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Effects of Dietary Docosahexaenoic Acid on Survival Time and Stroke-Related Behavior in Stroke-Prone Spontaneously Hypertensive Rats

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ABSTRACT. 1. Dietary docosahexaenoic acid (DHA) suppressed the age-dependent increase in systolic blood pressure and prolonged the average survival time of stroke-prone spontaneously hypertensive rats (SHRSP).

2. Dietary DHA (1% and 5% in diets) altered the circadian rhythm of SHRSP, causing significant increases in ambulatory activity during the dark period. At the onset of stroke, desynchronization with light and dark phases and new biological rhythms were noted in all of the control SHRSP (DHA 0%). DHA-treated SHRSP did not show such behavioral changes.

3. These effects were accompanied by the increase of DHA and the decrease of AA levels in plasma and brain cortex.

4. It was concluded that dietary DHA suppresses the development of hypertension and stroke-related behavioral changes, resulting in prolongation of the SHRSP's life span. GEN PHARMAC 29;3:401–407, 1997. **© 1997 Elsevier Science Inc.**

KEY WORDS. Docosahexaenoic acid (DHA), stroke-prone spontaneously hypertensive rats (SHRSP), life span, ambulatory activity, circadian rhythm

INTRODUCTION

Because the lethal course of stroke in stroke-prone spontaneously hypertensive rats (SHRSP) developed by Okamoto *et al.* (1974) coincides well with that of patients with cerebrovascular lesions (Saito *et al.*, 1995; Yamori *et al.*, 1976), SHRSP have also been used as an animal model for vascular dementia.

Dietary marine oils rich in n-3 fatty acids, eicosapentaenoic acid (EPA; 20: 5n-3) and docosahexaenoic acid (DHA; 22: 6n-3), have beneficial effects on cardiovascular disorders (Shimokawa *et al.*, 1988; Yamori *et al.*, 1982). We have demonstrated that, with aging, the renal phospholipid A_2 activity of SHRSP increases, and that their membranous phospholipids decrease (Okamoto *et al.*, 1989) along with the arachidonic acid (AA) in the phospholipids (Okamoto *et al.*, 1989). Functional abnormalities such as decreased membrane fluidity and increased Ca^{2+} permeability have been observed in cell membranes of SHR and SHRSP (Devynck *et al.*, 1981; Kawaguchi *et al.*, 1986, 1987; Montanay-Garestier *et al.*, 1981; Tsuda *et al.*, 1987; Yamori *et al.*, 1982). These results suggest that the stroke-prone nature of this strain may be modulated by dietary fatty acids.

We previously demonstrated that dietary DHA significantly suppresses the age-dependent increase in systolic blood pressure of SHRSP in a dose-dependent manner, by 17% and 26% with 1% and 5% DHA in their diets, respectively (Kimura *et al.*, 1995). Using chronobiological analysis, we have recorded the behavioral changes throughout the life span of SHRSP, including the periods in which hypertension develops and the onset of stroke and death (Minami *et al.*, 1985). At the onset of cerebral stroke, SHRSP exhibited behavioral abnormalities including increased ambulatory activity and disrupted circadian rhythms. These behavioral abnormalities in SHRSP might correspond to the delirium state observed in patients with dementia after cerebrovascular accidents (Minami *et al.*, 1985).

Kalman *et al.* (1992) reported that dietary marine fish oil causes both a reciprocal replacement of n-6 fatty acids with n-3 fatty acids and a decreased formation of the cyclooxygenase and lipoxygenase products of the arachidonate cascade in the brain capillary endothelial cells in rats. Furthermore, Umemura *et al.* (1995) showed that dietary DHA produced antithrombotic effects and caused a reduction in the size of ischemic cerebral lesions in a middle-cerebral-artery-thrombosis model in the rat.

The purpose of this study is to elucidate the effects of dietary DHA on the stroke-related behavioral changes and life span of SHRSP.

MATERIALS AND METHODS

Animals and feeding

SHRSP and normotensive Wistar–Kyoto rats (WKY) were maintained in our laboratory. Six-week-old male SHRSP were subjected to a 12-hr light–dark alternation cycle (lights on 19:00 to 7:00). Food and water were given *ad libitum*. Illumination was provided by fluorescent light (100 lux). Room temperature was maintained at 22±2°C throughout the experiment. The plethysmographic tail-cuff method (KN-0090; Natsume Co. Ltd., Tokyo) was used for

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TABLE 1. Characteristics of SHRSP compared with those of WKY

	SHRSP	WKY
SBP (mmHg) at age of:		
15 weeks	186.6 ± 4.9[a] (15)	137.3 ± 4.4 (15)
34 weeks	220.0 ± 5.3[a] (69)	141.1 ± 1.8 (69)
Average survival time (weeks)	35.5 ± 2.9[a] (27)	82.7 ± 8.2 (10)

Mean ± SEM (*n*). SHRSP = stroke-prone spontaneously hypertensive rats; WKY = Wistar–Kyoto rats; SBP = systolic blood pressure.
[a]$P < 0.01$ vs. WKY.

noninvasive measurements of blood pressure and heart rate. For the purpose of comparing SHRSP and WKY with respect to systolic blood pressure, survival time (Table 1) and pathological studies, rats were fed a conventional laboratory diet containing 5% lipids (CE-2, used as a normal diet; Clea Japan Co. Ltd., Tokyo). When the effects of DHA were examined, a semipurified diet (Clea) was used, which consisted of 24.5% milk casein; 46.5% corn starch; 5.0% cellulose; 10.0% sucrose; 1.0% vitamin mixture; 7.0% mineral mixture; 6% safflower oil; and 0%, 1% or 5% [w/w] DHA (DHA ethylester, 98% pure; Harima Chemicals Co. Ltd., Japan). The fatty acid composition of the basal diet was 16:0 (8.6% of the total fatty acids), 18: 2 (2.2%), 18: 1n-9 (10.4%), 18: 2n-6 (78%) and 18: 3n-3 (0.05%), when fatty acids were designated by the carbon chain. The number of double bonds, and the position of first double bond numbered from methyl terminals were designated as n-9, n-6 or n-3 (Shimokawa *et al.*, 1988). Diets with peroxide values below 30 mEq/kg were served throughout the experiments. Rats were randomly assigned to groups receiving 1% DHA, 5% DHA or to a control group without DHA administration (DHA 0%) throughout their life span.

Determination of ambulatory activity

An automatic Ambulo-Drinkometer (Ohara & Co., Ltd., Tokyo) was used to determine ambulatory activity. This apparatus is composed of ten separated steel cages equipped with microswitches which are activated by the tilting of the floor as the rats move around (Minami *et al.*, 1985; Togashi *et al.*, 1982). Ambulatory activity data were obtained at hourly intervals and were subjected to autocorrelation and power spectral analysis. The average period of the rhythm (τLD) (Stephan, 1983) was determined by power spectral analysis. We used only male rats for the behavioral study because female rats had a 3- or 4-day rhythm due to the estrus cycle in addition to a circadian rhythm.

Lipid analysis

In the study of chronic administration of DHA, tissue and plasma samples were obtained from SHRSP at 20 weeks of age after a 14-week DHA treatment. Plasma was obtained by centrifugation of the blood samples with EDTA supplemented as an anticoagulant. Each preparation was kept frozen at −80°C until lipid analysis. After the extraction of lipids with chloroform/methanol (Bligh and Dyer, 1959), neutral lipids and phospholipids were separated by thin layer chromatography (Silica Gel HE, Merck) with developing solvents of petroleum ether/diethylether/acetic acid (80:30:1, v/v) and chloroform/methanol/water (70:30:5, v/v), respectively. Spots were visualized by sparing first with a Rhodamine 6G solution and then with 28% NH_4OH. Lipids were extracted from the corresponding spots twice with chloroform/methanol/3% NH_4OH (6:5:1, v/v) and then once with chloroform/methanol (2:1, v/v). Fatty acids were converted to methylesters by treatment with 5% HCl in methanol and then analyzed by gas liquid chromatography (GLC) using a capillary column (DB-225, J & W Scientific, Folsom, CA, USA) essentially as described previously (Yamamoto *et al.*, 1987). Heptadecanoic acid was added as an internal standard. Protein concentration was determined by the method of Lowry *et al.* (1951).

Pathological examination

After death, brain tissues were fixed in 10% formalin solution. The fixed brain slices were then stained by the hematoxylin-eosin staining method and examined by light microscopy. In addition to cerebral softening, encephalomalacia was diagnosed by the presence of hypertrophic astrocytes and by the invasion of neutrophils and/or macrophages around or inside the foci. The staining of rat brains was performed by Dr. Takeuchi of Sapporo Toxicological Laboratory, and the pathological diagnoses were made by Professor Nakamura of the Department of Pathology, Hokkaido University School of Medical Technology.

Statistical analysis

Values were expressed as the means±SE. The Student's *t*-test was used to analyze differences between two groups. When more than two groups were compared, the significance of the difference between groups was evaluated by ANOVA and, where applicable, was followed by Tukey's test. The Bonferroni adjustment was used for testing at two points (Wallenstein *et al.*, 1980). $P<0.05$ was considered significant.

RESULTS

Causes of death and mean survival time of SHRSP compared with WKY in normal diet

Table 1 compares the characteristics of the SHRSP and WKY used in this study. The systolic blood pressure of SHRSP that were fed a normal diet from an early age was significantly higher than those of WKY (Table 1). There was a significant difference between the mean survival times of SHRSP and WKY ($P<0.001$). After killing with urethane and α-chloralose, autopsies and pathological studies were carried out to determine the type of cerebral accident. The results obtained so far in our laboratory for a total of 669 rats (from 6 to 45 weeks of age), 192 WKY and 477 SHRSP, are summarized as follows. Stroke was observed in 75 of 477 SHRSP. The incidence of stroke was 7.5% in SHRSP under the age of 20 weeks and 42.5% in those over the age of 20 weeks.

Effects of DHA on blood pressure

Systolic blood pressure in the control SHRSP (DHA 0%) increased progressively from 120.2±2.2 mmHg at 6 weeks of age to 202.9±5.7 mmHg at 20 weeks ($n=10$). On the other hand, the systolic blood pressure of 1% DHA-treated SHRSP was 117.2±1.6 mmHg at 6 weeks of age and then rose to 167.8±7.1 mmHg at 20 weeks ($n=11$). The systolic blood pressure of 5% DHA-treated SHRSP increased from 119.3±1.4 mmHg at 6 weeks of age to 149.8±1.4 mmHg at 20 weeks ($n=10$). A significant difference in systolic blood pressure was noted between the control SHRSP and the DHA-treated SHRSP at 20 weeks of age. DHA significantly sup-

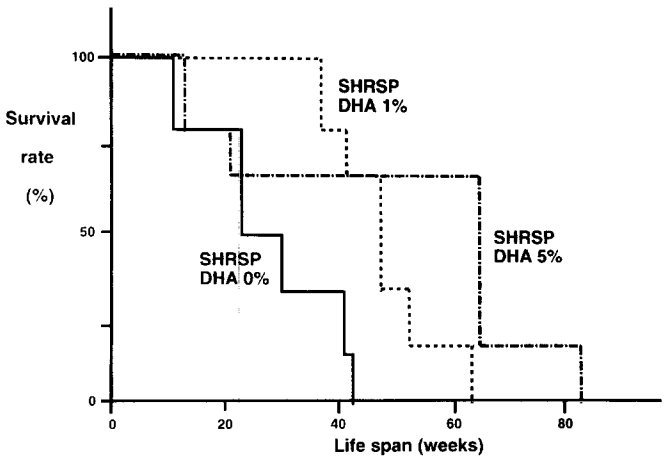

FIGURE 1. Effect of DHA on the average survival time of SHRSP.

pressed the increase in systolic blood pressure of SHRSP in a dose-dependent manner.

Effect of DHA on life span

Death occurred at 29.0±4.9 weeks in six male control SHRSP (DHA 0%), four from cerebral infarction and two from cerebral infarction with cerebral bleeding. The average survival time of DHA-treated SHRSP (1%) was 48.3±3.7 weeks (n=6). They died of senility. There was a significant difference between the average survival time of control SHRSP and DHA-treated SHRSP (1%) (P<0.05). DHA-treated (5%) SHRSP died at 51.8±11.5 weeks, two died from cerebral bleeding at a young age (13.7 and 19.3 weeks of age) (Fig. 1). There was no significant difference between the average survival time of control SHRSP and DHA-treated (5%) SHRSP.

Effects of age and DHA on ambulatory activity of SHRSP

Figure 2 compares the ambulation of SHRSP (DHA 0%, 1%, 5%) at 15 and 30 weeks of age. Ambulatory activity was measured during continuous 3-hr intervals. Activity counts of ambulation in the dark period were higher than those in the light period, a pattern typical of nocturnal animals. DHA supplementation tended to increase ambulatory activity of SHRSP at 15 weeks of age, but the difference was not statistically significant. At 30 weeks of age, ambulation counts of DHA-treated SHRSP (DHA 1% and 5%) in the dark phase were significantly greater than those of the control SHRSP (DHA 0%).

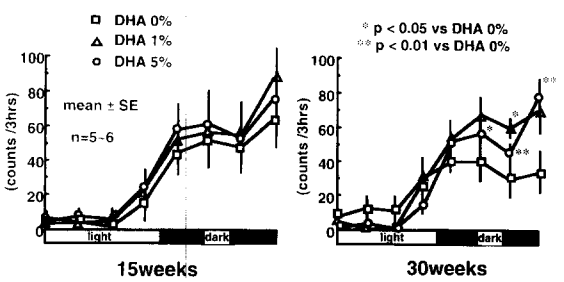

FIGURE 2. Effects of age and DHA on ambulatory activity in SHRSP. DHA was orally administered for 14 weeks from 15 to 30 weeks of age. Values indicate ambulatory activity during 3-hr periods.

On the contrary, the total ambulatory activity during the light phase (percent of 24 hr ambulation) in the control group (DHA 0%: 35.5±7.0%) was significantly greater than that in the DHA-treated groups (DHA 1% and 5%: 19.9±3.3%, P<0.05 and 14.0±1.3%, P<0.01, respectively). Desynchronization with the dark and light phase was demonstrated in the control SHRSP at 30 weeks of age. DHA ameliorated this rhythm disturbance.

Effect of DHA on behavioral changes at the onset of stroke in SHRSP

The upper panel of Figure 3 shows the typical behavioral changes before death in a control SHRSP that died of cerebral bleeding. When this rat was 16–18 weeks old, ambulatory activity in the dark phase was greater than that in the light phase. Synchronization with the light and dark phases was observed and circadian rhythm was noted. The middle portion of Figure 3 shows an abrupt increase in ambulatory activity in the light phase and desynchronization with the light and dark phases followed. SHRSP that died of cerebral infarction (lower panel) showed similar behavioral changes including desynchronization with the light and dark phases and disturbance in biological rhythm.

According to the power spectral analysis of the behavioral changes, this same SHRSP had a 24-hr τ-value for ambulation at 20 weeks. At the onset of stroke, however, a much longer periodicity was observed in addition to the circadian (24-hr) periodicity. On the other hand, WKY that died of senility showed only a slight desynchronization in ambulatory activity and circadian rhythm still existed (data not shown).

Figure 4 shows the behavioral changes in DHA-treated SHRSP that died of senility; for instance, without apparent cerebral bleeding or cerebral infarction but with long survival times. Synchronization with the light and dark phases was maintained in these DHA-treated SHRSP. All of the six control SHRSP that died of cerebral stroke showed a much longer periodicity in addition to their circadian rhythms. On the other hand, none of the DHA-treated SHRSP showed significant new rhythms (much longer periodicity in addition to the circadian rhythm). There was a significant difference (P<0.01) between the frequencies of the new biological rhythms in both the control (DHA 0%: 6 of 6) and the DHA-treated groups (DHA 1%: 0 of 6 and DHA 5%: 0 of 6).

Plasma and cortex AA and DHA

DHA treatment decreased the plasma total fatty acids from 1.66±0.71 at 0% DHA to 1.32±0.24 at 1% DHA and to 0.87±0.14 mg/ml plasma at 5% DHA at 20 weeks of age. Brain cortex total fatty acid content was not affected by the diets. Depending on the dietary fatty acid compositions, high linoleate (18: 2n-6)-based diet or DHA-supplemented basal diets, DHA treatment significantly decreased the plasma AA and cortex AA levels as compared with those in control SHRSP (DHA 0%) (Fig. 5). On the other hand, plasma and cortex DHA levels increased significantly in a dose dependent manner after DHA ingestion (Fig. 5). The marked decrease in plasma AA contents could also be due to the feedback inhibition of desaturation–elongation activities by DHA, because the plasma 18: 2n-6/AA (20: 4n-6) ratio increased from 0.51 at 0% DHA to 0.99 at 1% DHA and to 3.68 at 5% DHA.

DISCUSSION

In this study, dietary DHA have suppressed the development of hypertension, leading to a prolongation of the life span of SHRSP.

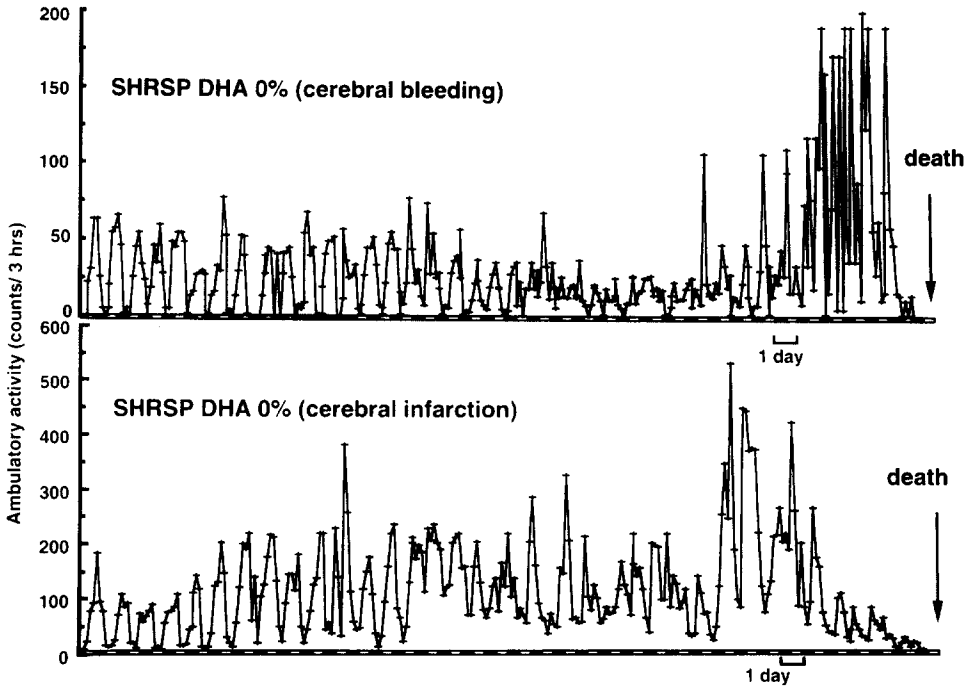

FIGURE 3. Typical behavioral changes in ambulation of SHRSP that died of cerebral bleeding (upper part) and cerebral infarction (lower part).

DHA caused a significant inhibition in blood pressure rise in SHRSP. We previously indicated that DHA induced an inhibition in the loss of kidney function in SHRSP (Kimura *et al.*, 1995). An improvement in renal hemodynamic behavior induced by DHA may be attributable in part to the prevention of lipid alteration. Yamori *et al.* (1982) and Whitmer *et al.* (1986) proposed that the cell membrane abnormalities in SHRSP could be observed in the vascular smooth muscle, the kidney and the heart as well as in the erythrocytes. α-Linolenate (n-3)-rich and linoleate (n-6)-rich diets modify the fatty acid composition of tissue lipids in SHR (Wirth *et al.*, 1984; Yamamoto *et al.*, 1987). Along with aging, both the renal phospholipase A_2 and the AA in the renal phospholipids decrease in SHRSP. These decreases may be involved in the development of hypertension (Okamoto *et al.*, 1989). Recently, we found that serum creatinine concentration and blood urea nitrogen were decreased significantly in DHA-treated SHRSP (Kimura *et al.*, 1995). These results suggest that n-6 and n-3 fatty acids are involved in the renal function, the time course of hypertension and apoplexy in

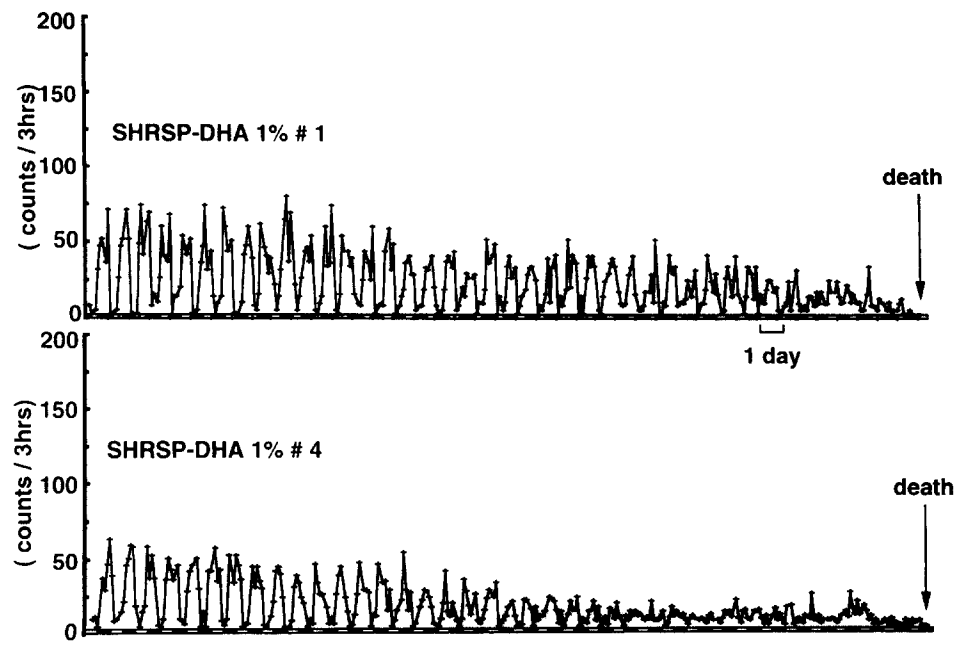

FIGURE 4. Effects of DHA (1%) on the behavioral changes before death of SHRSP in two typical examples.

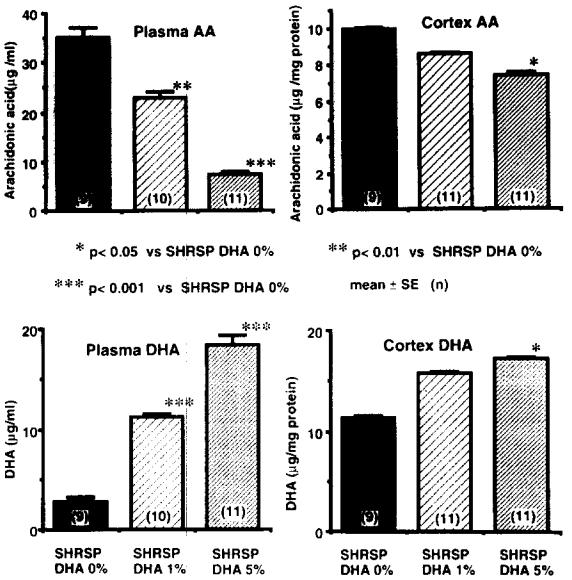

FIGURE 5. Effects of DHA on plasma AA, DHA, brain AA and DHA concentrations in SHRSP.

SHRSP. Dietary DHA increases tissue DHA levels and plasma phospholipids in rats (Bruckner *et al.*, 1984; Takahashi *et al.*, 1987). We reported that antihypertensive treatment inhibited the decreases in membranous phospholipids (Okamoto *et al.*, 1989). Although the actions of antihypertensive agents differ, all drugs that increase membrane phospholipids are known to increase renal blood flow (Richer *et al.*, 1983; Romero *et al.*, 1987).

Effects of n-3 fatty acids on similar hemodynamic properties may be explained in terms of eicosanoid balance: increasing AA in membrane phospholipids results in increased TXA_2/PGI_2 ratios (Abeywardena *et al.*, 1992; Fisher and Weber, 1986; Lee *et al.*, 1989). DHA inhibits TXA_2 production via the cyclooxygenase pathway (Hadjiagapiou and Spector, 1987). The existence of an enzyme system which converts DHA to EPA has been reported (Nettleton, 1991). Numerous studies have shown that dietary (n-3) fatty acids modify the tissue lipid composition and change the prostanoid synthesis (Kinsella *et al.*, 1990a; Nordøy and Dyerberg, 1989; Simopoulos *et al.*, 1991). Brown *et al.* (1984) reported replacement of n-6 by n-3 fatty acids and decreased synthesis of PGI_2 and $PGF_{2\alpha}$ after dietary manipulation with linseed oil (rich in n-3) in rats. Yerram *et al.* (1989) provided evidence of reduced PGI_2 and $PGF_{2\alpha}$ formation in cultured and isolated mouse brain capillary endothelial cells after preincubation with EPA and DHA, respectively. (n-3) Fatty acids competitively inhibit the incorporation of AA into phospholipids (Murase *et al.*, 1988) and the formation of eicosanoids from AA (Kinsella *et al.*, 1990a; Lands *et al.*, 1985; Von Schacky *et al.*, 1985). EPA is usually converted to eicosanoids including TXA_3 with lower proaggregatory and vasoconstrictive activities than TXA_2. In this study, dietary DHA significantly decreased plasma and brain cortex AA levels while increasing DHA levels of SHRSP. Thus, dietary n-3 fatty acids, such as DHA, may ameliorate eicosanoid-mediated diseases such as atherosclerosis and inflammatory diseases by reducing the tissue AA content and by inhibiting eicosanoid synthesis (Kinsella *et al.*, 1990b).

DHA induced the prolongation of life span in SHRSP. However, it has been suggested that an excess dietary intake of the n-3 series may have a detrimental effect on several diseases (Kinsella *et al.*, 1990b). Epidemiological studies originating with Greenlanders and Danes showed that dietary fish oil containing EPA and DHA reduces thrombotic diseases (Dyerberg, 1986; Dyerberg *et al.*, 1987; Kramhout *et al.*, 1985; Leaf and Weber, 1988). However, the incidence of apoplexy was higher in the Greenlanders than in the Danes, raising the question of whether excess intake of n-3 fatty acids might stimulate cerebral bleeding. In fact, two of six of our DHA (5%)-treated SHRSP underwent cerebral bleeding at a young age. A balanced intake of dietary n-6 and n-3 fatty acids is necessary to avoid the potential adverse effect of excess n-6-derived eicosanoid production (Kinsella *et al.*, 1990b). However, a fish oil diet containing 1% DHA (this study) and 2.6% DHA (Kobayashi *et al.*, 1996) prolonged the life span of SHRSP. Furthermore, perilla seed oil with n-6/n-3 ratios of 0.2–0.3 prolonged the survival time of a conventional strain of rat (Yamamoto *et al.*, 1991) as well as SHRSP (Shimokawa *et al.*, 1988). These studies indicate that an increase in dietary n-3 fatty acids may be more efficient in the reduction of n-6-derived eicosanoids. DHA supplementation resulted in a significant decrease in plasma and cortex AA levels of SHRSP (Fig. 5). A decrease in the AA levels in tissue phospholipids is known to decrease the ratio of vasoconstrictor TXA_2 to vasodilator PGI_2 (Abeywardena *et al.*, 1992; Fisher and Weber, 1986; Lee *et al.*, 1989). DHA also suppressed thrombotic tendency, carcinogenesis and metastasis (Okuyama, 1992), indicating a healthy nutritional nature of the n-3 fatty acids.

Loss of synchronization with the light and dark alternation cycle usually occurred after the onset of stroke in control SHRSP (DHA 0%). Furthermore, SHRSP that died of cerebral stroke showed a much longer periodicity (a new rhythm) in addition to the 24-hr periodicity (circadian rhythm) in accordance with our previous study (Minami *et al.*, 1985). In contrast, DHA-treated SHRSP maintained synchronization with the light and dark phases (Figs. 1 and 3). None of the DHA-treated SHRSP, including stroke cases, showed significant rhythm disturbance. This is the first report that DHA ameliorates the chronobiological disturbance observed in control SHRSP. Dietary DHA passes through the blood–brain barrier. Brain utilization of unesterified DHA bound to albumin has been shown (Anderson and Connor, 1988; DeGeorge, *et al.*, 1991; Onuma *et al.*, 1984; Thies *et al.*, 1994). Orally administered DHA seems to be redistributed to the retina and brain from the intestine (Li *et al.*, 1992). The brain contains a large amount of DHA, mainly in the gray matter and synaptosomes (Bourre *et al.*, 1989). Membranes of retinal and pineal cells contain large amounts of DHA, which may exert a stimulatory effect at the level of phospholipid synthesis (Delton *et al.*, 1995). DHA plays a structural and metabolic role in the retina and also seems to be involved in the development of learning ability and exploratory behavior in young animals (Bourre *et al.*, 1989; Nakashima *et al.*, 1993; Wainwright, 1991; Yamamoto *et al.*, 1987).

The disturbance of biological rhythms before death in SHRSP might correspond to behavioral changes such as the delirium-state observed in patients with dementia caused by cerebrovascular lesions. The mechanisms underlying sleep–wake rhythm disorders (desynchronization with the light and dark phase) are not yet well understood, and treatment of such rhythm disorders is often very difficult. Hypnotic drugs are not usually effective for these disorders. Our previous data demonstrated that dopa decarboxylase (DDC) activity and plasma DA concentration increased in DHA-treated SHRSP (Kimura *et al.*, 1995). It is suggested that DHA increases dopaminergic tone in the central nervous system and modulates behavioral changes. Similar results were obtained with methylcobalamin ($V\text{-}B_{12}$)-treated SHRSP in our laboratory (Minami *et al.*, 1991). Kamgar-Parsi *et al.* (1983) first reported that a patient with a

non–24-hr sleep–wake cycle was able to follow a normal 24-hr sleep–wake regimen after V-B_{12} treatment. V-B_{12} curtailed the abrupt increase of ambulatory activity and only 1 of 10 SHRSP treated with V-B_{12} demonstrated a new rhythm in addition to circadian rhythm (Minami *et al.*, 1991). Hakim *et al.* (1983) suggested that the reduced activity of methionine synthetase, which was shown to be induced by V-B_{12} deficiency causes defective methylation in the liver, which leads to insufficient formation of choline, the necessary precursor of acetylcholine in the brain.

SUMMARY

Dietary DHA dose-dependently suppressed the age-dependent increase in systolic blood pressure of SHRSP. Dietary ingestion of DHA significantly decreased plasma and brain cortex arachidonic acid levels while increasing DHA levels in a dose-dependent manner. The plasma total lipid values were also decreased by the dietary DHA.

In this study, the effects of DHA on stroke-related behavior and survival time were also examined. Six-week-old male SHRSP were fed diets with DHA (0%, 1% and 5%) throughout their life span. Dietary DHA altered the circadian rhythm, causing significant increases in ambulatory activity during the dark period and a tendency for decreased ambulation during the light period. At the onset of stroke, the ambulation of SHRSP abruptly increased. Desynchronization with light and dark phases and new biological rhythms were noted in all of the control SHRSP (DHA 0%). DHA-treated SHRSP did not show such behavioral changes. Furthermore, DHA administration significantly prolonged the mean survival time of SHRSP.

In conclusion, dietary DHA suppressed the age-dependent increase in systolic blood pressure and prolonged the average survival time of SHRSP. Furthermore, DHA ameliorated desynchronization with the dark and light phases and helped to prevent the new biological rhythms noted in control SHRSP.

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