Influence of docosahexaenoic acid on cerebral lipid peroxide level in aged rats with and without hypercholesterolemia

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Abstract

Female Wistar rats, 100 weeks old, were divided into four groups: one group was fed a high-cholesterol diet, one received a non-cholesterol diet, and the others were fed either a non- or a high-cholesterol diet plus docosahexaenoic acid. The level of lipid peroxide (LPO) in brain tissue was measured with a LPO assay kit. Fatty acid concentrations were analyzed by gas chromatography. Brain LPO in the aged and hypercholesterolemic rats fed docosahexaenoic acid decreased in the cerebrum but not in the brain stem or cerebellum. In the cerebrum, LPO showed a decrease, with an increase in the ratio of docosahexaenoic acid to arachidonic acid. The cerebrum, unlike the other areas of the brain, was more sensitive to docosahexaenoic acid as the concentrations of LPO decreased. © 1998 Elsevier Science Ireland Ltd.

Keywords: Cerebral lipid peroxide; Docosahexaenoic acid; Arachidonic acid; Hypercholesterolemia; Aged rats

The fatty acids, arachidonic acid (AA, 20:4n-6) and docosahexaenoic acid (DHA; 22:6n-3), account for approximately 50% of fatty acids in the gray matter of the brain [10]. AA potentiates synaptic transmission in the rat hippocampus [17], and DHA improves the spatial learning deficit in rats produced by occlusion [11]. An increase in DHA and AA reportedly enhances lipid peroxidation [6,13]. Since the central nervous system is highly susceptible to the effects of reactive oxygen species [12], such tissues would show an increase in the concentration of lipid peroxide (LPO) that could damage the membrane. The role of the administration of polyunsaturated fatty acids (PUFA) in the improvement of cerebral function merits evaluation. Since LPO is increased with age [2] and in the presence of hypercholesterolemia [16], we monitored the levels of PUFA and LPO in the brains of aged rats fed a high-cholesterol diet or a non-cholesterol diet.

Female Wistar rats, 100 weeks old, were randomly divided into four groups according to diet: one group (n = 10) was fed a high-cholesterol diet (HC) consisting of a semisynthetic diet without fish oil that contained 1% cholesterol and 1% cholic acid (Funabashi Farm, Chiba, Japan), another group (n = 10) was fed a non-cholesterol diet (F1) consisting of a semisynthetic diet without fish oil (F1 group) (Funabashi Farm, Chiba, Japan), and the others were fed a non- or a high-cholesterol diet plus 300 mg/kg daily of oral DHA-95E, which is an ethyl ester derivative of all cis-4,7,10,13,16,19-docosahexaenoic acid (Harima Chemicals, Tokyo, Japan) (F1 + DHA, n = 10 and HC + DHA, n = 10). DHA-95E, which is free of vitamin E, was gently emulsified in 5% gum arabic solution in ice-cold water by use of an ultrasonic cell homogenizer (Taisei VP-5, Taisei, Tokyo, Japan). The rats were housed for 12 weeks and were fasting overnight before the experiment.

After the induction of anesthesia with pentobarbital sodium, 65 mg/kg injected intraperitoneally, the rats were killed in accordance with the procedures outlined in the Guidelines for Animal Experimentation of Shimane Medical University. The entire brain, together with the brain stem, cerebellum and cerebrum, was excised and these separate regions were dissected free. Tissues were weighed and homogenized in cold 25 mM Tris–HCl buffer (pH 7.4) containing 137 mM NaCl, 5.4 mM KCl and 1.1 mM glucose, by use of a Polytron homogenizer (PCU 2–110, Kinematica GmbH, Steinhofhale, Switzerland). The homogenates were

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adjusted to a final concentration of 100 mg of tissue weight per milliliter of buffer and were immediately subjected to the following assays.

Protein concentration was estimated by the method of Lowry et al. [8]. The level of lipid peroxide in brain tissue was measured by use of the LPO assay kit (Wako, Osaka, Japan). Fatty acid concentrations were determined by the one-step analysis of Lepage and Roy [7], after gas chromatography (Hewlett-Packard model 5890 II, Avondale, PA, USA).

Data are reported as the mean ± SE. Statistical analysis of the results utilized Student’s t-test or by one-way analysis of variance (ANOVA) followed by Fisher’s protected least significant difference test for post hoc comparisons. A level of $P < 0.05$ was accepted as statistically significant.

Table 1

|                  | Brain stem | Cerebrum | Cerebellum | |  
|------------------|------------|----------|------------| |  
|                  | HC (F1)    | HC + DHA (F1 + DHA) | HC (F1) | HC + DHA (F1 + DHA) | |  
| 20:4n-6          | 44.6 ± 2.03b | 48.3 ± 1.50a | 47.1 ± 3.50a | 51.8 ± 2.30a | 76.9 ± 1.70b | 70.0 ± 1.75b | |  
| 20:5n-3          | 0.95 ± 0.08b | 0.84 ± 0.10a | 0.98 ± 0.09b | 0.90 ± 0.12a | 0.95 ± 0.02b | 0.85 ± 0.10b | |  
| 22:6n-3          | 0.74 ± 0.18b | (0.76 ± 0.55)a | (1.00 ± 0.10)a | (0.99 ± 0.10)a | (0.60 ± 0.08)b | (0.70 ± 0.70)b | |  
| EPA/AA           | (0.017 ± 0.005)b | (0.019 ± 0.002)a | (0.016 ± 0.003)a | (0.014 ± 0.003)b | (0.011 ± 0.006)b | (0.010 ± 0.006)b | |  
| DHA/AA           | 1.45 ± 0.088b | 1.42 ± 0.084a | 1.66 ± 0.037b | 1.55 ± 0.065b | 1.21 ± 0.018b | 1.39 ± 0.019b | |  

Numerals in parentheses represent values for the F1/DHA groups. HC, aged rats (n = 10) fed a high-cholesterol diet; F1, aged rats (n = 10) fed a non-cholesterol diet; HC + DHA, aged rats (n = 10) fed a high-cholesterol diet plus oral DHA-95E; F1 + DHA, aged rats (n = 10) fed a non-cholesterol diet plus oral DHA-95E; EPA/AA, ratio of EPA (20:5n-3) to AA (20:4n-6); DHA/AA, ratio of DHA (22:6n-3) to AA (20:4n-6). Superscripts a,b,c,d: values in the same row that do not share a common superscript are significantly different ($P < 0.05$).
of DHA than the 300 mg/kg per day administered to rats was found to diminish the AA concentration, both in the hippocampus and in the frontal cortex, which caused a significant increase in the DHA/AA ratio in the frontal cortex [11]. This finding suggests that the cerebral cortex is sensitive to the DHA supplement used to reduce the cerebral concentration of AA. Levels of EPA were very low in the areas of the brain studied, and remained relatively unchanged. Several studies suggest that DHA acts as a pro-oxidant [3,4,15]. Thus, the enrichment of DHA in the aged rat brain would be expected to enhance the production of LPO. However, high levels of cerebral DHA in the hypercholesterolemic HC + DHA rats and in the F1 + DHA group significantly reduced the cerebral level of LPO (P < 0.05) (Fig. 1). Since the cerebral levels of DHA and LPO were inversely correlated with the combined HC/HC + DHA group (r = -0.79, P < 0.05), we speculate that cerebral DHA served as an antioxidant. The cerebral DHA/AA ratio in this study was inversely correlated with the corresponding level of cerebral LPO (P < 0.05) (Table 2). Moreover, the whole-brain content of LPO (Y) in the HC/HC + DHA and F1/F1 + DHA groups (Fig. 1) was inversely correlated with the respective DHA/AA ratio (X), \(Y = -0.33X + 0.82, r = -0.67, P < 0.001\), in the HC/HC + DHA group, and \(Y = -0.09X + 0.42, r = -0.55, P < 0.05\), in the F1/F1 + DHA group. Thus, the DHA/AA ratio may be a marker for host defense capability against damage induced by oxidants.

A deficiency in brain DHA has been detected in patients with Alzheimer's disease [14]. Such a deficiency is reported to induce a loss of learning ability for discrimination in rats [5]. Taking these findings into account, we suggest that an increase in cerebral DHA in mammals is important in depressing the cerebral production of LPO and in improving cerebral dysfunction such as a cognitive deficit. Further studies are needed to elucidate the possible interactions of DHA and LPO in different areas of the brain.

### Table 2

<table>
<thead>
<tr>
<th>X</th>
<th>Y (LPO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC/HC + DHA (n = 20)</td>
<td>F1/F1 + DHA (n = 20)</td>
</tr>
<tr>
<td>DHA/AA</td>
<td>(Y = -1.27X + 2.07)</td>
</tr>
<tr>
<td>AA</td>
<td>NS</td>
</tr>
<tr>
<td>DHA</td>
<td>(Y = -0.018X + 2.18)</td>
</tr>
<tr>
<td></td>
<td>(r = -0.79^*)</td>
</tr>
</tbody>
</table>

LPO, nmol of lipid peroxide per mg of protein; AA, \(\mu g\) of arachidonic acid per mg of protein; DHA, \(\mu g\) of docosahexaenoic acid per mg of protein; DHA/AA, ratio of DHA to AA; NS, not significant. *P < 0.05.


