepam group 40 min before the start of the test to give a total dose of diazepam 1.0 mg/kg body weight. Similarly, rats in the control and OLS groups received intraperitoneal vehicle (0.9% NaCl, 2% TWEEN20) at a total dose of 2.0 m*l*/kg body weight. The elevated plus-maze was set at a height of 70 cm from the floor. The two closed arms were 40 cm high, 50 cm long, and 10 cm wide. The two open arms with a ledge of 5 mm

were 50 cm long and 10 cm wide. The illumination intensity was 3 lux on the surfaces of both the closed and open arms. At the start of the test, a rat was put at the center of the plus-maze and positioned facing an open arm; behavior was then recorded for 15 min with a CCD camera. Data from rats that could not be positioned facing an open arm were excluded. Some animals in the diazepam group fell from an open arm. These data were excluded. After completion of the test, the recorded images were analyzed. The behavioral indicators of anxiety included % open time, % closed time, % center time, number of open arm entries, and number of total arm entries [15]. In addition to these indicators, measurements were made of other indicators such as grooming duration as an indication of approach/avoidance conflict and vertical activity, total rears as an indication of vertical activity, and head-dips as an indication of exploration [15].

Data were expressed as mean ± standard error (SE) and were analyzed using one-way analysis of variance. Multiple comparisons were performed using the Tukey-Kramer test at a significance level of 5%.

Table 1 shows the body weights of each group at the time of raising and at the time of the test. No significant between-group differences in body weights at the time of raising and at the time of the test were observed.

Figure 1 shows results for % open time, % closed time, % center time, number of open arm entries, and number of total arm entries in (0–5 min) and (5–15 min) observation intervals. In the (0–5 min) interval, the % open time increased in the order of the control, OLS, and diazepam groups, respectively. The % closed time was opposite to the result for % open time and there was no significant difference in the % center time between the 3 groups. In the (5–15 min)interval, no significant difference in % open time was observed between the 3 groups. The % closed time in the (5–15 min) interval was lower in the diazepam group than in the control and OLS groups and the % center time was the highest in the diazepam group. In the (0–5 min) interval, more open arm entries were seen in the diazepam group than in the control group and the OLS group had an intermediate value; there was no significant difference in total arm entries between the 3 groups. In the (5–15 min) interval, the number of open arm entries and total arm entries were not significantly different between the control and OLS groups but the diazepam group had a lower value than the control and OLS groups.

Figure 2 shows results for other indicators. A comparison of the indicators between the (0–5 min) and (5–15 min) intervals showed that in any of the three groups, grooming duration was higher in the (5–15 min) interval than in the (0–5 min) interval and that head-dips and rears were lower in the (5–15 min) interval than in the (0–5 min) interval. No significant difference in grooming duration was observed between the 3 groups in both the (0–5 min) and (5–15 min) intervals. The number of total rears was lower in the diazepam group than in the other two groups in both the (0–5 min) and (5–15 min) intervals. For head-dips in the (0–5 min) interval, the diazepam and OLS groups had a significantly higher value than the control group. For head-dips in the (5–15 min) interval, the OLS group had a significantly higher value than the other two groups.

An elevated plus-maze test is considered to be an approach/avoidance conflict model in which approach behavior (curiosity) based on an exploratory drive that animals have inherently is in equilibrium with avoidance behavior driven by anxiety and fear [15]. The anti-anxiety effect was observed remarkably in the (0–5 min) interval in the diazepam group. However, in the (5–15 min) interval in that group, decreased locomotor activity (a lower number of total arm entries) probably due to the hypnotic effect of diazepam was found and a decrease in exploration (decreased head-dips) as a drive for approach was observed. Furthermore, vertical activity (total rears) was lower in the diazepam group than in the control group. In contrast, OLS seemed to have a continuous anti-anxiety effect within the time prescribed in this study although the effect was less strong than diazepam. Head-dips were significantly higher in the OLS group than in the control group in the (0–5 min) interval and than in the control and diazepam groups in the (5–15 min) interval. This suggests that OLS suppresses a decrease over time in exploration inherent in animals under the conditions of anxiety and fear. In addition, unlike the diazepam group, the OLS group showed a decrease in vertical activity. These data may suggest the possibility that the anti-anxiety effect is different between OLS and diazepam.

In conclusion, this study showed that OLS does not have such a fast-acting and strong anti-anxiety effect as diazepam but that continuous ingestion of OLS causes an anti-anxiety effect in animals.

In future, we intend to conduct further study into the consistency of the anti-anxiety effect of OLS and the effect of its combination with anti-anxiety drugs.

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Table 1. Body weight of rats

Body weight		Control	Diazepam	OLS
Initial	(g)	59.3 ± 0.4	60.9 ± 0.7	60.5 ± 0.8
Final	(g)	211.4 ± 2.6	215.3 ± 3.9	219.8 ± 2.4

Control: control group, n=14. Diazepam: diazepam group, n=11. OLS: group that received ovary lipid from skipjack tuna, n=13.

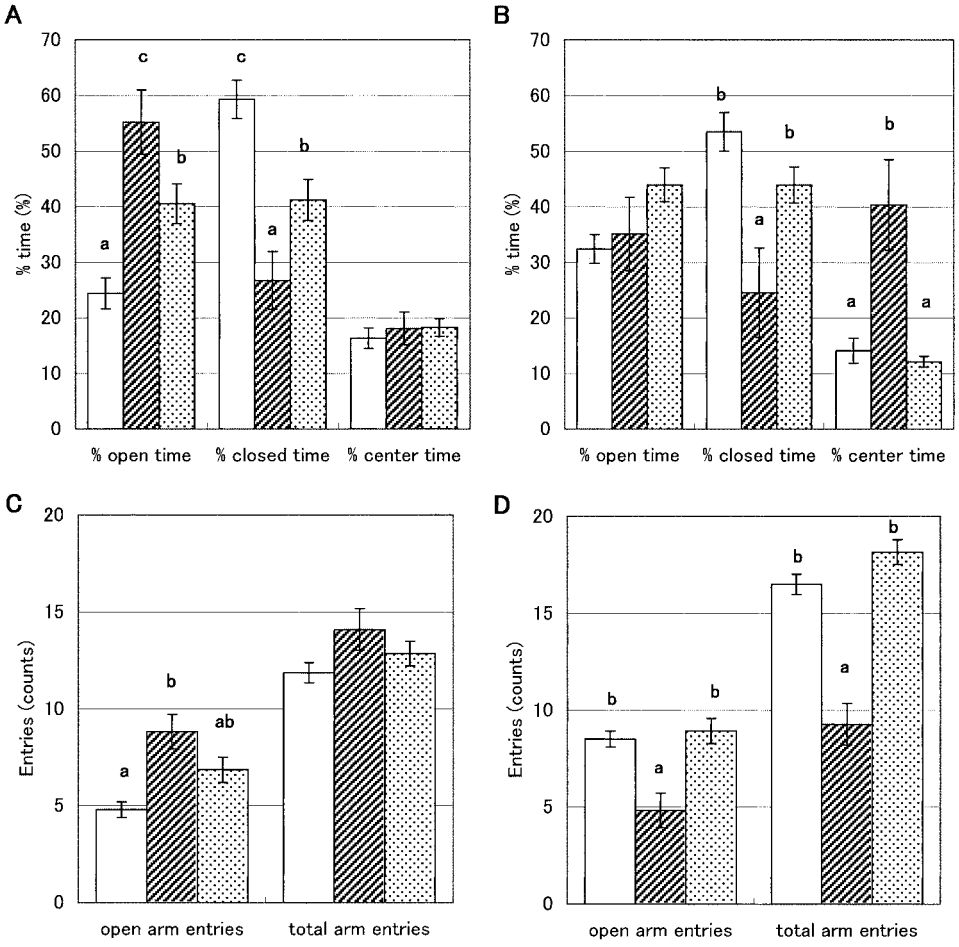

Fig. 1. Evaluation of the anti-anxiety effect of ovary lipid from Skipjack tuna (OLS) using an elevated plus-maze test. A, % time in 0–5 min intervals; B, % time in 5–15 min intervals; C, open arm entries and total arm entries in 0–5 min intervals; and D, open arm entries and total arm entries in 5–15 min intervals. Data are expressed as mean ± SE. Data were analyzed using a one-way analysis of variance and multiple comparisons were made using the Tukey-Kramer method. Means within the same line that do not share a common superscript letter were significantly different ($P<0.05$). □, control group (n=14); ▨, diazepam group (n=11); and ⁝⁝, group that received ovary lipid from skipjack tuna (n=13).

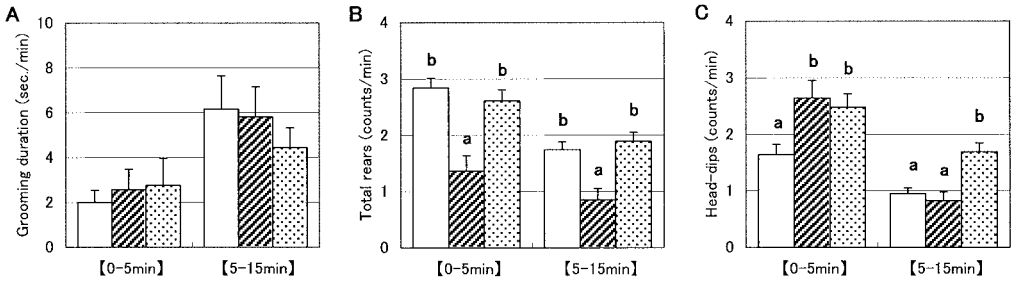

Fig. 2. Other behavioral indicators effect of ovary lipid from Skipjack tuna (OLS) using an elevated plus-maze test. A, grooming duration; B, total rears; and C, head-dips. Data are expressed as mean ± SE. Data were analyzed using a one-way analysis of variance and multiple comparisons were made using the Tukey-Kramer method. Means within the same line that do not share a common superscript letter were significantly different ($P<0.05$). □, control group (n=14); ▨, diazepam group (n=11); and ⁝⁝, group that received ovary lipid from skipjack tuna (n=13).

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