Dietary Supplementation with n-3 Polyunsaturated Fatty Acids Attenuates the Depression of Food-Motivated Behavior during Zymosan-Induced Peritonitis

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Peripheral inflammation is accompanied by neurobehavioral alterations such as depression of feeding, exploratory and sexual behaviors. Our previous investigation reported that dietary enrichment with n-3 polyunsaturated fatty acids (PUFA) attenuated the depression of food-motivated behavior and social exploration, but not endocrinological and metabolic disturbances in the mice with systemic inflammation induced by lipopolysaccharide (LPS). We here demonstrate that dietary n-3 PUFA also attenuate the reduction of food-motivated behavior during zymosan-induced peritonitis in mice without influencing plasma leakage into peritoneum and writhing response. Our results suggest that the common mechanism is involved in the attenuation of behavioral depression during systemic and local inflammation by dietary n-3 PUFA.

Key words sickness behavior; zymosan; n-3 polyunsaturated fatty acid (PUFA); peritonitis; prostaglandin; mouse

It is known that peripheral inflammation is accompanied by a series of neurobehavioral alterations such as depression of feeding, exploratory and sexual behaviors.[1,2] Although the mechanism of the neurobehavioral alterations during peripheral inflammation are largely unknown, this process involves the generation of proinflammatory cytokines as well as prostaglandins, cyclooxygenase (COX) metabolites of arachidonic acid, in the peripheral tissues or central nervous systems.[3—8]

Our prevous investigation demostrated that dietary n-3 polyunsaturated fatty acids (PUFA) attenuated the reduction of food-motivated behavior and social exploration in the mice with systemic inflammation induced by lipopolysaccharide (LPS).[9] It is known that dietary supplementation with n-3 PUFA inhibits prostaglandins generation from arachidonic acid[10,11] and modulates the synthesis of proinflammatory cytokines,[12,13] which may explain the attenuation of the behavioral depression upon LPS administration. However, it has been not yet examined whether dietary n-3 PUFA attenuate the behaviroal depression under different inflammatiory conditions.

Intraperitoneal injection of zymosan induces acute peritonitis in the experimental animals, which is associated with the increased vascular permeability and viceral pain response assessed as writhing. It is known that vasoactive and nociceptive prostaglandins generated locally contribute to these inflammatory reactions.[14—17] In addition, zymosan-induced peritonitis is reported to be associated with behavioral depression such as the reduction of food intake.[18,19] Although the role of inflammatory mediators involved in the behavioral depression during zymosan-induced peritonitis has not been investigated, it is possible that endogenous proinflammatory cytokines or prostaglandins also play an important role. In the present study we examined the effect of dietary n-3 PUFA on the reduction of food-motivated behavior as well as plasma leakage and writhing response during zymosan-induced peritonitis in mice.

MATERIALS AND METHODS

Animals and Dietary Protocols Male ddY mice at 4 weeks of age (SLC, Shizuoka, Japan) were used throughout the experiments. Test diets were prepared by supplementing a laboratory chow (Labo MR Stock, Nihon Nosan, Yokohama, Japan) with an n-3 PUFA-rich perilla oil (Ohta Oil Mill, Okazaki, Japan) or an n-6 PUFA-rich safflower oil (NOF, Tokyo) at 10% (w/w). The details in the fatty acid composition of these test diets were described in our previous paper.[9] The mice fed the n-3 or n-6 PUFA-rich diet for 4 weeks were used for the following experiments. The mice were housed under a 12 h : 12 h light–dark cycle, in which the light was turned off at 08:00 h This experiment had been approved by the Committee of Animal Care and Experiments of Toyama Medical and Pharmaceutical University (Protocol #2001-33).

Food-Motivated Behavior Group-housed mice (4—6 mice in a cage) were fed each test diet for 2 weeks and then were individually housed for an additional 2 weeks for food restriction in order to maintain their mean body weights at approximately 85% of the mean body weights of unrestricted controls (4 mice in each dietary group). During the period of food restriction, the mice were trained using an operant conditioning chamber to determine food-motivated behavior. The mice were trained in two Plexiglas operant chambers (Muromachi Kikai, Tokyo) each measuring 159×140×127 mm. Each chamber was equipped with two adjacent levers located 8 cm apart and a pellet dispenser. Responses on the retractable levers were recorded and reinforced by the delivery of a pellet (20 mg) (Bioserve, Holton Industries Co., Frenchtown, NJ, U.S.A.) from a hole situated midway between the two levers. The training of food-restricted mice was started at the fixed ratio 1 (FR1), which means that one pellet is given after 1 press of the lever, and was performed for 15 min per day. Then the schedule was upgraded to FR2, FR5 and FR10, and the training was considered complete when the mice exhibit constant responses at FR10 within 5 min. On the first day of the 5th week of feeding, the trained

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mice were tested using the FR10 schedule for 5 min 1 h before and 1, 2, 4, 8 and 24 h after intraperitoneal (i.p.) zymosan injection. Zymosan (Zymosan A, Sigma, St. Louis, MO, U.S.A.) was suspended in physiological saline and injected between 08:00 and 10:00 h at a dose of 100 mg/kg. The lever pressing behavior was expressed as the percentages of basal values measured 1 h before zymosan injection. The diets were inaccessible to the mice after they were returned to their original cages between sessions.

Plasma Leakage into Peritoneum and writhing Response The n-3 PUFA or n-6 PUFA diet was fed to the mice for 4 weeks. For the assessment of plasma leakage upon zymosan-induced peritonitis, 0.2 ml of 0.5% Evans Blue dye dissolved in saline was intravenously injected just before i.p. injection of zymosan (100 mg/kg). Mice lightly anesthetized were decapitated 20 min after i.p. zymosan injection and blood was collected to obtain serum. Peritoneal cavity was then washed with 4 ml of ice-cold PBS and the peritoneal lavage fluid was centrifuged briefly to obtain supernatants. The content of Evans Blue dye in the peritoneal lavage fluid was determined at 620 nm by spectrophotometer. The concentration of Evans Blue dye in the serum was determined similarly. The volume of plasma leaked (μl) for 20 min after zymosan injection was estimated by dividing the amount of Evans blue dye in the peritoneal lavage fluid (μg) by the concentration of Evans blue dye in serum (μg/μl). For the assessment of writhing response, mice were treated with i.p. zymosan and then the number of writhing response was counted for 20 min.

Statistical Analysis of Data An unpaired Student's *t*-test was used for the comparison of data between the two dietary groups. A StatView version 5.0 (Abacus Concepts Inc., Berkeley, CA, U.S.A.) was used for the statistical analysis of data.

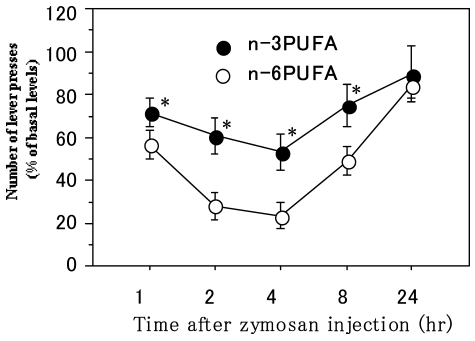

Fig. 1. Effect of Dietary n-6 and n-3 PUFA on the Food-Motivated Behavior after Intraperitoneal Injection of Zymosan

Each point and bar present the mean±S.E.M. of the percentages of basal values measured 1 h before zymosan injection (13 and 14 mice in the n-3 PUFA and n-6 PUFA groups, respectively). Asterisks indicate a significant difference ($p<0.05$, unpaired Student's *t*-test) between the dietary groups at each time point.

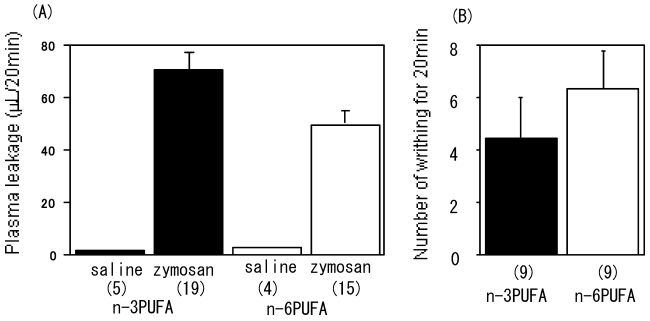

Fig. 2. Effects of Dietary n-6 and n-3 PUFA on the Plasma Leakage (A) and Writhing Response (B) upon Intraperitoneal Injection of Zymosan

The column and bar represent the mean±S.E.M. The number of mice used in each group was indicated in the parentheses. The increased rate upon intraperitoneal zymosan injection in the n-3 PUFA group was marginally larger than in the n-6 PUFA group, although this difference was not statistically significant ($t=1.878$, $p=0.07$) (A). There was no statistically significant difference in the number of writhing between the two dietary groups ($t=-0.885$, $p=0.39$) (B).

RESULTS

Details in the changes in the fatty acid composition in the liver and brain of mice fed the n-3 or n-6 PUFA diet have already been described[9] elsewhere. Feeding the n-3 PUFA-rich diet for 4 weeks resulted in the reduction of arachidonic acid (20:4n-6) and the elevation of eicosapentaenoic acid (20:5n-3) as compared with the n-6 PUFA-rich diet in the liver phospholipids. However, only a small but signifcant reduction of arachidonic acid was observed in the brain phospholipids in the mice fed the n-3 PUFA diet as compared with the n-6 PUFA-rich diet.

The numbers of lever presses in 5 min 1 h before zymosan injection were similar between the two dietary groups (265±22 and 222±18 in the n-3 and n-6 fatty acid-rich groups, respectively, $p=0.14$) (Fig. 1). Lever pressing behavior was reduced after i.p. zymosan injection maximally 4 h after zymosan injection and subsequently recovered in both dietary groups. However, the reduction of lever pressing behavior after i.p. zymosan injection was significantly attenuated in the n-3 PUFA group than in the n-6 PUFA group at 1, 2, 4 and 8 h after the injection ($p=0.037$, 0.011, 0.014, 0.0152, respectively). The number of lever pressing was recovered almost to the basal levels 24 h after zymosan injection and was not significantly different between the two dietary group ($p=0.19$).

The rate of plasma leakage was measured based on the exudation of Evans blue dye into the peritoneum (Fig. 2A). The rate in the mice treated with i.p. injection of saline was quite small ($<3\mu$l/20 min) and not different between the two dietary groups. In the mice treated with i.p. zymosan, the rate markedly increased. However, the increased rate in the n-3 PUFA group was marginally larger than in the n-6 PUFA group ($t=1.878$, $p=0.07$).

The local pain response during zymosan-induced peritonitis was assessed as writhing responses (Fig. 2B). There was no significant difference in the number of writhing during 20 min after i.p. zymosan injection ($t=-0.885$, $p=0.39$)

DISCUSSION

I.p. administration of LPS and zymosan primarily induces systemic and local inflammatory reactions, respectively. Therefore, the proinflammatory mediators involved in LPS- and zymosan-induced inflammation are supposed to be different. However, it is demonstrated that pretreatment with COX inhibitors and the gene depletion of COX-2 attenuate behavioral depression induced by peripheral LPS administration.[7,8] In addition, prostaglandin generation during zymosan-induced peritonitis was reported to be suppressed by the supplementation with n-3 PUFA-rich diet.[20] Accordingly,

the suppression of prostaglandin generation by dietary n-3 PUFA is a common mechanism to account for the attenuation of LPS- and zymosan-induced behavioral depression. We have also observed that dietary supplementation with docosahexaenoic acid (22:6n-3, DHA)-rich fish oil attenuated the depression of food-motivated behavior in LPS-treated mice as compared with the supplementation with palm oil containing low n-3 PUFA (unpublished data). DHA-enriched diet similarly reduces tissue level of arachidonic acid and suppresses the generation of prostaglandins from arachidonic acid.[13] Furthermore, recent several investigations have shown that the role of prostaglandins generated in the endothelial cells lining brain microvessels play an important role in neurobehavioral alterations induced by LPS administration.[21,22] In contrast, the role of prostaglandins in the brain and peripheral tissues are not clear yet in the behavioral depression during zymosan-induced peritonitis and should be investigated especially in connection with dietary n-3 PUFA.

It is also possible that modulation of cytokine generation may be implicated to the attenuation of depressed food-motivated behavior during zymosan-induced peritonitis. Proinflammatory cytokines such as tumor necrosis factor α, interleukin-1 and interleukin-6 are known to be generated in the local site of peritonitis.[23,24] However, we have not examined the effect of dietary n-3 PUFA on the contents of these cytokines in the peritoneal cavity. Furthermore, in addition to local cytokines, these generated in central nervous system also play an important role in the neurobehavioral alterations upon peripheral inflammation. Therefore, the effect of dietary n-3 PUFA on the expression of cytokines in brain regions is needed to be examined.

It is very interesting that dietary n-3 PUFA attenuated behavioral depression during zymosan-induced peritonitis without influencing local inflammatory events like plasma leakage and writhing response (Fig. 2). These local inflammatory reactions during the zymosan-induced peritonitis are known to be attenuated effectively by the pretreatment with COX inhibitors.[15,16] In particular, prostacyclin (prostaglandin I_2) is generated in the inflamed peritoneum, and plays a crucial role in the vascular permeabilization and local pain responses.[14,17] There is a possibility that prostaglandins might be involved in the behavioral depression to a greater extent than in the local inflammatory responses during zymosan-induced peritonitis. In our previous studies, dietary supplementation with n-3 PUFA was effective to inhibit prostaglandin generation but its effect was not complete.[25] Therefore, it is interpreted that the behavioral depression might be preferentially attenuated by dietary n-3 PUFA over the local inflammatory reactions during zymosan-induced peritonitis.

We previously reported that dietary enrichment with n-3 PUFA attenuated behavioral depression but not endocrinological and metabolic disturbances in the mice treated with systemic LPS.[9] In the present study, the behavioral depression was attenuated by dietary n-3 PUFA without influencing the local inflammatory reactions during zymosan-induced peritonitis. These observations suggest that the neurobehavioral alterations during peripheral inflammation are preferential targets of n-3 PUFA. Further investigations are needed to clarify how the neurobehavioral alterations are induced during peripheral inflammation and the dietary n-3 PUFA preferentially influences this process.

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