Beta-Cryptoxanthin, Plentiful in Japanese Mandarin Orange, Prevents Age-Related Cognitive Dysfunction and Oxidative Damage in Senescence-Accelerated Mouse Brain

Keiko UNNO,* Minoru SUGIURA, Kazunori OGAWA, Fumiyo TAKABAYASHI, Masateru TODA, Midori SAKUMA, Ken-ichi MAEDA, Keisuke FUJITANI, Hideaki MIYAZAKI, Hiroyuki YAMAMOTO, and Minoru HOSHINO

Laboratory of Bioorganic Chemistry, School of Pharmaceutical Sciences, University of Shizuoka; 52–1 Yada, Suruga-ku, Shizuoka 422–8021, Japan. Research Team for Health Benefit of Fruit, National Institute of Fruit Tree Science; Shimizuokitsunakacho, Shizuoka 424–0292, Japan; and * Junior College, University of Shizuoka; 2–2–1 Oshika, Suruga-ku, Shizuoka 422–8021, Japan.

Received November 22, 2010; accepted December 20, 2010; published online December 22, 2010

Increased oxidative stress is known to accelerate age-related pathologies. Beta-cryptoxanthin (β-CRX, (3R)-β,β-carotene-3-ol) is a potent antioxidant that is highly rich in Satsuma mandarin orange (mandarin), which is the most popular fruit in Japan. We investigated the antioxidative and anti-aging effects of β-CRX and mandarin using senescence-accelerated mice (SAMP10), which were characterized by a short lifespan, high generation of superoxide anions in the brain and poor learning ability with aging. β-CRX (0.5—5.0 μg/ml) or mandarin juice (3.8—38.0%) was added to drinking water of SAMP10 one to 12 months of age. β-CRX was dose-dependently incorporated into the cerebral cortex and the contents were similar to the concentration of β-CRX in the human frontal lobe. These mice also had higher learning ability. The level of DNA oxidative damage was significantly lower in the cerebral cortex of mice that ingested β-CRX and mandarin than control mice. In addition, the mice that ingested β-CRX (>1.5 μg/ml) and mandarin (>11.3%) exhibited a higher survival when 12 month-old, the presenile age of SAMP10, than control mice. These results suggest that β-CRX is incorporated into the brain and has an important antioxidative role and anti-aging effect.

Key words β-cryptoxanthin; mandarin orange; learning; oxidative damage; survival; senescence-accelerated mouse

The brain is highly susceptible to oxidative damage because it consumes a large amount of oxygen and generates many free radicals as normal products of cellular metabolism.1—3 In addition, the brain contains diverse lipid components and fewer antioxidant enzymes than other organs.4 Although the antioxidant defense system responds to an increase in the production of reactive oxygen species (ROS) or peroxide under normal conditions, it gives rise to oxidative stress in the brain if an imbalance occurs between the production of ROS and the cellular defense capability. Aging is an important risk factor of this imbalance. Accumulation of oxidative damage is the most probable cause of the decline in learning and memory with aging.5 Preventing the accumulation of ROS-causing oxidative damage might suppress cognitive dysfunction.

Several studies have demonstrated that dietary antioxidants from fruits and vegetables prevent oxidative stress in the brain.6—10 Neuroprotective dietary compounds have an essential role in reducing aging of the brain.11,12 Beta-cryptoxanthin (β-CRX, (3R)-β,β-carotene-3-ol, Fig. 1) is the main precursor of vitamin A in citrus juices.13 This carotenoid is not fully cleaved in vitamin A in the gut and it is therefore recovered in human plasma.14 Recent clinical studies indicated that the frequent intake of citrus fruits increases plasma β-CRX concentrations and reported β-CRX to be a biomarker of mandarin consumption.15 The concentrations of carotenoids, tocopherols and retinols were investigated in an aged human brain.16 An age-related decline in retinol, total tocopherols, total xanthophylls (oxygenated carotenoids) and total carotenoids was observed in frontal but not in occipital lobes. The antioxidative activity of β-CRX has been reported to scavenge radicals,17 and to suppress lipid peroxidation18 and nitrogen oxide production.19 The important role of antioxidants in the brain is thus expected. In this study, we investigated the effect of consumption of β-CRX (Fig. 1) and Satsuma mandarin orange (mandarin, Citrus unshiu MARC.) juice on brain function because β-CRX is rich in mandarin, the most popular fruit in Japan.

We investigated the effect of β-CRX and mandarin juice on the brain using senescence-prone mouse strain 10 (SAMP10), which is a useful model of brain aging with a short life span and declined learning ability in later life.20,21 Moreover, the production of superoxide anion was higher in SAMP10 than in other mice with normal longevity and brain function.22 In addition, our previous data indicates that the consumption of green tea catechin (catechin), a potent antioxidant in green tea, suppressed brain dysfunction and DNA oxidative damage in aged SAMP10.23—25 These data strongly suggest that chronic intake of other antioxidants in fruits and vegetables also play a role in reducing the risk of age-related brain dysfunction. In this study, we investigated the effect of chronic consumption of β-CRX and mandarin juice, which contains a similar amount of β-CRX, on brain function using SAMP10 mice.

Fig. 1. Structure of β-CRX

* To whom correspondence should be addressed. e-mail: unno@u-shizuoka-ken.ac.jp © 2011 Pharmaceutical Society of Japan
MATERIALS AND METHODS

Animals All experimental protocols were performed in accordance with the guidelines for the care and use of laboratory animals of the University of Shizuoka. Male SAMP10/TaSlc (SAMP10) mice, which are senescence-prone, were purchased from Japan SLC Co., Ltd. (Shizuoka, Japan) and bred under conventional conditions in a temperature- and humidity-controlled room with a 12-h light/dark cycle. Mice were housed 5 per cage. Experimental mice had free access to a normal diet (CE-2; Clea Co., Ltd., Tokyo, Japan) and tap water containing 0.5% Tween 20 or 2% CMC.

Experimental Design The concentration of β-CRX was fixed at 0.5—5.0 μg/ml. The amount of β-CRX in mandarin (Ehime Beverage, Inc., Ehime, Japan) was 14.9 μg/ml. This was analyzed by reverse-phase HPLC using β-apo-8’-carotenol as the internal standard, as described previously.26 Therefore, mandarin juice was diluted with tap water to be 3.8—38.0% (v/v).

Eighty mice were divided into four groups containing a control for the β-CRX experiments (20 mice/group). Mice of each group were fed 0 (control), 0.5, 1.5 and 5.0 μg/ml of β-CRX, respectively. Another 80 mice were divided into four groups containing a control for the mandarin experiments. Mice of each group were fed 0 (control), 3.8, 11.3 and 38.0% mandarin juice, respectively. Mice 1 to 12 months of age drank tap water containing β-CRX or mandarin, i.e., for a total of 11 months.

The ingestion volume of β-CRX and mandarin per cage was measured 3 times/week for 11 months (143 times) and calculated as the average consumption/mouse/d. Body weight was measured once a month. When mice died, the lifespan was recorded. The learning ability (memory acquisition test) was measured in 11-month-old mice. Mice were sacrificed when 12-month-old. Cerebral cortex, cerebellum and liver samples were stored at −80 °C until analyses. Four samples of the cerebral cortex that were randomly selected were used to measure oxidative damage in DNA. The remaining cerebral cortex of each group was combined and used to measure oxidative damage in DNA. The ratio of the peak area of 8-oxodeoxyguanosine (8-oxodG) against that of deoxyguanosine (dG) was obtained. The amount of 8-oxodG was measured as a marker of oxidative damage to DNA.

Measurement of β-CRX in Brain and Liver The cerebral cortices, cerebellums and livers of each group were combined and homogenized with 1 ml of saturated saline solution and 2 ml of water in a polypropylene centrifuge tube (15 ml) using a homogenizer (Ultra Turrax T25, IKA Works Inc., Wilmington, NC, U.S.A.). After adding 6 ml of t-butyl-methylether (TBME, Wako Pure Chemical Industries, Ltd., Osaka, Japan) with 0.1% 2,6-di-t-butyl-4-methylphenol (BHT, Wako Pure Chemical Industries, Ltd., Osaka, Japan), the solution was homogenized and refrigerated at −30 °C. After centrifugation (10000 g, 20 min, 4 °C), the organic phase was recovered and the extraction procedure from the aqueous phase was repeated twice. The combined TBME phase was concentrated at 30°C in vacuo. The residue was re-dissolved in 150 μl of ethanol with 0.1% BHT, and subjected to HPLC (HP1100 system, Agilent Technologies, Santa Clara, CA, U.S.A.) analysis. Each sample of mice that ingested β-CRX (0.5 μg/ml) and mandarin juice (3.8%) was all used for one time measurement. The samples of mice that ingested β-CRX (1.5, 5.0 μg/ml) and mandarin juice (11.3, 38.0%) were used to measure two times, respectively.

HPLC analysis was carried out under the following conditions: column, YMC carotenoid column (3 μm, 3 mm i.d.× 150 mm, YMC Co., Ltd., Kyoto, Japan); solvent system, A) methanol:acetone:nitrite:H₂O (7 : 1 : 2) and B) TBME:methanol (9 : 1); elution schedule, an initial 0.5 min of 22.5% of A in B followed by a linear gradient with increasing concentration of B from 22.5 to 65% in 44.5 min; column temperature, 30°C; flow rate, 1.0 ml/min; injection volume, 50 μl; detection, UV absorption at 450 nm. The quantity of carotenoids was estimated by the absolute calibration method. The peak for each carotenoid was identified by its UV spectrum obtained by a diode-array detector. Detection limit of the analysis was 0.018 pmol/injection. All chemicals used for the measurement procedure of β-CRX were of reagent or HPLC grade.

Statistical Analyses Data were expressed as mean±S.E. The effect of mandarin and β-CRX intake was determined by one-way analysis of variance followed by the Bonferroni t-test for multiple comparisons. In the test for a linear trend, the association among continuous variables across four groups (β-CRX; 0, 0.5, 1.5, 5.0 μg/ml) and mandarin (0, 3.8, 11.3, 38.0%) was carried out by regression analysis. Survival
differences were compared using the logrank test. The concentrations of β-CRX in cerebral cortex and liver were expressed as mean±S.D.

RESULTS

Consumption of β-CRX and Body Weight Each mouse drank about 11—13 ml of water containing β-CRX in a day. The ingestion volume of mandarin juice was 12—14 ml (Tables 1A, B). Those volumes did not change significantly with aging. The average ingestion of water containing β-CRX ranged from 5.4 to 58.7 μg/d/mouse in mice that drank water-containing β-CRX (0.5—5.0 μg/ml), and from 7.7 to 68.0 μg/d/mouse in mice that drank mandarin juice (3.8—38.0%) (Tables 1A, B). Body weight did not change among these groups (Figs. 2A, B). The growth curve indicates that mice matured until about 3—4 months of age.

Table 1A. Consumption of β-CRX in Drinking Water

<table>
<thead>
<tr>
<th>β-CRX in water (μg/ml)</th>
<th>Volume (ml/d/mouse)</th>
<th>β-CRX (μg/d/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>11.7±0.28</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>10.9±0.21</td>
<td>5.4±0.01</td>
</tr>
<tr>
<td>1.5</td>
<td>12.5±0.29</td>
<td>18.8±0.05</td>
</tr>
<tr>
<td>5.0</td>
<td>11.7±0.25</td>
<td>58.7±0.56</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E. (n=143).

Table 1B. Consumption of β-CRX in Mandarin Juice

<table>
<thead>
<tr>
<th>Mandarin (%)</th>
<th>Volume (ml/d/mouse)</th>
<th>β-CRX (μg/d/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13.7±0.21</td>
<td>0</td>
</tr>
<tr>
<td>3.8</td>
<td>13.8±0.33</td>
<td>7.7±0.10</td>
</tr>
<tr>
<td>11.3</td>
<td>13.1±0.13</td>
<td>22.2±0.48</td>
</tr>
<tr>
<td>38.0</td>
<td>12.1±0.25</td>
<td>68.0±1.60</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E. (n=143).

Learning Ability The time for learning not to enter the dark room was measured at 11 months using a step-through passive avoidance task. A longer learning time implies lower learning ability. The learning time was significantly shorter in mice that drank a higher concentration of β-CRX than control mice that drank water without β-CRX (Fig. 3A) (F(3, 55)=2.140, p=0.1056). Similarly, the learning time was shorter in mice that drank 38% mandarin juice than control mice (Fig. 3B) (F(3, 51)=3.225, p=0.0301). In the test for a linear trend, the association among continuous variables across four groups of β-CRX (0, 0.5, 1.5, 5.0 μg/ml), and mandarin juice (0, 3.8, 11.3, 38%) was carried out by regression analysis. The p values for the trend were 0.027 (β-CRX) and 0.014 (mandarin), indicating that learning ability improved in a dose-dependent manner by consuming β-CRX and mandarin juice.

DNA Oxidative Damage in Brain As the learning ability improved in mice that drank β-CRX and mandarin juice, the level of DNA oxidative damage was investigated in their cerebral cortex where the level of 8-oxodG has been found to increase in aged SAMP10.23 The level of 8-oxodG was significantly lower in 12-month-old SAMP10 that drank water containing more than 1.5 μg/ml β-CRX than in age-matched control mice that did not consume β-CRX (Fig. 4A) (F(3, 12)=9.999, p=0.0014). Similarly, the level of 8-oxodG was lower in mice that drank more than 11.3% mandarin juice than in control mice (Fig. 4A) (F(3, 13)=7.230, p=0.0042). The level of 8-oxodG in the cerebral cortex was significantly higher in mice that drank water without β-CRX and mandarin juice, suggesting that the intake of β-CRX suppressed oxidative damage in the cerebral cortex.

The Concentration of β-CRX in Brain and Liver To confirm the incorporation of β-CRX into the brain and body, the concentrations of β-CRX in cerebral cortex, cerebellum and liver were measured. Each value was obtained from the combined organ samples of each group. The level was high in the cerebral cortex and liver of mice that ingested a higher...
concentration of $\beta$-CRX and mandarin juice (Table 2). Strong correlations were observed between the levels of $\beta$-CRX in the organs and the concentrations of $\beta$-CRX in drinking water and mandarin juice ($R^2=0.859-1.000$, Fig. 5). In addition, the level of $\beta$-CRX tended to be higher in mice that ingested mandarin juice than of mice that ingested $\beta$-CRX. In the cerebellum, $\beta$-CRX was not detected.

As another main carotenoid, $\beta$-carotene was detected at 0.04—0.08 $\mu$g/g in the liver of mice that ingested $\beta$-CRX, and 0.06—0.15 $\mu$g/g in the liver of mice that ingested mandarin juice. However, $\beta$-carotene was not detected in the cerebral cortex of mice that ingested either $\beta$-CRX or mandarin juice. $\beta$-Carotene is thought to come from the diet and mandarin juice.

**Survival** The lifespan of mice ingesting each concentration of $\beta$-CRX and mandarin was plotted. The starting number of mice was 20 in each group. Only one group, $\beta$-CRX (1.5 $\mu$g/ml), consisted of 19 mice because one mouse died just before the experiment. Mice that ingested no (control) or a low concentration of $\beta$-CRX (0.5 $\mu$g/ml) began to die from about 4 months of age. The survival rate of mice that ingested $1.5 \mu$g/ml $\beta$-CRX was 0.872 at 12 months of age, tending to be higher than 0.675 in those mice that ingested $<0.5 \mu$g/ml $\beta$-CRX ($p=0.13$, logrank test) (Fig. 6A). Simi-
Fig. 5. The Content of β-CRX in Cerebral Cortex and Liver of Mice That Drank β-CRX (A) or Mandarin Juice (B)

The cerebral cortex and liver samples of each group were combined and the levels of β-CRX were measured (cerebral cortex, circle; liver, square) (mean±S.D.).

Table 2A. β-CRX in Cerebral Cortex and Liver of Mice That Ingested β-CRX

<table>
<thead>
<tr>
<th>β-CRX in water (µg/ml)</th>
<th>Times of measurement</th>
<th>β-CRX (ng/g)</th>
<th>Cerebral cortex</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>4.14</td>
<td>11.69</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>1</td>
<td>3.99</td>
<td>10.12</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>2</td>
<td>4.74±0.32</td>
<td>22.43±15.88</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>2</td>
<td>8.56±0.93</td>
<td>61.21±4.77</td>
<td></td>
</tr>
</tbody>
</table>

Each value was obtained from the combined organ samples of each group (mean±S.D.).

Table 2B. β-CRX in Cerebral Cortex and Liver of Mice That Ingested Mandarin Juice

<table>
<thead>
<tr>
<th>Mandarin (%)</th>
<th>Times of measurement</th>
<th>β-CRX (ng/g)</th>
<th>Cerebral cortex</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>4.01</td>
<td>16.67</td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td>1</td>
<td>5.43</td>
<td>22.53</td>
<td></td>
</tr>
<tr>
<td>11.3</td>
<td>2</td>
<td>13.61±4.25</td>
<td>35.45±7.90</td>
<td></td>
</tr>
<tr>
<td>38.0</td>
<td>2</td>
<td>18.91±10.42</td>
<td>85.52±5.68</td>
<td></td>
</tr>
</tbody>
</table>

Each value was obtained from the combined organ samples of each group (mean±S.D.).

Fig. 6. The Survival Ratio of Mice That Drank β-CRX (A) or Mandarin Juice (B)

Mice drank different concentrations of β-CRX [0 µg/ml (control, open square), 0.5 µg/ml (closed square), 1.5 µg/ml (closed triangle) and 5.0 µg/ml (closed circle in 0.5% Tween20 (A)) and different concentrations of mandarin juice [0% (control, open square), 3.8% (closed square), 11.3% (closed triangle) and 38.0% (closed circle) in 2% CMC (B)]. The starting number of mice was 20 in each group. Only one group, β-CRX (1.5 µg/ml), consisted of 19 mice because one mouse died just before the experiment.
larly, the survival ratio at 12 months of age was 0.825 in mice that ingested \( \geq 11.3\% \) mandarin, being significantly higher than 0.625 in mice that ingested \(< 3.8\% \) mandarin \((p<0.05, \text{logrank test})\) (Fig. 6B).

DISCUSSION

A potent antioxidative activity of carotenoids is at least in part suggested to contribute to beneficial health effects.\(^{28-30}\) In this study, we demonstrated that the chronic intake of \( \beta \)-CRX and mandarin, in which \( \beta \)-CRX was the main carotenoid, prevented the decrease in learning ability and the increase in DNA oxidative damage in the cerebral cortex of aged SAMP10 mice (Figs. 3, 4). In addition, the survival rate at the presenile age of SAMP10 was higher in mice that ingested \( \beta \)-CRX and mandarin than in control mice (Fig. 6). How then do these effects/phenomena interact with each other? We found two relationships; one is between DNA oxidative damage and the survival rate, and the other is between learning ability and the content of \( \beta \)-CRX in the cerebral cortex.

DNA Oxidative Damage and Survival Various mice models with genetic manipulations in their antioxidant defense system have been used to investigate whether altering oxidative stress/damage change life span. These mice showed that increased oxidative stress accelerated age-related pathology and reduced oxidative stress retarded pathology, suggesting that oxidative stress plays a major role in a healthy life span.\(^{31}\) Furthermore, it has been reported that the lifespan of various mice was prolonged by ingestion of various antioxidants.\(^{32-36}\)

In the brain of SAMP10, the generation of superoxide anion was higher than in mice of normal lifespan.\(^{22}\) Furthermore, the level of 8-oxodG was 1.4—1.5 times higher in the kidney and liver of 12-month-old mice than that in 6-month-old SAMP10 and age-matched control mice (unpublished data), suggesting that oxidative damage increased in various tissues of SAMP10. The average lifespan (50% survival time) of SAMP10 was 15.5 months and the survival ratio at 12 months of age was about 0.6—0.7 in our laboratory.\(^{23}\) In SAMP10 mice, 11 and 12 months of age represent the presenile and geriatric age.

In both mice that ingested \( \beta \)-CRX (>1.5 \( \mu \)g/ml) and mandarin (>11.3%), oxidative damage in the cerebral cortex was significantly suppressed (Fig. 4). In addition, we found that SAMP10 mice that ingested mandarin (>11.3%) exhibited a significantly higher survival than mice that ingested mandarin (<3.8%) \((p<0.05, \text{logrank test}, \text{Fig. 6B}).\) The survival rate of mice that ingested \( \beta \)-CRX (>1.5 \( \mu \)g/ml), 0.872, was as high as that of mice that ingested mandarin (0.825). The mice with low oxidative damage following the ingestion of \( \beta \)-CRX (>1.5 \( \mu \)g/ml) and mandarin (>11.3%) exhibited an increased survival ratio.

On the other hand, it was thought that the improved learning ability was, in part but not fully, explained by the suppression of DNA oxidative damage, although another marker of oxidative damage in protein or lipid might need to be investigated.

Learning Ability and the Content of \( \beta \)-CRX in the Cerebral Cortex The consumption of \( \beta \)-CRX effectively suppressed a decline in learning ability in aged mice (Fig. 3). Learning ability improved more in mice that consumed higher concentrations of \( \beta \)-CRX and mandarin juice. In the test for a linear trend by regression analysis, the dose-dependent associations were observed among continuous variables across four groups of \( \beta \)-CRX (0—5.0 \( \mu \)g/ml) and mandarin juice (0—38%).

Incidentally, \( \beta \)-CRX incorporated into liver through the intestine is thought to be delivered into brain and other organs. The levels of \( \beta \)-CRX in the cerebral cortex and liver were closely related to the concentrations of \( \beta \)-CRX in drinking water and mandarin juice (Fig. 5). These results suggest that the dose-dependent increase of \( \beta \)-CRX content in the cerebral cortex was related to the dose-dependent improvement in learning ability. A hypothesis was proposed in which the late onset of Alzheimer’s disease was influenced by availability in the brain of retinoic acid, the final product of the vitamin A metabolic cascade.\(^{37}\) Since \( \beta \)-CRX has a role as a natural RAR (retinoic acid receptor) ligand, \( \beta \)-CRX incorporated into the brain might have a role in learning ability as not only an antioxidant but also as a provitamin A.

The contents of \( \beta \)-CRX in the cerebral cortex were higher in mice that ingested mandarin juice than those that ingested water containing \( \beta \)-CRX (Table 2). Although the regression of learning ability tended to be more suppressed in mice that ingested mandarin juice than in mice that ingested \( \beta \)-CRX (Fig. 4), the difference was not significant. \( \beta \)-CRX might have been fully supplied in these mice that ingested a high dose of \( \beta \)-CRX and mandarin juice. Alternatively, further behavioral analysis might be needed. \( \beta \)-CRX is found in mandarin in both free and esterified forms. An investigation about the effect of different forms of \( \beta \)-CRX on absorption and stability might also be needed.

Separately, synapse formation (synaptic plasticity) has been found to be associated with memory storage and learning.\(^{39-41}\) In aged SAMP10, a decreased level of synaptophysin, a presynaptic vesicular protein, and synaptic loss have been observed in the atrophied anterior cerebral cortex,\(^{21}\) and cognitive performance has been found to be associated with alterations in cerebral atrophy,\(^{24,42}\) and the level of synaptophysin.\(^{23}\) In mice that ingested \( \beta \)-CRX, however, the wet weight of the cerebrum did not change significantly and an increase of synaptophysin was observed but it was not significant (data not shown). These results suggest that \( \beta \)-CRX might not mainly affect synaptic plasticity.

A Possibility of \( \beta \)-CRX in Prolonging Healthy Life Span and Suppressing Brain Dysfunction in Humans It has been reported that \( \beta \)-CRX is a major carotenoid in the human brain and that the level of \( \beta \)-CRX in the frontal lobe was 23.0±8.5 and 17.5±3.2 pmol/g in white and gray matter, respectively, in the elderly human brain (67—90 of age). In addition, the frontal but not occipital lobes exhibited an age-related significant decline in total tocopherols, carotenoids and xanthophylls.\(^{16}\) Patients with dementia have also been reported to have lower levels of carotenoids containing \( \beta \)-CRX than controls in plasma.\(^{43}\)

The concentrations of \( \beta \)-CRX in the human frontal lobe mentioned above are about 8—17 ng/g. In the cerebral cortex of mice that ingested \( \beta \)-CRX and mandarin juice, the level of \( \beta \)-CRX was ca. 8.56 and ca. 18.91 ng/g, respectively (Table 2). This result indicates that the level of \( \beta \)-CRX in mice was similar to that in humans. In our experiments using mice, the
effective dose of β-CRX was 0.5—2.0 mg/kg body weights for suppressing DNA oxidative damage and cognitive dysfunction. As rodents were classified as non-accumulators of carotenoids, a high concentration of β-CRX was needed for the delivery of β-CRX into the cerebral cortex of mice. However, the requirement of β-CRX is thought to be very low in the case of humans because humans readily accumulate dietary or supplemental carotenoids. Recently, Sugira et al. reported that the geometric mean of the daily intake of β-CRX among a middle-aged Japanese population was estimated at about 0.3 mg/d. Using the data from this population, they found some inverse associations of serum β-CRX with risks for insulin resistance, metabolic syndrome, and atherosclerosis. In addition, the serum levels of carotenoids have been reported to be inversely associated with all-cause mortality and lung cancer. These epidemiological studies support the beneficial effect of β-CRX on a healthy life span.

In conclusion, we found that the consumption of β-CRX and mandarin juice suppressed the decline of learning ability and DNA oxidative damage in the cerebral cortex of aged mice. In addition, the survival rate was higher in the presenile age of mice that ingested β-CRX and mandarin juice than in control mice.

Acknowledgements We gratefully thank Ehime Beverage Inc. for providing us with Satsuma mandarin extract. The study was supported in part by the Nestlé Nutrition Council of Japan and the grant for the Council for Advancement of Fruit Tree Science.

REFERENCES