

Antinociceptive Effects of Docosahexaenoic Acid against Various Pain Stimuli in Mice

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Docosahexaenoic acid (DHA), an omega-3 polyunsaturated fatty acid (n-3 PUFAs), is an essential polyunsaturated fatty acid in the central nervous system, and possesses many physiological functions in neurodegenerative diseases. Previously, there are some reports that n-3 PUFAs contribute to pain relief. As the antinociceptive effect of DHA alone has not been reported, this study examined the antinociceptive effect of DHA on various pain stimuli. To evaluate the antinociceptive effect of DHA on thermal and chemical nociception, we employed the tail flick test, acetic acid writhing test and formalin test in mice. DHA was orally administrated at 5, 15 and 25 mmol/kg at 30 min before measurement. DHA administration dose-dependently exerted an antinociceptive effect against thermal and chemical stimulation in comparison to the control olive oil administration. These effects of DHA were abolished when mice were pretreated with naloxone, an opioid receptor antagonist. These findings suggest that DHA has opioid receptor-mediated pain control activities, and may provide valuable information towards an advanced therapeutic approach for pain control.

Key words docosahexaenoic acid; opioid; antinociceptive effect

Docosahexaenoic acid (DHA) is a predominant omega-3 polyunsaturated fatty acid (n-3 PUFAs) in marine fish and is highly concentrated in the brain and the retina in humans.¹⁾ Aside from the guaranteed safety and efficacy of DHA as a nutritional supplement, many recent reports indicate that DHA has numerous beneficial physiological effects, including antioxidant and anti-inflammation properties. In addition, DHA plays a crucial role in the development and function of brain neurons.²⁾ Furthermore, dietary supplementation with DHA is known to be effective in various neurodegenerative disorders, including Alzheimer's disease,³⁾ attention deficit hyperactivity disorder,⁴⁾ anxiety,⁵⁾ bipolar disorder⁶⁾ and depression.⁷⁾

Recently, several studies have shown that intake of a large amount of n-3 PUFAs is associated with reduction of pain in rheumatoid arthritis,⁸⁾ inflammatory bowel disease⁹⁾ and dysmenorrhea.¹⁰⁾ In addition, rats treated with a high n-3/n-6 PUFA ratio diet exhibit a markedly increased threshold for thermal pain¹¹⁾ and neuropathic pain.¹²⁾ From these findings, it is likely that n-3 PUFAs have an antinociceptive effect. In a human study, it is known that n-3 PUFAs alter the plasma levels of opioid peptides.¹³⁾ Moreover, other reports suggest that n-3 PUFAs could compete with arachidonic acid,¹⁴⁾ leading to reduction in the inflammatory eicosanoids. These findings indicate that n-3 PUFAs might exert antinociceptive effects *via* an endogenous opioidergic system or anti-inflammatory system. It is possible that DHA also has antinociceptive effects although there is no direct evidence regarding DHA-induced antinociception. The aim of our study is to elucidate the antinociceptive effect of DHA on various pain stimuli in mice and specifically on the mechanistic involvement of opioidergic systems.

MATERIALS AND METHODS

Animals The experiments were performed on male ddY

mice (5 week old) obtained from SLC (Hamamatsu, Japan). The animals were housed at 23–24 °C with a 12-h light–dark cycle (lights on 8:00 a.m. to 8:00 p.m.). Food and water were available *ad libitum*. The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, adopted by the Japanese Pharmacological Society. In addition, all experiments were approved by the ethical committee for animals of Kobe Gakuin University (Approval #: A090602-21).

Drug Administration DHA was kindly donated by Ikeda Tohka Industrials Co. (Fukuyama, Japan). DHA (5, 15, 25 mmol/kg) or the control oil (olive oil) was orally given to mice. Naloxone (NLX; 1 mg/kg) (Sigma, St. Louis, MO, U.S.A.), a nonselective opioid receptor antagonist, was intraperitoneally administered 10 min before DHA administration.

Tail-Flick Test The antinociceptive response against thermal stimuli was assessed with the tail-flick test.¹⁵⁾ Mice were gently held with the tail positioned in the tail-flick apparatus (MK-330B; Muromachi Kikai Co., Ltd., Tokyo, Japan) for radiant thermal stimulation of the dorsal surface of the tail. The intensity of the thermal stimulus was adjusted to cause the animal to flick its tail within 2.5 to 3 s as the baseline of the tail-flick latency. The tail-flick latency was measured before and 30, 60, 90 and 120 min after oral administration. The cutoff time that was set at 10 s to minimize tissue damage. The tail flick latency was converted to represent the percent maximum possible effect (%MPE) according to the following formula: %MPE = 100 × (each latency – baseline latency) / (10 – baseline latency). The area under the curve (AUC) value for the antinociceptive action on each mouse was calculated for the relevant experiments.

Acetic Acid Writhing Test The writhing activity was evaluated by the method of Hayashi and Takemori.¹⁶⁾ The number of stretches or writhes was counted for 30 min after administration of 0.6% (v/v) acetic acid (10 ml/kg, intraperi-

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toneally (i.p.).

Formalin Test in Mice The formalin test was carried out as described by Hunskaar and Hole.¹⁷ Mice were injected with 10 μ l of 5% formalin [formaldehyde solution 37% (w/w) diluted in saline] into the subplantar space of the right hind paw 30 min after the treatment of DHA (p.o.). The times spent in licking, biting and shaking behaviors were measured during 0 to 5 min (early phase) and 5 to 30 min (late phase) after formalin injection.

Statistical Analysis Data are shown as the mean \pm S.E.M. The statistical significance of differences between the control and DHA-treated group was analyzed using one-way ANOVA followed by Dunnett's multiple-comparison test for the tail flick test, acetic acid writhing test and formalin test. The statistical significance of differences between the DHA-treated groups and NLX-pretreated group was analyzed using one-way ANOVA followed by Scheffe's multiple-comparison test for the tail flick test, acetic acid writhing test and formalin test. The differences were regarded as statistically significant when the *p* value was less than 0.05.

RESULTS

Effect of DHA on Thermal Nociception in Mice DHA (5–25 mmol/kg, p.o.) significantly increased the tail-flick latency in a dose-dependent manner, and the maximal effect was observed at 60 min after DHA administration (Fig. 1A). Also, the *AUC*_{0–120} in the DHA treated group was significantly greater than that in the control group (Fig. 1B). On the

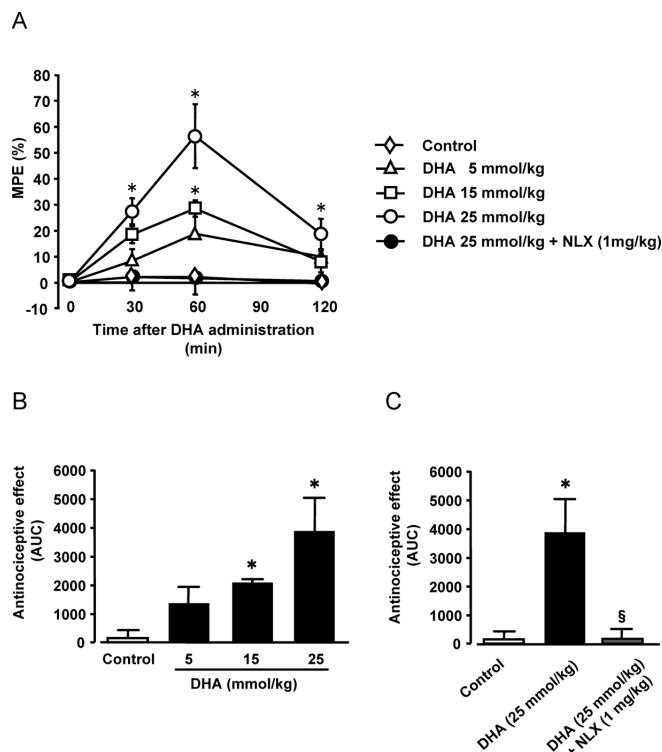


Fig. 1. Time Course of the Antinociceptive Effects of DHA (A), Effect of DHA on Thermal Nociception in Mice (B) and Effect of NLX on DHA-Induced Antinociceptive Activity (C)

The cut-off time was set at 10 s in the test to avoid injury to the tail. Data are shown as the mean \pm S.E.M. (*n*=8). **p*<0.05, compared with the control group (one-way ANOVA and Dunnett's test). §*p*<0.05, compared with 25 mmol/kg DHA-treated group (one-way ANOVA and Scheffe's test).

other hand, the antinociceptive effect of DHA was significantly attenuated by pretreatment of NLX (1 mg/kg, i.p.) 10 min before DHA administration (Fig. 1C).

Effect of DHA on Chemical Nociception in Mice DHA significantly reduced the number of writhes compared with the control group in a dose-dependent manner (Fig. 2A). The antinociceptive effect of DHA was completely suppressed by pretreatment of NLX (1 mg/kg, i.p.) 10 min before DHA administration (Fig. 2B).

Furthermore, in the formalin test, a high dose of DHA (25 mmol/kg) significantly reduced licking, biting and shaking behavior in both the early and late phases in the formalin-induced nociceptive response. The antinociceptive effect of DHA was completely suppressed by pretreatment of NLX (1 mg/kg, i.p.) 10 min before DHA administration. On the other hand, a lower dose of DHA (5 mmol/kg) had no effect in the formalin test (Fig. 3).

DISCUSSION

In this study, we focused on the antinociceptive effect of DHA on various pain stimuli in mice. We demonstrated that DHA (5–25 mmol/kg) exhibited a significant antinociceptive effect in a dose-dependent manner. In particular, DHA (25 mmol/kg) suppressed the pain behavior induced by ther-

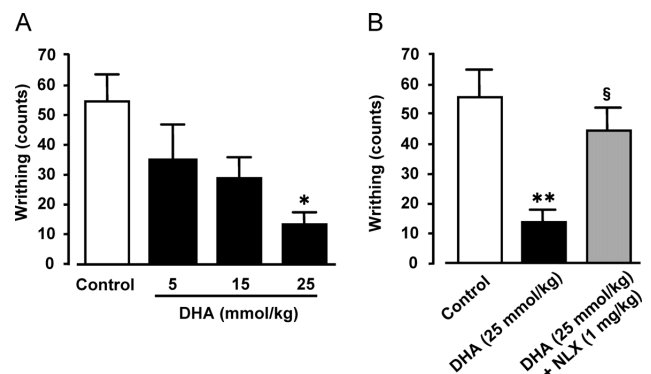


Fig. 2. Antinociceptive Effect of DHA on Acetic Acid Writhing Behavior in Mice (A) and Effect of NLX on DHA-Induced Antinociceptive Activity in Mice (B)

Data are shown as the mean \pm S.E.M. (*n*=8). ***p*<0.01, **p*<0.05, compared with the control group (one-way ANOVA and Dunnett's test). §*p*<0.05, compared with the 25 mmol/kg DHA-treated group (one-way ANOVA and Scheffe's test).

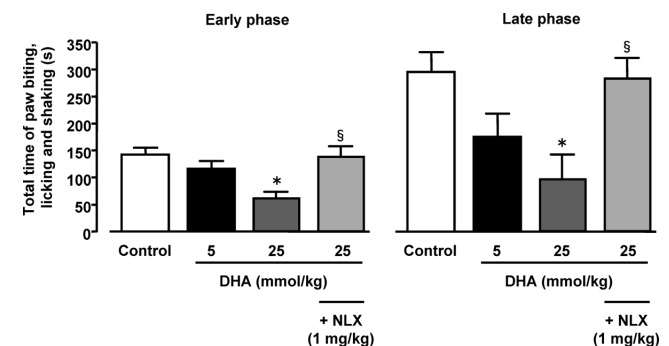


Fig. 3. Antinociceptive Effect of DHA on Formalin-Induced Nociceptive Behavior in Mice

Data are shown as the mean \pm S.E.M. (*n*=8). **p*<0.05, compared with the control group (one-way ANOVA and Dunnett's test). §*p*<0.05, compared with the 25 mmol/kg DHA-treated group (one-way ANOVA and Scheffe's test).

mal and chemical nociceptive stimuli in mice. The dose of DHA employed in this study was similar to those used in previous reports showing DHA-induced decrease of triacylglycerol and cholesterol in rodents.¹⁸⁾ In addition, the toxicological profile of DHA demonstrates that this oil is safe in rats (up to a consumption level of 10 mmol/kg/d) in a 90 d toxicology evaluation.¹⁹⁾ Furthermore, DHA (60, 120 mmol/kg) suppressed the up-regulation of inflammatory cytokine mRNA without DHA-induced toxicity in nephropathy model mice.²⁰⁾ In this study, DHA (25 mmol/kg) administered mice did not show any abnormal behavior, and their drinking volume and food ingestion did not altered at all when compared to control mice. Therefore, it is suggested that the toxicity of DHA used here might be negligible.

It is widely known that opioidergic nervous systems regulate the various pain. Since the antinociceptive effect of DHA was completely inhibited by pretreatment of NLX (1 mg/kg, i.p.), it is possible that DHA accelerates the release of endogenous opioid peptides or directly acts on opioid receptors. The previous reports suggesting that n-3 PUFAs affects the binding of opioid peptides including enkephalin and β -endorphin to their receptors,^{21–23)} support our results in the present study. However, a further study is necessary whether DHA directly binds to the opioid receptors.

In the formalin test, DHA (25 mmol/kg) seems to reduce pain-related behavior in both the early phase (direct stimulation to primary afferent nerve) and the late phase (indirect stimulation to primary afferent nerve caused by inflammatory substances such as prostaglandins).¹⁷⁾ In particular, the antinociceptive effect of DHA was clearly observed in the late phase of formalin-induced nociceptive behavior. Such chemical nociceptive stimuli induced by formalin or by acetic acid cause the release of endogenous pronociceptive substances such as bradykinin and prostanoids.²⁴⁾ It is reported that DHA ethyl ester decreased the inflammatory cytokine such as interferon- γ , interleukin (IL)-1 β , IL-2 and IL-6.²⁵⁾ Accordingly, it is possible that the antinociceptive effect of DHA may be partly due to attenuation of the central sensitization induced by some inflammatory substances. In the thermal stimulus test, it has been reported that the firing of many cationic channel,^{26–28)} including transient receptor potential vanilloid-1 (TRPV-1), are associated with pain. Recently, it is reported that n-3 PUFAs such as DHA and EPA can bind to TRPV-1 and regulate them in protein kinase C-dependent manner.²⁹⁾ Therefore, it is possible that DHA might relieve the pain by modulating the activity of such receptors or channels.

In conclusion, this study is the first report proposing that DHA exhibits antinociceptive effects against various pain stimuli in mice. Furthermore, the antinociceptive effect of DHA is likely related to the activation of the opioidergic system.

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