Central Nervous System Migration of Astaxanthin and Adonixanthin Following Their Oral Administration in Cynomolgus Monkeys

Shinsuke Nakamura1, Takashi Maoka2, Shohei Tsuji1, Masahiro Hayashi3, Masamitsu Shimazawa1,4 and Hideaki Hara1,4

1 Molecular Pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University, Gifu 501–1196, Japan
2 Research Institute for Production Development Division of Food Function and Chemistry, Kyoto 606–0805, Japan
3 ENEOS Corporation, Tokyo 108–8005, Japan
4 Biomedical Research Laboratory, Gifu Pharmaceutical University, Gifu 501–1196, Japan

(Received April 15, 2020)

Summary

Astaxanthin, which has been shown to have significant antioxidant activity, is rapidly spreading as a health functioning ingredient in the health food and cosmetics sectors worldwide. It is well known that astaxanthin acts on the brain; however, there is little evidence of brain translocation due to the difficulty in identifying astaxanthin in tissues. Therefore, in this study, we investigated the concentrations of astaxanthin and adonixanthin, the latter being a biosynthetic intermediate from β-carotene to astaxanthin, in the brain after oral administration in primates. Cynomolgus monkeys were orally administered astaxanthin or adonixanthin at a dose of 50 mg/kg for 10 d, through a disposable catheter inserted into the stomach via the nasal passage. Following euthanization, the monkeys’ brains and various other organs were collected. The carotenoid content in serum and individual organs was analyzed by high-performance liquid chromatography. Adonixanthin was found to accumulate at a higher concentration than astaxanthin in monkey brain tissues. Also, both astaxanthin and adonixanthin were found to be distributed in the heart, spleen, liver, and kidneys. These findings indicate that astaxanthin and adonixanthin can enter the central nervous system of primates following their oral administration. This provides important evidence for the activity of astaxanthin and adonixanthin on the central nervous system.

Key Words

blood concentration, brain distribution, carotenoid, primate, tissue absorption

Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) which is biosynthesized as shown in Fig. 1 (1), is a carotenoid with potent antioxidant (2); it occurs in haematococcus algae and aquatic animals such as salmon, shrimps, and crabs (3–6). Kotake-Nara and Nagao has reported that carotenoids with a 3-hydroxy-β-end group in xanthophylls such as β-carotene were oxidized to carotenoids with a 3-oxo-β-end group through an intermediate with a 3-oxo-β-end group by a NAD+ dependent dehydrogenase (7). According to previous reports, astaxanthin exhibits physiological and pharmacological actions, possessing not only antioxidant but also anti-inflammatory, anti-apoptotic, and anti-tumor properties (8–11). Interestingly, astaxanthin has been reported to improve human cognitive function (12). In fact, there have been several reports showing the effects of astaxanthin in in vitro experimental systems. For example, following treatment with astaxanthin, the rat neuronal cell line, PC12, was protected from neurotoxicity induced by β-amyloid peptide (13). Furthermore, it has been reported that astaxanthin inhibits 6-hydroxydopamine (OHDA)-induced apoptosis in human neuroblastoma SH-SY5Y cells, via the attenuation of intracellular reactive oxygen species (ROS) generation and p38 mitogen-activated protein kinase (MAPK) activation (14). In addition to in vitro studies, there have been several reports of astaxanthin acting on the central nervous system in some animal models. Specifically, astaxanthin at 30 mg/kg (i.p.) was significantly attenuated due to the number of coagulated and damaged pyramidal neurons in ischemia-reperfusion model mice (15). We have also previously reported that astaxanthin has anxiolytic-like effects (16) and that both astaxanthin and its derivative, adonixanthin (3,3′-dihydroxy-β,β-carotene-4-one), exert protective effects against hemorrhagic brain injury in mice (17). Adonixanthin is known to have a stronger antioxidant effect than astaxanthin (18). Together, these reports indicate that astaxanthin and adonixanthin can pass through the blood–brain barrier (BBB) to act on cranial
nerve cells and cerebral blood vessels.

The BBB limits paracellular permeability by providing a meshwork of non-fenestrated microvascular endothelial cells enclosed by neurons, pericytes, and astrocytes (19). As a result, the brain can selectively absorb useful compounds, such as amino acids, glucose, vitamins, and inorganic compounds, but exerts tight control over any other substrates attempting to cross the BBB (20). Recently, Manabe et al. reported that dietary astaxanthin accumulates in the hippocampus and cerebral cortex of rats (21), suggesting that astaxanthin can penetrate the BBB in rodents; as yet, however, there is no evidence for this in primates. Hence, we examined the penetration of astaxanthin and adonixanthin into the

Fig. 1. Astaxanthin biosynthesis pathways.

Fig. 2. The chemical structures of astaxanthin, adonixanthin, and other carotenoids found in monkey.
brain of cynomolgus monkeys following its oral administration.

Materials and Methods

Reagents. Astaxanthin and adonixanthin (Fig. 2) obtained from Paracoccus carotinifaciens were both provided by JXTG Nippon Oil & Energy Corporation (Tokyo, Japan). Adonixanthin is an intermediate compound between zeaxanthin and astaxanthin (22, 23).

Olive oil was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

Animals and treatments. Experiments using cynomolgus monkeys (Macaca fascicularis) were approved by the Institutional Animal Care and Use Committee of Shin Nippon Biomedical Laboratories, Ltd. (SNBL; Kagoshima, Japan), which is licensed by the Association for Assessment and Accreditation of Laboratory Animal Care International (approval number: IACUC730–002). Two male monkeys were used in this study, aged 3 years and weighing 3.36 and 3.45 kg. The monkeys were housed in a room maintained at 24–28°C with 30–70% humidity, ventilated 15 times/h, with artificial lighting for 12 h (7:00 to 19:00) and provided ad libitum access to water and approximately 108 g of solid food (HF Primate J 12G 5K9J, Purina, Mills LLC) daily. This solid food have no carotenoids. After 1 wk of acclimation, the monkeys were euthanized by exsanguination under anesthesia, and their brains and eyeballs were collected. The retina was removed from each eyeball. The following structures were dissected out from the whole brains: cerebral cortex, cerebral medulla, cerebellum, mesencephalon, striatum (putamen), striatum (caudate nucleus), hippocampus, medulla oblongata, and diencephalon. All samples, including both serum and tissues, were stored at −80°C until being analyzed for astaxanthin and adonixanthin content. This study was performed in accordance with the animal welfare by laws of the Drug Safety Research Laboratories of SNBL, which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Analysis of astaxanthin and adonixanthin content. Carotenoid analysis, including testing for astaxanthin and adonixanthin content, in the serum and organs of cynomolgus monkeys was performed as follows (24). Ethanol (2 mL) was added to 1 mL serum and stirred. Carotenoids were extracted from this mixture by adding 5 mL diethyl ether; hexane (2 : 8, v/v) solution and stirring. The mixture was left to stand for 10 min, then the upper layer was filtered through a polyvinylidene difluoride membrane (0.45-μm pore size, Millipore Corp, Massachusetts, USA), followed by evaporation to dryness. The residue was dissolved in acetone : hexane (2 : 8, v/v) and subjected to high-performance liquid chromatography (HPLC). To extract carotenoids from organs or tissues, each organ sample was homogenized, and repeated extraction with acetone was performed until a colorless extract was obtained. The extract was filtered, the acetone was evaporated, and carotenoids were extracted from the concentrated acetone extract using a diethyl ether : hexane (2 : 8, v/v) solution. After evaporation of the extract to dryness, the residue was dissolved in acetone : hexane (2 : 8, v/v) and subjected

| Carotenoid (ng/mL)   | Astaxanthin                       | Adonixanthin                       |
|---------------------|-----------------------------------|-----------------------------------|
|                     | Pre-treatment                      | After 10 d of repeated administration | Pre-treatment                      | After 10 d of repeated administration |
| β-Carotene          | 10.6                              | 8.5                               | 9.5                                | 18.9                                |
| β-Cryptoxanthin     | 21.0                              | 21.8                              | 25.8                                | 45.4                                |
| e,e-Caroten-3,3′-dione | 1.7                              | 1.5                               | 2.5                                | 38.9                                |
| 3-OH-β,e-Caroten-3-one | 4.2                              | 4.0                               | 5.8                                | 23.8                                |
| Lutein              | 13.7                              | 21.8                              | 23.0                                | 38.7                                |
| Zeaxanthin          | 13.8                              | 21.8                              | 22.2                                | 35.9                                |
| Sum of common carotenoids | 65.0                              | 79.4                              | 88.8                                | 201.7                               |
| Astaxanthin         | N.D.                              | 36.2                              | N.D.                                | 1,387.4                             |
| Adonixanthin        |                                   |                                   |                                     |                                     |
| Sum of total carotenoids | 65.0                              | 115.6                             | 88.8                                | 1,589.0                             |

n=1. N.D.: not detected.
Brain Distribution of Astaxanthin and Adonixanthin in Primates

Results

Concentration of carotenoids in serum

Cynomolgus monkeys generally do not have either astaxanthin or adonixanthin in their serum (24). Both carotenoids were undetectable prior to their administration, but were noticeably present in serum by the 10th day of repeated administration at 50 mg/kg (Table 1). There was no apparent change in the normal carotenoid concentration in serum following the administration of astaxanthin, but administration of adonixanthin markedly increased them (Table 1).

Concentration of carotenoids in the brain and retina

Next, we investigated the concentration of carotenoids in the brain and retina of astaxanthin- or adonixanthin-treated monkeys. Carotenoid concentrations in the brain and retina of cynomolgus monkeys have been analyzed previously (24), but this is the first detailed measurement of carotenoid concentrations in specific regions of the brain (Table 2). Indeed, Nishino et al. reported that both astaxanthin and adonixanthin are usually absent in the brain of monkeys (24). Here, we showed that astaxanthin or adonixanthin could be detected in the brain following their oral administration (Table 2). In the astaxanthin-treated monkey, the ratio of astaxanthin to total carotenoids in the cerebral cortex, cerebral medulla, cerebellum, mesencephalon, striatum (putamen), striatum (caudate nucleus), hippocampus, medulla oblongata, diencephalon, and retina was 14%, 30%, 38%, 21%, 16%, 22%, 21%, 39%, 21%, and 17%, respectively (Table 2). The ratio of adonixanthin to total carotenoids in the cerebral cortex, cerebral medulla, cerebellum, mesencephalon, striatum (putamen), striatum (caudate nucleus), hippocampus, medulla oblongata, diencephalon, and retina in the adonixanthin-treated monkey was 72%, 62%, 62%, 37%, 51%, 50%, 54%, 65%, 76%, and 63%, respectively (Table 2). Therefore, compared with the level of astaxanthin, adonixanthin was present at a level approximately 40 times higher in the serum, approximately 9 to 10 times higher in the hippocampus and cerebral cortex, and about 28 times higher in the retina.

Concentration of carotenoids in tissues other than the brain and retina

We also examined the concentration of carotenoids in other tissues of the astaxanthin- and adonixanthin-treated monkeys. As in the brain, astaxanthin and adonixanthin have been reported to usually be absent in other organs (24); however, following the oral administration of astaxanthin or adonixanthin they were seen to accumulate in the heart, spleen, kidneys, and liver (Table 3). In all organs, adonixanthin was present at

### Table 2. Carotenoid concentration in astaxanthin- or adonixanthin-treated brain and retina.

| Carotenoid (ng/g) | Cerebral cortex | Cerebral medulla | Cerebellum | Mesencephalon | Striatum (Putamen) | Striatum (Caudate nucleus) | Hippocampus | Medulla oblongata | Diencephalon | Retina |
|------------------|----------------|-----------------|------------|---------------|-------------------|---------------------------|-------------|-------------------|--------------|--------|
| β-Carotene       | 3.9            | 3.4             | 3.8        | 7.7           | 2.1               | 1.9                       | 6.6         | 3.5               | 2.1          | 3.2    |
| Χ-Cryptoxanthin  | 10.1           | 2.9             | 11.1       | 11.1          | 2.1               | 1.5                       | 1.1         | 0.3               | 0.3          | 0.3    |
| Χ-Caroten-3,3′-dione | 2.1           | 0.1             | 2.0        | 1.2           | 1.5               | 0.6                       | 0.3         | 0.3               | 0.3          | 0.3    |
| ΧOH-β-Caroten-3-one | 6.1           | 4.9             | 4.9        | 6.7           | 6.2               | 6.7                       | 3.1         | 3.5               | 4.2          | 7.5    |
| Zeaxanthin       | 5.7            | 4.6             | 5.7        | 6.0           | 6.1               | 6.0                       | 3.0         | 3.2               | 4.0          | 7.1    |
| Sum of common carotenoids | 29.0 | 19.4            | 33.1       | 35.4          | 39.3              | 40.7                      | 35.6        | 38.5              | 39.2         | 46.3   |
| Astaxanthin      | 4.8            | 4.0             | 4.2        | 4.2           | 4.2               | 4.2                       | 4.2         | 4.2               | 4.2          | 4.2    |
| Adonixanthin     | 90.0           | 49.0            | 53.2       | 53.2          | 57.5              | 57.5                      | 50.0        | 50.0              | 50.0         | 50.0   |
| Sum of total carotenoids | 33.8 | 68.4            | 82.7       | 86.3          | 88.1              | 92.9                      | 92.9        | 92.9              | 92.9         | 92.9   |

n=1, AST: astaxanthin, ADX: adonixanthin.

HPLC was performed using a Hitachi L-6000 intelligent pump, an L-4250 UV-VIS detector set to 450 nm, and a 5 μm Cosmosil 5SL-II (250x4.6 mm i.d.) column (Nacalai Tesque, Japan). Acetone : hexane (2 : 8, v/v) was used as the mobile phase at a flow rate of 1.0 mL/min. The authentic samples of carotenoids were used as our carotenoid samples.
higher concentrations than astaxanthin. Compared with the level of astaxanthin, adonixanthin was present at a level 19 times higher in the heart, 59 times higher in the spleen, 7 times higher in the kidneys, and 30 times higher in the liver. Although it has previously been reported that carotenoids, including astaxanthin, migrate to the peripheral tissues (25), we found that, in primates, astaxanthin and adonixanthin penetrated to the heart, spleen, kidneys, and liver following their oral administration, and that adonixanthin accumulated at higher concentrations than astaxanthin.

The effects of astaxanthin and adonixanthin on body weight

To investigate the systemic influence that resulted from the administration of astaxanthin and adonixanthin, changes in the monkeys’ body weight were measured. The weight of the astaxanthin- and adonixanthin-treated monkeys pretreatment was 3.53 and 3.50 kg, respectively. On the 8th day of administration, the weight of the astaxanthin- and adonixanthin-treated monkeys was 3.53 and 3.47 kg, respectively. Therefore, there were no significant changes in body weight in either the astaxanthin- or adonixanthin-treated monkeys following treatment.

Discussion

Our main finding in this study is that, following the oral administration of carotenoids such as astaxanthin and adonixanthin, these substances are absorbed into the brains of monkeys. There have been many reports about the effects astaxanthin can have on the central nervous system (26–30), and we recently reported an interesting finding that the intake of astaxanthin improved cognitive function in humans (12). We have also previously reported that dietary astaxanthin and adonixanthin have protective effects against brain hemorrhage in mice (17). The results of the present study provide additional evidence to support these previous reports. Another important point to note is that the present study is the first example of measuring the brain’s permeability to adonixanthin. Together, these findings provide strong support for the effectiveness of astaxanthin and adonixanthin on the central nervous system.

As shown in Table 1, neither astaxanthin nor adonixanthin are usually present in the serum of monkeys; however, both were detected in the serum following their oral administration. Interestingly, the concentration of adonixanthin in the serum was much higher than that of astaxanthin. The molecular structures of astaxanthin and adonixanthin are similar, except for the presence of a carbonyl (C=O) at the C-4′ of astaxanthin. Unlike astaxanthin, adonixanthin has a free hydroxy group and a hydrogen-bonding hydroxy group. At least, these differences may be related to the greater affinity of adonixanthin than that of astaxanthin for aqueous solvents. Adonixanthin may affect the metabolism of common carotenoids such as β-carotene, β-cryptoxanthin, and lutein, although the details remain unknown. It has been reported that a scavenger receptor class B type 1 (SR-B1) is involved in intestinal carotenoid transport for β-carotene, β-cryptoxanthin, lutein, and zeaxanthin (29). Adonixanthin may have a stronger involvement of these transporters than astaxanthin. Carotenoid distribution may be comprehensively regulated by some mechanisms including simple diffusion, facilitated diffusion, and excretion. At present, there have been no reports that both astaxanthin and adonixanthin affect the metabolism or accumulation of other carotenoids. Further experiments will be needed to clarify the detailed mechanisms. Furthermore, followed by a dose of 100 mg astaxanthin equivalents after 4 wk, the plasma level of astaxanthin were reached 0.28±0.1 mg/L (Cmax) in the middle-aged male (30). Therefore, astaxanthin may accumulate in blood more efficiently in humans than in monkeys.

The analysis of carotenoid concentrations in normal

| Tissue (ng/g) | Heart | Spleen | Kidney | Liver |
|--------------|-------|--------|--------|-------|
|              | AST   | ADX    | AST    | ADX   | AST    | ADX   | AST    | ADX   |
| β-Carotene   | 10.9  | 11.3   | 48.7   | 80.7  | 6.6    | 26.2  | 7.2    | 14.8  |
| β-Cryptoxanthin | 25.2   | 38.1   | 53.4   | 113.5 | 15.7   | 63.3  | 11.8   | 38.6  |
| e,e-Caroten-3,3′-dione | 0.2 | 7.4 | 5.7 | 68.7 | 0.7 | 3.9 | 3.1 | 19.0 |
| 3-OH-β,e-Caroten-3-one | 0.6 | 7.3 | 8.8 | 10.3 | 0.8 | 3.3 | 3.2 | 5.2 |
| Lutein       | 7.2   | 18.7   | 32.9   | 121.1 | 19.6   | 24.0  | 30.0   | 40.3  |
| Zeaxanthin   | 6.2   | 18.3   | 32.1   | 86.1  | 18.4   | 23.0  | 28.1   | 36.3  |
| Sum of common carotenoids | 50.3 | 101.1 | 181.6 | 480.4 | 61.8 | 143.7 | 83.4 | 154.2 |
| Astaxanthin  | 15.7  | 88.8   | 229.7  | 37.8  |       |       |       |       |
| Adonixanthin | 305.4 |       | 5,280.6 | 1,565.6 |       |       |       |       |
| Sum of total carotenoids | 66.0 | 406.5 | 270.4 | 5,761.0 | 291.5 | 1,709.3 | 121.2 | 1,278.3 |

n=1. AST: astaxanthin, ADX: adonixanthin.
cynomolgus monkey brains has been reported (24), The concentrations of some standard carotenoids, such as \( \beta \)-carotene, \( \beta \)-cryptoxanthin, \( \alpha,\beta \)-carotene-3,3\'dione, 3-OH-\( \beta,\beta \)-caroten-3\' -one, lutein, and zeaxanthin (Fig. 2) in the brains of monkeys treated with astaxanthin or adonixanthin were not notably different from those previously reported (24). Despite only astaxanthine or adonixanthine was administered during the experimental period, the other carotenoids were detected in the brain. The distribution of their carotenoids in this study was similar to that found in the brains of normal monkeys, as previously reported (24). The reason for the accumulation of carotenoids in the brains may be due to carotenoids in foods given before the experiment. As shown in Table 2, both astaxanthin and adonixanthin were taken up into the brain. The reason adonixanthin showed a significantly higher value than astaxanthin absorbed into the brain may be due to the difference in serum concentration between the two carotenoids. On the other hand, differences in localization within the brain were also observed. For example, astaxanthin and adonixanthin were more abundant in the cerebellum and the striatum and in the diencephalon and the hippocampus, respectively. Further studies are needed to clarify the differences between astaxanthin and adonixanthin in terms of both their distribution and metabolism. The present study revealed that astaxanthin and adonixanthin can reach all regions of the brain following their oral administration. Recent research has shown that dietary astaxanthin can cross the BBB in rats (21, 31). The present data show that astaxanthin can also transfer into the brain of primates, which is consistent with the rodent data (21).

The transfer of both astaxanthin and adonixanthin to the retina was also confirmed (Table 2). There have previously been some reports that the oral or intragastric administration of astaxanthin can have a protective effect on the retina (32–34). The present results indicate that astaxanthin can translocate to the retina in primates and that it may be useful as an effective oral supplement or drug in human retina, as is adonixanthin. These results provide further evidence for the effects of astaxanthin and adonixanthin on both the retina and the central nervous system in primates. The systemic accumulation of astaxanthin and adonixanthin was also demonstrated (Table 3). Although it will be necessary to conduct various efficacy evaluations in the future, the effects of astaxanthin and adonixanthin on the whole body, including the brain, may be expected. This study provides valuable data in relation to primates, having used cynomolgus monkeys; however, it should be noted that the sample size was small, representing one of the limitations of the study.

In conclusion, we showed that astaxanthin and adonixanthin could penetrate the central nervous system in an experiment using a primate model. Notably, this is the first paper to report tissue penetration, including to the brain, by adonixanthin following its oral administration. The results from the present study may be useful in predicting the effective clinical concentrations of astaxanthin and adonixanthin.

Authorship

Research conception and design: SN, TM, MH, and HH; experiments: SN, TM, and ST; quantitative analysis: TM; interpretation of the data: SN, TM, MH, MS, and HH; writing of the manuscript: SN, MH, and HH.

Disclosure of state of COI

This research was funded in part by ENEOS Corporation (Tokyo, Japan).

Acknowledgments

We thank Mr. Ken Endo (ENEOS Corporation) for providing valuable information about astaxanthin and adonixanthin.

REFERENCES

1) Maoka T. 2020. Carotenoids as natural functional pigments. J Nat Med 74: 1–16.
2) Zaituaga M, Gueguen V, Letourneur D, Pavar-Djadid G. 2018. Astaxanthin-antioxidant impact on excessive Reactive Oxygen Species generation induced by ischemia and reperfusion injury. Chemico-Biol Interactions 279: 145–158.
3) Lotan T, Hirschberg J. 1995. Cloning and expression in Escherichia coli of the gene encoding beta-C-4-oxygenase, that converts beta-carotene to the ketocarotenoid canthaxanthin in Haematococcus pluvialis. FEBS Lett 364: 125–128.
4) Turujman SA, Wamer WG, Wei RR, Albert RH. 1997. Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin. J AOAC Int 80: 622–632.
5) Armenta RE, Guerrero-Lagarreta I. 2009. Stability studies on astaxanthin extracted from fermented shrimp byproducts. J Agric Food Chem 57: 6095–6100.
6) Coral-Hinoestroza GN, Bjerkeng B. 2002. Astaxanthin from the red crab langostilla (Pleuroncodes planipes): optical R/S isomers and fatty acid moieties of astaxanthin esters. Comp Biochem Physiol B Biochem Molec Biol 133: 437–444.
7) Kotake-Nara E, Naguo A. 2011. Absorption and metabolism of xanthophylls. Marine Drugs 9: 1024–1037.
8) Ohgami K, Shiratori K, Kotake S, Nishida T, Mizuki N, Yazawa K, Ohno S. 2003. Effects of astaxanthin on lipo-poly saccharide-induced inflammation in vitro and in vivo. Invest Ophthalmol Visual Sci 44: 2694–2701.
9) Kim YJ, Kim YA, Yokozawa T. 2009. Protection against oxidative stress, inflammation, and apoptosis of high-glucose-exposed proximal tubular epithelial cells by astaxanthin. J Agric Food Chem 57: 8793–8797.
10) Chew BP, Park JS, Wong MW, Wong TS. 1999. A comparison of the anticancer activities of dietary beta-caro- tene, canthaxanthin and astaxanthin in mice in vivo. Anticancer Res 19: 1849–1853.
11) Shao Y, Ni Y, Yang J, Lin X, Li J, Zhang L. 2016. Asta- xanthin inhibits proliferation and induces apoptosis and cell cycle arrest of mice H22 hepatoma cells. Med Sci Monit Int Med J Exp Clin Res 22: 2152–2160.
12) Hayashi M, Ishibashi T, Maoka T. 2018. Effect of astaxanthin-rich extract derived from Paracoccus carotini- faciens on cognitive function in middle-aged and older individuals. J Clin Biochem Nutr 62: 195–205.
13) Chang C-H, Chen C-Y, Chiou J-Y, Peng RY, Peng C-H. 2010. Astaxanthine secured apoptotic death of PC12 cells induced by beta-amyloid peptide 25–35: its molecular action targets. *J Med Food* 13: 548–556.

14) Ikeda Y, Tsuji S, Satoh A, Ishikura M, Shirasawa T, Shimizu T. 2008. Protective effects of astaxanthin on 6-hydroxydopamine-induced apoptosis in human neuroblastoma SH-SY5Y cells. *J Neurochem* 107: 1730–1740.

15) Lee D-H, Lee YJ, Kwon KH. 2010. Neuroprotective effects of astaxanthin in oxygen-glucose deprivation in SH-SY5Y cells and global cerebral ischemia in rat. *J Clin Biochem Nutr* 47: 121–129.

16) Nishioka Y, Oyagi A, Tsuruma K, Shimazawa M, Ishibashi T, Haru H. 2011. The anxiolytic-like effect of astaxanthin extracted from Paracoccus carotinifaciens. *Biofactors* 37: 25–30.

17) Iwata S, Imai T, Shimazawa M, Ishibashi T, Hayashi M, Haru H, Nakamura S. 2018. Protective effects of the astaxanthin derivative, adonixanthin, on brain hemorrhagic injury. *Brain Res* 1698: 130–138.

18) Maoka T, Yasui H, Ohmori A, Tokuda H, Suzuki N, Osawa A, Shindo K, Ishibashi T. 2013. Anti-oxidative, anti-tumor-promoting, and anti-carcinogenic activities of adonirubin and adonixanthin. *J Oleo Sci* 62: 181–186.

19) Daneman R, Prat A. 2015. The blood-brain barrier. *Cold Spring Harb Perspect Biol* 7: a020412.

20) Banks WA. 2016. From blood-brain barrier to blood-brain interface: new opportunities for CNS drug delivery. *Nat Rev Drug Discov* 15: 275–292.

21) Manabe Y, Komatsu T, Seki S, Sugawara T. 2018. Dietary astaxanthin can accumulate in the brain of rats. *Biosci Biotechnol Biochem* 82: 1433–1436.

22) Breitenbach J, Misuwa N, Kajiwara S, Sandmann G. 1996. Expression in Escherichia coli and properties of the carotene ketolase from Haematococcus pluvialis. *FEBS Microbiol Lett* 140: 241–246.

23) Rhodes A. 2006. Dietary effects on carotenoid composition in the marine harpacticoid copepod Nitokra lacustris. *J Plankton Res* 29(Suppl 1): 173–183.

24) Nishino A, Ichilhara T, Sugimoto K, Kuriki T, Yasui H, Maoka T. 2019. Predicting organ carotenoids levels from analysis of plasma could lead to errors: A study in cynomolgus monkeys. *Natr Res (New York, NY)* 61: 95–101.

25) Jewell C, O’Brien NM. 1999. Effect of dietary supplementation with carotenoids on xenobiotic metabolizing enzymes in the liver, lung, kidney and small intestine of the rat. *Br J Nutr* 81: 235–242.

26) Abadie-Guedes R, Guedes RC, Bezerra RS. 2012. The impairing effect of acute ethanol on spreading depression is antagonized by astaxanthin in rats of 2 young-adult ages. *Alcoholism Clin Exp Res* 36: 1563–1567.

27) Wen X, Huang A, Hu J, Zhong Z, Liu Y, Li Z, Pan X, Liu Z. 2015. Neuroprotective effect of astaxanthin against glutamate-induced cytotoxicity in HT22 cells: Involvement of the Akt/GSK-3beta pathway. *Neuroscience* 303: 558–568.

28) Ji X, Peng D, Zhang Y, Zhang J, Wang Y, Gao Y, Lu N, Tang P. 2017. Astaxanthin improves cognitive performance in mice following mild traumatic brain injury. *Brain Res* 1659: 88–95.

29) During A, Dawson HD, Harrison EH. 2005. Carotenoid transport is decreased and expression of the lipid transporters SR-BI, NPC1L1, and ABCA1 is downregulated in Caco-2 cells treated with ezetimibe. *J Nutr* 135: 2305–2312.

30) Coral-Hinostroza GN, Ytrestøyl T, Ruyter B, Bjerkeng B. 2004. Plasma appearance of unesterified astaxanthin geometrical E/Z and optical R/S isomers in men given single doses of a mixture of optical 3 and 3′R/S isomers of astaxanthin fatty acyl diesters. *Comp Biochem Physiol Toxicol Pharmacol CBP* 139: 99–110.

31) Manabe Y, Komatsu T, Seki S, Sugawara T. 2018. Dietary astaxanthin can accumulate in the brain of rats. *Biosci Biotechnol Biochem* 82: 1433–1436.

32) Otsuka T, Shimazawa M, Inoue Y, Nakano Y, Ojino K, Izawa H, Tsuruma K, Ishibashi T, Haru H. 2016. Astaxanthin protects against retinal damage: Evidence from in vivo and in vitro retinal ischemia and reperfusion models. *Curr Eye Res* 41: 1465–1472.

33) Yeh PT, Huang HW, Yang CM, Yang WS, Yang CH. 2016. Astaxanthin inhibits expression of retinal oxidative stress and inflammatory mediators in Streptozotocin-induced diabetic rats. *PLoS One* 11: e0146438.

34) Otsuka T, Shimazawa M, Nakamichi T, Ohno Y, Inoue Y, Tsuruma K, Ishibashi T, Haru H. 2013. Protective effects of a dietary carotenoid, astaxanthin, against light-induced retinal damage. *J Pharmacol Sci* 123: 209–218.