Effects of Repeated Administrations of Facteur Thymique Sérique (FTS) on Biochemical Changes Related to Aging in Senescence-Accelerated Mouse (SAM)

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Abstract—Superoxide dismutase (SOD) activity, malondialdehyde (MDA) content and monoamine oxidase B (MAO-B) activity were measured in the brain, liver and kidney of a normal aging strain (R/1) and an accelerating aging strain (P/8) senescence-accelerated mice (SAM) at 9–10 months of age, and the effects of facteur thymique sérique (FTS) were examined. The activity of Cu,Zn-SOD in the kidney and MAO-B in the liver was significantly low and high in SAM-P/8 compared to SAM-R/1. FTS enhanced the activity of Mn-SOD and Cu,Zn-SOD in the kidney of SAM-P/8 and Cu,Zn-SOD activity in the brain of both SAM-P/8 and SAM-R/1. It decreased the activity of MAO-B in the liver and the contents of malondialdehyde (MDA) in the brain and kidney of SAM-P/8. Thus, FTS affects the biochemical factors related to senescence in SAM-P/8, a particular senescent animal model, and may thus possibly be effective as an anti-senescent medicine.

Facteur thymique sérique (FTS) is a nonapeptide which was first isolated from pig serum by Bach et al. (1–3) and then from the thymus (4). Several studies indicate that FTS exerts an immunobiological effect: 1) functional activation and differentiation in T cells (5), 2) suppression in experimental allergic encephalomyelitis (EAE) (6), 3) improvement of rheumatoid arthritis (7, 8) and cellular immunity (9, 10), 4) stimulation of spontaneous DNA synthesis in thymocytes (11), and 5) increase of neurotransmitter content in the brain (12). FTS receptors have been found to be present in certain T cells (13). Its levels in the body progressively decline, eventually becoming null with aging (14–16).

Senescence-accelerated mouse (SAM), a murine model of accelerating aging, was established by Takeda et al. (17) in the following forms: senescence-accelerated prone mouse (SAM-P) and senescence-accelerated resistant mouse (SAM-R). SAM-P shows accelerated changes with many signs of aging, such as a short life span, changes in general behavior, loss of skin glossiness, increased skin coarseness, hair loss, periorbital lesion, cataract, increased lordokyphosis of the spine (17), rapid appearance of dysfunction in learning and memory (18, 19), enhanced systemic amyloidosis (20–22), loss of bone mass (23), and others.

Nomura et al. (24) have demonstrated changes in several biochemical parameters related to the degree of senescence in SAM. For instance, at 11–12 months, the plasma level of testosterone and hepatic activity of superoxide dismutase (SOD) decreased, but the hepatic content of malondialdehyde (MDA) and the activity of monoamine oxidase type B (MAO-B) increased in SAM-P/8 as compared with those in SAM-R/1.

In the present study, we measured SOD, MAO-B activity and MDA content in the brain, liver and kidney of female SAM-P/8
and SAM-R/1 at 9–10 months of age. The effects of FTS on these parameters are also discussed.

Materials and Methods

Animals and FTS administration

SAM-P/8 and SAM-R/1 mice were originally obtained from Professor Toshio Takeda (Kyoto University). They were bred under conventional conditions, housed at 23±1 °C with an alternating 12 hr light/dark cycle, with food and water ad libitum. At 9 or 10 months of age, 3 female mice were used in each group. FTS dissolved in saline was administered at doses of 0.1 and 1.0 mg/kg/day, s.c., to SAM-P/8 and SAM-R/1 continuously for 21 days. Saline was administered as the control. One hour following the final administration, the animals were decapitated and their brain, heart, liver and kidney were quickly dissected out and stored at -80°C until use.

Assay of activity of SOD

The liver and kidney were homogenized (25) (1:9 w/v) with 0.25 M sucrose and the brain, with 0.32 M sucrose, both containing 1 mM EDTA and 10 mM Tris-HCl (pH 7.4), in a teflon-glass homogenizer. The homogenates were subsequently centrifuged at 300 x g for 15 min, and the supernatant was centrifuged at 10,000 x g for 15 min at 4°C. The pellet fractions were used for the assay of Mn-SOD activity in the presence of 2 mM potassium cyanide, in which Cu,Zn-SOD was selectively inhibited; and the supernatant was used to assay Cu,Zn-SOD activity following the inactivation of Mn-SOD by sodium dodecyl sulfate (SDS).

SOD activity was measured by the modified method of McCord and Fridovich (26). The assay mixture contained: 2.4 ml of 50 mM Na2CO3-NaHCO3 buffer (pH 10.3), 5 μl of 1 U/ml xanthine oxidase, 0.1 ml of 3 mM xanthine monosodium salt, 3 mM EDTA, 0.15% bovine serum albumin (BSA) and 0.75 mM nitroblue tetrazolium (NBT). The 0.1 ml sample containing SOD was reacted with the mixture at 25°C for 10 min, and 0.1 ml of 6 mM CuCl2 was added to terminate the reaction. Monitoring was then conducted spectrophotometrically at 560 nm. These assays were carried out using purified SOD protein (3200 U/mg) as the standard.

Measurement of MDA contents

The MDA content was measured by the TBA method (27). Brain, liver and kidney were homogenized with ice-cold 1.15% KCl to prepare a 10% homogenate. The homogenate (0.5 ml) pipetted into a centrifuge tube was mixed with 3 ml of 1% phosphoric acid and 1 ml of 0.6% thiobarbituric acid (TBA) aqueous solution, followed by heating in a boiling water bath for 45 min. After cooling and adding 4 ml of n-butanol, the system was mixed vigorously, and the butanol layer was separated by centrifugation. The optical density of the butanol layer was determined at 535 and 520 nm. The difference in optical density for these determinations was calculated and used as the TBA value. 1,1,3,3-tetramethoxypropane served as the standard.

Assay of MAO-B activity

The activity of MAO-B was measured using the modified radiometric method (28, 29). In brief, about 0.2 g brain or liver tissue was homogenized in 2 ml of ice-cold 0.1 M phosphate buffer (80.7 mM Na2HPO4, 19.1 mM KH2PO4, pH 7.4) and centrifuged at 600 x g for 10 min. The supernatant was further centrifuged at 15,000 x g for 10 min. The pellet thus obtained was suspended in 0.1 M phosphate buffer. As an in vivo experiment, 50 μl of the suspension (containing 1 mg protein/ml), which served as a crude mitochondrial fraction, were added to the incubation tubes containing 50 μl of 0.1 M phosphate buffer; and as an in vitro experiment, the crude mitochondria in the liver of SAM untreated by FTS was preincubated with FTS (total of 100 μl) at several concentrations at 37°C for 20 min. The mitochondria of both the in vivo and in vitro experiments were then reacted with 1 μM of [14C]phenylethylamine (PEA) in a 37°C shaking water bath for 20 min. The reaction was terminated by the addition of 100 μl of 2 N HCl. The metabolites were extracted with 2 ml of toluene by vigorous shaking. Radioactivity in the upper toluene phase was measured by a Packard liquid-scintillation spectrometer.

Assay of protein, DNA and RNA content

Protein assay: The tissues of the heart, liver and kidney were homogenized with distilled water to prepare a 10% homogenate, followed
by adding the same volume of 10% trichloroacetic acid (TCA). After centrifugation at 3,000 × g for 5 min, the pellet was incubated in 95°C water for 20 min in 10 times the volume of 5% TCA and centrifuged at 3,000×g for 5 min. The supernatant was used for the assays of DNA and RNA, and the pellet was dissolved in 1 N NaOH for measurement of protein by the method of Lowry et al. (30) using BSA as the standard.

**DNA assay (31):** A 0.5-ml sample was incubated with 4 ml of diphenylamine reagent (diphenylamine, 1 g; acetic acid, 100 ml; HClO₄, 10 ml; 1 ml of 1.6% acetaldehyde added immediately before use) and 1.5 ml distilled water at 60°C for 60 min, followed by measurement of the absorbance at 595 nm.

**RNA assay (32):** A 0.2 ml of sample was incubated with 2.5 ml of 0.1% orcinol reagent (0.1% orcinol FeCl₃-HCl solution) and 3.3 ml distilled water in boiling water for 20 min. Absorbance at 680 nm was measured after cooling.

**Statistical analysis**

The two way analysis of variance (ANOVA) was used to evaluate statistical significance.

**Drugs**

Facetteur thymique sérique (FTS) was synthesized at the Institute of Biological Sciences, Mitsui Pharmaceuticals, Inc.; [¹⁴C]phenyl (1-¹⁴C)ethylamine ([¹⁴C]PEA, 60 mCi/mmol) was from Amersham (Japan); superoxide dismutase (SOD), xanthine oxidase and RNA were from Sigma Chemical; xanthine monosodium salt was from NBCo Biochemical; nitroblue tetrazolium (NBT) and 1,1,3,3-tetramethoxypropane (TMP) were from Tokyo Kasei Organic Chemicals (Japan); 2-thiobarbituric acid was from Nacalai Tesque (Japan) and orcinol, diphenylamine and DNA were from Wako Pure Chemical Industries, Ltd. (Japan).

### Results

**SOD activity:** FTS significantly increased Cu,Zn-SOD activity at doses of 0.1 and 1.0 mg/kg/day for 21 days in the brain of both SAM-P/8 and SAM-R/1 (Fig. 1A, Table 1). The activities of Cu,Zn-SOD and Mn-SOD were expressed to a lesser degree in the kidney of SAM-P/8 than in SAM-R/1. The stimulative effect of FTS on Cu,Zn-SOD and Mn-SOD activities seems to be dose-dependent in SAM-P/8 but not in SAM-R/1. In contrast, the activity of Mn-SOD was higher in the liver and brain of SAM-P/8, and it could not be influenced by FTS (Fig. 1, A and B, Table 1).

**MDA content:** The content of MDA in the FTS untreated brain was not significantly different between SAM-P/8 and SAM-R/1. Interestingly, FTS reduced the content of MDA sharply at the doses of 0.1 and 1.0 mg/kg/day in the brain and at a dose of 1.0 mg/kg.

### Table 1. Comparisons of the activities of SOD between SAM-R/1 and SAM-P/8 treated with several doses of FTS

| Category | Tissues | Comparisons between strain treated with FTS | F₀ |
|----------|---------|------------------------------------------|----|
|          | Brain   | SAM-R/1 and SAM-P/8                      | 0.28 |
|          |         | FTS treatment                            | 8.61** |
| Cu,Zn-SOD| Liver   | SAM-R/1 and SAM-P/8                      | 0.15 |
|          |         | FTS treatment                            | 0.09 |
|          | Kidney  | SAM-R/1 and SAM-P/8                      | 5.12* |
|          |         | FTS treatment                            | 0.40 |
|          | Brain   | SAM-R/1 and SAM-P/8                      | 3.24 |
|          |         | FTS treatment                            | 0.90 |
| Mn-SOD   | Liver   | SAM-R/1 and SAM-P/8                      | 5.05* |
|          |         | FTS treatment                            | 0.58 |
|          | Kidney  | SAM-R/1 and SAM-P/8                      | 2.46 |
|          |         | FTS treatment                            | 3.47 |

Significance: *P<0.05 and **P<0.01. F₀ values were estimated by two-way analysis of variance.
in the kidney of SAM-P/8 (Fig. 2, Table 2).

MAO-B activity: The activity of MAO-B in the liver of SAM-P/8 was significantly higher than in SAM-R/1. FTS decreased MAO-B activity in the liver of SAM-P/8 at the doses of 0.1 and 1.0 mg/kg/day in vivo, and also the in vitro MAO-B activity in this tissue at a concentration range from 10 to 100 nM (Figs. 3 and 4, Table 3).

DNA, RNA and protein content: The effects of FTS on these parameters are shown in Fig. 5. The content of DNA in the heart of SAM-P/8 was lower than in SAM-R/1. FTS significantly increased DNA in the heart of SAM-P/8. In addition, FTS significantly increased the protein content in the heart and liver of SAM-P/8 and in the liver of SAM-R/1, but no effect was observed in the kidney at 1.0 mg/kg/day. RNA content was not influenced by FTS in either SAM-P/8 or SAM-R/1 (Fig. 5, Table 4).

Discussion

SOD activity, MAO-B activity, MDA and testosterone content significantly have been shown to differ in male SAM-P/8 and SAM-R/1 at 11–12 months (24). In the present study, the effects of repeated administrations
of FTS on the activity of SOD and MAO-B as well as the content of MDA were examined in several tissues of female SAM-P/8 and were found to differ significantly from the effects expressed in SAM-R/1.

Mn-SOD is located in the mitochondria (33), while Cu,Zn-SOD is mainly in the cytosol and lysosomes (34). The activity of both SODs diminishes with increase in age. The activity of Cu,Zn-SOD in the kidney of SAM-P/8 was significantly lower than that in SAM-R/1, but in the brain, they were essentially the same. FTS increased Cu,Zn-SOD activity in the brain of both SAM-P/8 and SAM-R/1 and in the kidney of SAM-P/8. The activity of Cu,Zn-SOD may thus decrease in the brain of either SAM-P/8 or SAM-R/1 and in the kidney of only SAM-P/8, and FTS may selectively increase the activity of Cu,Zn-SOD which diminishes with aging. The activity of Mn-SOD has also been considered to increase as a feed back effect when superoxide anion content rises with aging (35). The present results show low Mn-SOD activity in only the kidney of SAM-P/8, not in the brain and liver. That superoxide anions in the brain and liver increase earlier than that in the kidney with aging may be the reason for this. Mn-SOD gradually increases following that in superoxide anions and consequently FTS may be incapable of further increasing their activity in the brain and liver. Since the protein content did not significantly change in the kidney by the administrations of FTS, the activity of SOD in the kidney of SAM-P/8 may consequently be increased by this drug (FTS).

Peroxidized lipids present in animal tissues are generally recognized to be involved in...
Fig. 3. The effects of FTS on MAO-B activity of SAM. FTS was continuously injected subcutaneously in SAM-P/8 (*) and SAM-R/1 (○) for 21 days. Each value represents the mean±S.E.M. of 3 independent experiments. Statistical significance is shown in Table 3. PAA: phenylacetaldehyde.

Fig. 4. The influences of FTS on MAO-B activity in the liver of SAM in vitro. The crude mitochondria in the liver of SAM-P/8 (●) and SAM-R/1 (○) were used. Each value represents the mean±S.E.M. of 4 independent experiments. Statistical significance is shown in Table 3. PAA: phenylacetaldehyde.

Table 3. Comparisons of the activity of MAO between SAM-R/1 and SAM-P/8 treated with several doses of FTS

| Treatment | Tissues | Comparisons between | \( F_0 \) |
|-----------|---------|----------------------|-------|
| In vivo   | Brain   | SAM-R/1 and SAM-P/8  | 2.69  |
|           |         | FTS treatment        | 0.15  |
| In vitro  | Liver   | SAM-R/1 and SAM-P/8  | 23.71**|
|           |         | FTS treatment        | 27.71**|
|           | Liver   | SAM-R/1 and SAM-P/8  | 0.03  |
|           |         | FTS treatment        | 3.35* |

Significance: *P<0.05 and **P<0.01. \( F_0 \) values were estimated by two-way analysis of variance.
several cardiovascular, pulmonary, or hepatic diseases and a principal cause of aging. Lipid peroxidation progresses by absorbing molecular oxygen into activated free radicals produced from labile polyunsaturation in fatty acid chains. The present study has shown FTS to selectively reduce the level of MDA in the brain and kidney of SAM-P/8.

As described above, FTS was found to do the following: 1) increase the activity of SOD.

**Fig. 5.** The influences of FTS on contents of DNA, RNA and protein in SAM. The tissues of the heart, liver and kidney of SAM-P/8 (●) and SAM-R/1 (○) were used. Each value represents mean±S.E.M. of 3 independent experiments. Statistical significance is shown in Table 4.

**Table 4.** Comparisons of the contents of DNA, RNA and protein between SAM-R/1 and SAM-P/8 treated with several doses of FTS

| Category | Tissues | Comparisons between strain treated with FTS | \( F_0 \) |
|----------|---------|---------------------------------------------|--------|
| DNA      | Heart   | SAM-R/1 and SAM-P/8                        | 2.05   |
|          |         | FTS treatment                               | 5.11*  |
|          | Liver   | SAM-R/1 and SAM-P/8                        | 1.61   |
|          |         | FTS treatment                               | 1.42   |
|          | Kidney  | SAM-R/1 and SAM-P/8                        | 0.02   |
|          |         | FTS treatment                               | 0.30   |
| RNA      | Heart   | SAM-R/1 and SAM-P/8                        | 1.80   |
|          |         | FTS treatment                               | 1.85   |
|          | Liver   | SAM-R/1 and SAM-P/8                        | 1.26   |
|          |         | FTS treatment                               | 1.45   |
|          | Kidney  | SAM-R/1 and SAM-P/8                        | 1.53   |
|          |         | FTS treatment                               | 0.35   |
| Protein  | Heart   | SAM-R/1 and SAM-P/8                        | 0.03   |
|          |         | FTS treatment                               | 4.01*  |
|          | Liver   | SAM-R/1 and SAM-P/8                        | 8.51*  |
|          |         | FTS treatment                               | 19.40**|
|          | Kidney  | SAM-R/1 and SAM-P/8                        | 0.08   |
|          |         | FTS treatment                               | 1.63   |

Significance: *\( P<0.05 \) and **\( P<0.01 \). \( F_0 \) values were estimated by two-way analysis of variance.
thus possibly metabolizing activated free radicals, and 2) reduce the level of MDA. Both free radicals and MDA were produced by peroxidized lipids and are capable of impairing the body. Either the decrease in MDA or increase in the activity of SOD may be the means for preventing damage to the body, and in either case, this would give rise to the expression of an anti-aging effect in SAM-P/8.

MAO exists in at least two different forms in various of tissues (28), termed types A and type B, and they are distinguishable by substrate specificity and inhibitor sensitivity. The activity of MAO-B, the predominant MAO, increases with aging. In this study, MAO-B activity in the liver was higher in SAM-P/8 than in SAM-R/1. FTS inhibited MAO-B activity in the liver of SAM-P/8 both in vivo and in vitro. Thus possibly, FTS may inhibit the activity of MAO-B directly in the liver, consequently hindering accelerated senescence in SAM-P/8.

Accelerated age-related changes occurred to a greater extent in SAM-P/8 than in SAM-R/1 even at 10 months after birth, indicating SAM to be a useful experimental animal model for studying the molecular mechanisms of aging. FTS also lessened the accelerated biochemical changes, thus demonstrating its anti-aging effects. Additional research should be conducted to clarify in detail the anti-aging mechanisms of FTS.

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