Effects of Dietary Vegetable Oils on Behavior and Drug Responses in Mice

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Previously, we noted significant differences in the behavioral patterns of mice fed safflower oil with a very low α -linolenate/linoleate ratio and perilla oil with a high α -linolenate/linoleate ratio from mothers to offsprings. In this report, we compared the behavior and drug responses in mice fed diets containing six different vegetable oils—corn, rapeseed, soybean, safflower, perilla and a mixture of perilla and safflower oils— for a relatively short period: 8 months after weaning. Soybean oil is a component of most conventional diets and was used as a control. The α -linolenate/linoleate ratios of the oils appeared to affect the locomotor activities in a wheel cage: the activity decreased in the order of safflower, the mixture (1:1) and the perilla oil groups. However, the rapeseed oil group exhibited much higher locomotor activity than that expected from the α -linolenate/linoleate ratio. Additionally, the rapeseed oil group exhibited unusual behavior patterns, including higher ambulation and rearing activities, faster acquisition of the water maze task and slower habituation behavior as compared with the control group. Susceptibility to pentobarbital anesthesia tended to be higher in the rapeseed oil group. The differences in the α -linolenate/linoleate ratios of these oils alone do not account for the observed differences in the behavioral patterns among the six dietary groups. Although we cannot exclude the possibility that the observed behavioral anomaly is due to the unique fatty acid composition of rapeseed oil, we speculate that a factor(s) other than fatty acids in rapeseed oil affected nervous system functions.

Key words rapeseed oil; behavior; drug response; mouse

The question of whether food constituents affect the behavior of mammals has been controversial. The protein/carbohydrate ratio,¹⁾ amino acid precursors of neurotransmitters, and other food constituents^{2–4)} have been reported to affect the mood and behavior patterns of mammals.

Docosahexaenoic acid (22:6n–3, DHA) is enriched in the brain, nervous tissues, retina, testis and muscle. The essentiality of α -linolenic acid (18:3n–3), a precursor of docosahexaenoate, has been established relatively recently for the maintenance of brain and retinal functions in mammals.^{5–9)} Brightness-discrimination learning performance and water-maze learning performance, as well as retinal function, were found to be impaired in animals fed α -linolenate (n–3)-deficient but linoleate (n–6)-enriched diets.^{5,6,8,9)} Furthermore, general behavioral patterns as well as drug sensitivities were shown to be affected in n–3 deficient animals.^{10,11)} Thus, the n–3/n–6 ratio of foods has been proved to be an important factor which regulates nervous system function in mammals.

The precise mechanisms by which the n–3/n–6 fatty acid balance of foods affect brain functions remain to be elucidated. Mammals depend entirely on their diet for their supply of linoleate and α -linolenate. These fatty acids enter the central nervous system through the blood-brain barrier. While the fatty acid compositions of brain lipids are kept relatively constant under various nutritional conditions as compared with the liver and plasma lipids, extensive deficiencies of n–3 fatty acids, *e.g.*, through two generations, are known to induce a substantial decrease in the proportion of DHA in the brain, which in turn is

accompanied by a significant decrease in the brain and retinal functions.^{5–12)}

In the present study, we examined the behavior and drug responses in mice fed various vegetable oils for 8 months after weaning.

MATERIALS AND METHODS

Diets and Animals A conventional diet (CE2, Clea Japan Co., Ltd., Tokyo) was treated with hexane according to a method employed for the preparation of vegetable oils (Ohta Oil Co., Ltd., Okazaki) to remove oils, and then vegetable oils commercially available for human use—corn, rapeseed, soybean, safflower, perilla and a mixture (1:1) of safflower and perilla oils— and a vitamin mixture (Clea Japan Co., Ltd.) were supplemented at levels of 10 and 2% w/w, respectively. Each lot of the diets pelleted by the Clea Japan Co., Ltd. was assayed for peroxide content and fatty acid composition. The diets were fed to ICR male mice *ad libitum* for 8 months after weaning at 3 weeks of age. Animals were housed at $23 \pm 1^\circ\text{C}$ and at $50 \pm 5\%$ relative humidity, maintained on a 12 h alternating light-dark cycle with lights turned on at 0800 h.

Fatty acids were analyzed as methyl esters by gas chromatography. The fatty acid compositions of vegetable oils are shown in Table 1.

Behavioral Tests Details of the behavioral tests were described previously,^{11,13)} and brief accounts are given in the Results section. All tests were performed daily between 10:00 and 18:00 for 1 month after 8 months of dietary manipulation. Mice from the different dietary

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groups were tested alternately to minimize the variations associated with the time of tests.

Statistical Analyses The Student's *t*-test was used in experiments involving the pain threshold test, the elevated plus maze test, and the drug response test. Mann-Whitney's *U*-test was used for analysis of spontaneous locomotor activity, behavior test in open-field, habituation task, and Morris' water maze test. A two-way ANOVA was employed for all the tests. Soybean oil is the major lipid component of most conventional diets for mice and was used as a control.

RESULTS

Spontaneous Locomotor Activity A wheel cage was used for the measurement of spontaneous locomotor activity. The number of revolutions was recorded for 30 min once a day for 14 consecutive days.

The locomotor activity increased day by day in all the dietary groups (Fig. 1). The locomotor activity decreased in the order of safflower, the mixture (1:1) and the perilla oil groups, indicating that the α -linolenate/linoleate ratio affected the locomotor behavior in mice after 8 months of dietary manipulation. However, the locomotor activities were not positively correlated with the α -linolenate/linoleate

ratios when other dietary groups were included. From 2 to 13 d, the locomotor activities of the rapeseed oil group were the highest and were significantly higher than in the control (soybean oil) group (Mann-Whitney *U*-test). The difference between the rapeseed oil group and the control (soybean oil) group was also statistically significant in a two-way ANOVA, $F(5, 1596) = 32.63$, $p < 0.01$.

Behavior in Open Field The open field method was employed for the measurement of emotion.¹³⁾ The apparatus consisted of a wooden square open field (76 × 76 × 15 cm) with the inside surface painted mat dark gray. The field was divided into 36 blocks with 3 mm wide black lines and was lit evenly with a 100 W lamp located 70 cm above the field. The apparatus was placed in a ventilated, darkened, sound-attenuated room. The animal was placed in the center of the open field and was allowed to walk about and explore freely during a 1 min period. Scores for ambulation (the number of times the animal crossed the demarcation line) and rearing (the number of times the animal stood up on its hind legs) were recorded during the 1 min period.

Ambulation by the rapeseed oil group was significantly higher than in all other groups, except for the corn oil group, and that of the corn oil group was higher than that of the control group (Fig. 2). Rearing by the rapeseed oil group was significantly higher than the other groups except for the corn oil group. No significant differences were found in face washing, fur licking, defecation, or urination among the six dietary groups.

Behavior in Morris' Water Maze The spatial learning performance of mice was evaluated using Morris' water maze task¹⁴⁾ with some modifications. The apparatus consisted of a vinyl pool (80 cm in diameter) and a circular platform (8 cm in diameter). The pool was filled with water (kept at *ca.* 23 °C) to a level 1 cm above the platform, and India ink was poured into the pool to hide the platform. The apparatus was placed in a ventilated, sound-attenuated room in which there were many positional cues including a lamp and black and white boards on the wall. There were four starting points in the apparatus, and this point

Table 1. Fatty Acid Compositions of Vegetable Oils

Fatty acid	% of total fatty acids					
	Soybean	Mix (1:1)	Safflower	Corn	Rapeseed	Perilla
16:0	12.2	6.8	7.2	12.5	4.5	6.5
18:0	3.8	1.9	1.7	1.8	1.8	2.0
18:1n-9	22.8	12.8	9.8	27.4	57.4	14.4
18:2n-6	53.7	39.2	80.1	57.6	24.1	12.6
18:3n-3	7.5	39.2	0.1 >	0.7	11.3	63.2
n-3/n-6	0.14	1.0	0.01 >	0.01	0.46	5.0

The Mix, consisting of equimolar proportions of linoleate (18:2n-6) and α -linolenate (18:3n-3) was prepared by mixing perilla oil and safflower oil. Some small variations in fatty acid composition were noted among the lots.

Fig. 1. Locomotor Activity in a Wheel Cage

The number of animals in each group was 20–28, as shown in parentheses. The levels of significance were *, $p < 0.05$ and **, $p < 0.01$ vs. the control (soybean oil) group in Mann-Whitney's *U*-test. The difference between the rapeseed oil group and the control group was significant in a two-way ANOVA ($p < 0.01$).

was changed every day. The animal was put into the water at the starting point facing the wall of the pool. The goal latency, the elapsed time between the time the animal commenced swimming and the time the animal reached the platform, was measured. If the animal failed to find the platform within 100 s, it was placed on the platform by the experimenter. This task was performed twice a day for 5 consecutive days.

The goal latency was shortened by training day by day in all the dietary groups (Fig. 3). As to the acquisition of task, the control group tended to be inferior in the learning performance than the other dietary groups, except for the mixture (1:1) group, $F(5, 840)=14.49$, $p<0.01$ in a

two-way ANOVA. The rapeseed oil group appeared to be superior to the other groups in acquiring spatial memory.

Behavior in Elevated Plus Maze Task The apparatus for the elevated plus maze task consisted of two white open-arms (21.5×7.5 cm) and two black enclosed-arms ($21 \times 7.5 \times 20.5$ cm high) attached cross-wise to a white central platform (7.5×7.5 cm), which was set 50 cm above the floor. The mouse was placed on the central platform with its head toward the open arm; the time spent before entry of the four limbs into the enclosed arm (transfer latency) was measured. Retention of the acquired memory was estimated 24 h later.

On the first day, the transfer latency of the rapeseed group tended to be shorter. The ratio of the transfer latency on the second day to that on the first day was significantly higher in the rapeseed oil group than in the control group (Fig. 4). No significant differences were observed in these ratios of transfer latency among the other dietary groups.

Habituation Task A mouse was taken out of its home cage, put into a different cage ($30 \times 35 \times 16$ cm), and its locomotor activity was measured by an Optovarimex (Columbus Instrument) at 3 min intervals for 1 h.

The locomotor counts decreased with the time elapsed in all the dietary groups, indicating the formation of habituation. The ratio of the locomotor count for the final 3 min to that of the first 3 min was taken as an index of habituation. This value was significantly higher in the safflower oil group than in all other groups except the rapeseed oil group (Fig. 5). A similar result was also obtained when the ratio for the final 6 min to that of the first 6 min was compared (data not shown).

Pentobarbital-Induced Anesthesia Animals were injected intraperitoneally with pentobarbital-Na (50 mg/kg). The onset time and the anesthetic time in mice were measured immediately after the injection, using the loss of righting-reflex as an indicator of anesthesia.

The onset time was significantly shorter, and the anesthetic time tended to be longer in the rapeseed group than in the control group (Fig. 6). No significant differences were observed among the other dietary groups.

Pain Threshold with or without Eptazocine Administration The pain threshold was measured by a tail-flick

Fig. 2. Ambulation and Rearing Activities in an Open Field

Each column represents the mean $\pm$ S.E.M. for the number of animals shown in parentheses. The levels of significance in the Student's *t*-test were *, $p<0.05$; **, $p<0.01$, and ***, $p<0.001$ vs. the control group.

Fig. 3. Acquisition of Water Maze Task

The numbers of animals are shown in parentheses. The levels of significance were *, $p<0.05$ and **, $p<0.01$ vs. the control group in Mann-Whitney's *U*-test.

Fig. 4. Transfer Latency to the Enclosed Arm in an Elevated Plus Maze Task

The ratio of the transfer latency on the 2nd day to that of the first day is shown. In each column, the median for all mice tested is shown by the bold bar, while the range is shown after omitting the top and bottom quarters of the group. The numbers of animals tested are shown in parentheses. *, $p < 0.05$ vs. the control group in Mann-Whitney's *U*-test.

Fig. 5. Habituation Task

The locomotor activity of a mouse put into a new cage was followed for 60 min, and the ratio of the locomotor activity in the final 3 min to that of the first 3 min is shown as an index of habituation behavior. In each column, the median for all mice tested is shown by the bold bar, and the range is shown after omitting the top and bottom quarters. The numbers of animals tested are shown in parentheses. *, $p < 0.05$ vs. the control group in Mann-Whitney's *U*-test.

method. The tip of the tail was painted with a black marker, radiant heat was applied, and the time elapsed before the mouse moved its tail was measured.

The pain threshold after heat irradiation was significantly lower in the rapeseed oil group than in the control group (Fig. 7).

Eptazocine (8 mg/kg) was injected subcutaneously, and the pain threshold was measured at 15 min intervals for 90 min starting 15 min after the injection. The threshold increased from *ca.* 3 s to *ca.* 5 s, indicative of the analgesic action of eptazocine. No significant differences were observed among the six dietary groups (data not shown).

Behavioral Patterns Unaffected by the Dietary Oils
Motor and reflection functions were estimated by a traction test and a motor coordination test, respectively, but no significant differences were observed among the six dietary groups (data not shown).

Fig. 6. Pentobarbital-Induced Anesthesia

After injection of sodium pentobarbital (50 mg/kg, i.p.), the time before the onset of anesthesia and the anesthetic time were measured using the loss of righting-reflex as an indicator of anesthesia. Each column represents the mean $\pm$ S.E.M. for the number of animals shown in parentheses. *, $p < 0.05$ in Student's *t*-test vs. the control group.

Fig. 7. Pain Threshold in Tail-Flick Response

The tail was painted black, then heat-irradiated, and the time elapsed before the animal moved its tail was measured. Each column represents the mean $\pm$ S.E.M. for the number of animals shown in parentheses. **, $p < 0.01$ in Student's *t*-test.

DISCUSSION

The dietary α -linolenate/linoleate balance has been shown to affect the behavior patterns⁵⁻⁹⁾ and the sensitivity to drugs¹⁰⁾ of rats and mice; the α -linolenate deficiency becomes apparent when the deficient diets are given through two generations.¹²⁾ Apparently inconsistently, however, relatively short term feedings (*e.g.*, 1 month) of an α -linolenate deficient diet have been reported by a different group to affect the behavior of animals

(water maze learning).^{15,16)} In our studies, water maze learning ability and the sensitivity to pentobarbital were significantly different between the mice groups fed diets supplemented either with perilla oil or with safflower oil from mothers to offspring (through two generations),¹²⁾ but the difference was not apparent in the present experiments with mice fed diets supplemented with these oils for 8 months after weaning (Figs. 3 and 6).

On the other hand, the locomotor activity in a wheel cage decreased in the order of safflower, the mixture (1:1) and the perilla oil groups, suggesting that the α -linolenate/linoleate ratio affected the locomotor activity after 8 months of feeding with these oils. However, rapeseed oil, with the third highest α -linolenate/linoleate ratio of the vegetable oils examined, caused unusual behavior patterns under the conditions examined. Much higher locomotor activity, higher ambulation and rearing activities, higher learning ability in water maze tasks, slower habituation behavior, higher sensitivity to pain and higher sensitivity to pentobarbital all were characteristic behavioral response patterns of the rapeseed oil group as compared with the other dietary groups, which could not be accounted for by the difference in the α -linolenate/linoleate ratios.

One can argue that the anomalous behavior of the rapeseed oil group such as higher locomotor activity, higher ambulation and rearing activities, higher learning ability in water maze tasks and slower habituation behavior may be brought about through a common mechanism. However, α -linolenate deficiency (safflower oil vs. perilla oil) induced lower learning performance in water maze tasks and higher sensitivity to pentobarbital, while the rapeseed oil group exhibited relatively higher learning performance and higher sensitivity to this drug. Furthermore, catecholamine or serotonin neurons are supposed to be involved in emotionality, but pentobarbital exerts its effect through a γ -aminobutyric acid (GABA) receptor. Thus, the effects of rapeseed oil and α -linolenate deficiency appear to be pleiotropic; more than one type of neurons and receptors are affected.

We cannot exclude the possibility that the observed anomaly in behavioral patterns of the rapeseed oil group

is due to the unique fatty acid composition of rapeseed oil, but we speculate that a lipophilic factor(s) other than fatty acids may be present in the rapeseed oil and that this factor reaches the nervous system and induces the observed changes in behavior. This speculation is consistent with our observation that stroke-prone spontaneously hypertensive rat (SHR) fed rapeseed oil had unusually shorter mean survival times than groups fed perilla oil or soybean oil.¹⁷⁾ So far we have no clues as to the identity of the factor(s) in the rapeseed oil. Clearly, it will be important to pay more attention to the physiological activities of minor components present in vegetable oils.

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