Journal of Photoscience (2002), Vol. 9(2), pp. 418–420

Component analysis of the lipid hydroperoxide in the brain and peripheral organs of Senescence-Accelerated Mouse (SAM) model

Seiichi Matsugo[1]*, Fumihiko Yasui [1], Kazuo Sasaki [2]
[1]Department of Applied Chemistry and Biotechnology, Yamanashi University, Kofu 400-8511, Japan,
[2]Division of Bio-information Engineering, Toyama University, Toyama 930-8555, Japan

We measured previously the lipid hydroperoxides level in the brain and peripheral organs such as heart, liver, lung and kidney of senescence accelerated-prone (SAMP8) and –resistant (SAMR1) mice at 3,6 and 9 months of age. It was found that the lipid hydroperoxide levels in the brain did not show any age-dependent change, and that they were significantly higher in SAMP8 than in SAMR1 over the defined periods. In contrast, the lipid hydroperoxide levels in the peripheral organs, including liver, were increased with aging in both substrain, and they were significantly higher in SAMP8 than in SAMR1 at 3 and 6 months of age. In addition, the lipid hydroperoxide levels in the peripheral organs were higher than those in the brain in both substrains. To elucidate the difference of lipid hydroperoxide levels between the brain and the peripheral organs, we further carried out lipid component analysis in the brain and liver, one of the peripheral organs, of SAMP8 and SAMR1 at 6 months of age.

Keywords : Senescence-accelerated mouse, lipid hydroperoxide, lipid component

INTRODUCTION

Senescence-accelerated mice (SAM), established by Takeda *et al* as a murine model of accelerated aging, consists of senescence accelerated-prone and -resistant substrains (SAMP and SAMR, respectively) [1]. Of SAMP substrains, SAMP8 exhibits the age-related deficits in learning and memory observed at relatively early stage in their life span [2]. Thus, this substrain has been considered as a murine model to investigate the mechanism of age-related cognitive deficits.

The oxidative stress toward biological tissues by free radicals, especially those derived from molecular oxygen, has attracted much attention as a cause of aging and age-related disorders including Alzheimer's disease, atherosclerosis, cancer, ischemia-reperfusion. In accordance with this, it has been reported that the amount of various oxidative stress markers such as the carbonyl content in protein, 8-hydroxyguanidine in DNA and lipid hydroperoxide level in lipid are increased with aging and age-related disorders described above. In our previous report, we pointed out that the lipid peroxide level of the brain and peripheral organs of SAMP8 was significantly higher than those in SAMR1 (control mice for SAMP) at earlier ages (3-6 months) [3]. However, we could not explain the exact reason why the lipid hydroperoxide levels in the peripheral organs was much higher than those in the brain in both substrains over the defined time. In the present study, we carried out the quantification of the lipid components in the brain and liver of SAMP8 and SAMR1 at 6 months of age.

* To whom correspondence should be addressed.
E-mail : matsugoh@ab11.yamanashi.ac.jp

MATERIALS AND METHODS

Animals. Male SAMP8 and male SAMR1 mice at 6 months of age were used for the present study. Mice were anesthetized with diethyl ether and killed by decapitation. The brain and liver were rapidly removed and frozen under liquid nitrogen, and then stored at -196°C before use. All experiments were carried out according to the Guidelines for Animal Experimentation issued by Toyama

University.

Measurement of the lipid contents in tissue. The phospholipid contents in the brain and liver of SAMP8 and SAMR1 at 6 months of age were measured with the kit for clinical inspection according to the manufacture's instruction which developed molybdenum blue methods (Wako pure chemical Industries. Ltd., Japan). Briefly, the lipid contents in the tissue homogenate were extracted with $CHCl_3$ / CH_3OH (2:1) mixtures containing 0.01% (w/v) of butylated hydroxytoluene. The $CHCl_3$ layer was evaporated at room temperature and the residue was re-dissolved in $CHCl_3$ (3 ml). An aliquot of the lipid solution (0.5 ml) was removed and evacuated. One ml of distilled water and 1 ml of perchloric acid were added to the residue, respectively, and the reaction mixtures were heated at 110°C for 1 h. After addition of 30% hydrogen peroxide (0.2 ml) to this solution, the heat treatment was further continued for 6 h and then the solution was mixed with the reagent of kit for the determination of phospholipid, and measured absorbance at 750 nm.

On the other hand, the total lipid amount in the tissue was estimated gravimetrically as described previously [5].

RESULTS AND DISCUSSION

The amount of thiobarbituric acid-reactive substance (TBARS) was used as a common method for the determination of total lipid hydroperoxide levels in the biological samples. However, the several drawbacks are pointed out for the use of the TBARS method [4]. That is, TBA reacts with the other short chain aldehydes such as alkenals, alkanals, or alkadienals as well as malondialdehyde produced by the thermal decomposition of lipid peroxides, and also gives the visible absorption band around 532 nm, which is considered to represent the 1:2 adduct of TBA and malondialdehyde. It is known that the amount of TBARS, which has an absorption band at 532 nm, is increased with prolonged thermal treatment. In addition, the production of TBARS is enhanced by the cleavage of O-O bond, when metal ion such as ferrous ion is present in the reaction medium or in the tissue homogenate. These results show that TBARS values are strongly dependent to some extent on the experimental conditions and procedures employed, leading to the different results in the TBA values. In fact, the TBA values of the brain and liver of SAMP8 were inconsistent among the investigators with regard to the age

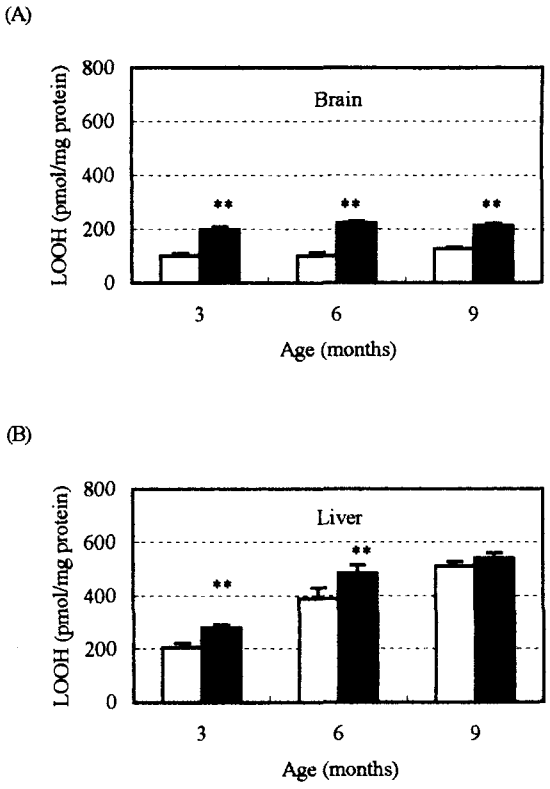
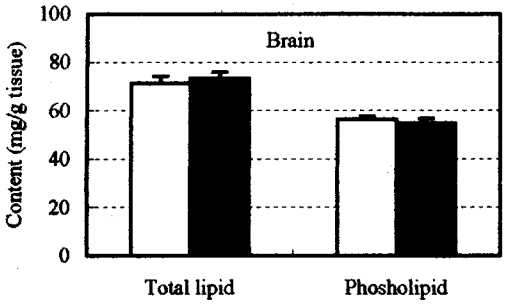


Fig. 1 Changes of Lipid hydroperoxide levels in the brain (A) and liver (B) of SAM mice with aging. (□) SAMR1, (■) SAMP8. Each value is the mean ± S.E. (n=5). ** P<0.01 and *p<0.05 indicate significant difference as compared to the same age of SAMR1. ††p<0.01 indicates significant difference as compared to 1 month-age SAMP8. Asterisks indicate significant difference between SAMP8 group and SAMR1 group of the same age (ANOVA followed by Fisher's PLSD test; p<0.05, **p<0.01).

[6-8]. In our preliminary study, the lipid hydroperoxide levels in the brain of SAMP8 and SAMR1 at 3, 6 and 9 months of age were measured using TBA method, and did not show any significant differences between SAMP8 and SAMR1 during experimental periods at 3, 6 and 9 months of age (data not shown). On the other hand, the method used in our previous study [3] reflects clearly the lipid hydroperoxide levels in the biological tissues, because this method is based on the direct chemical reaction between lipid peroxide and reactant (1-naphtyldiphenylphosphine; NDPP). As shown in Fig. 1 (A), the lipid hydroperoxide levels in the brain did not show any age-dependent change in either SAMP8 or SAMR1, it was significantly higher for SAMP8 than for SAMR1 at each age examined. In contrast, the lipid hydroperoxide levels in the liver

(A)



(B)

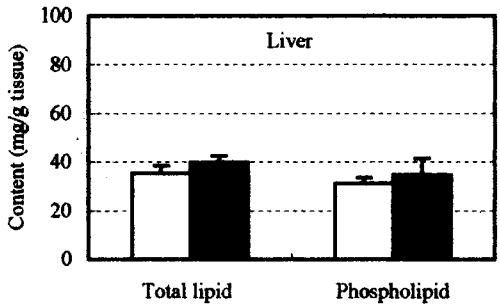


Fig. 2 Total lipid and phospholipid contents in the brain (A) and liver (B) of SAM mice at 6 months of age. (□) SAMR1, (■) SAMP8. Each value is the mean ± S.E. (n=3).

were increased with aging in both substrains, and the level were significantly higher for SAMP8 than for SAMR1at both 3 and 6 months of age (Fig.1 (B)). These results suggest that the brain and peripheral organs of SAMP8 may be exposed to an abnormal oxidative stress at an early stage prior to 3 months of age, and that such abnormal oxidative stress in the brain of SAMP8 is somewhat related to learning and memory deficits observed in SAMP8. However, it was still remained unclear the reason why the lipid peroxide level in the brain did not change with aging in either SAMP8 or SAMR1 and the lipid hydroperoxide level in the liver was significantly higher than those in the brain in both substrains after 3 months of age. To investigate the differences of lipid components rate in the brain and liver of SAMP8 and SAMR1, we measured the amounts of total lipid and phospholipid contents in the brain and liver of both substrains at 6 months of age by gravimetrical method [5] and commercially available kit for clinical examination, respectively. As shown in Fig. 2 (A and B), total lipid and phospholipid contents in the brain and liver did not show any significant difference between SAMP8 and SAMR1. The percentages of phospholipid contents / total lipid contents in the brain and liver in both substrains were more than 70% and 80%, respectively. So, it is quite reasonable to consider that lipid hydroperoxide level is strongly correlated with the phospholipid hydroperoxides. Further studies are now in progress in this laboratory.

REFERENCES

1. Takeda, T., et al. (1981) A new murine model of accelerated senescence. *Mech. Ageing Dev.* 17, 183-194.
2. Nomura, Y. and Okuma, Y. (1999) Age-related defects in lifespan and learning ability in SAMP8 mice. *Neurobiol. Aging*, 20, 111-115.
3. Matsugo, S., et al. (2000) Age-dependent changes in lipid peroxide levels in peripheral organs, but not in brain, in senescence-accelerated mice. *Neurosci. Lett.* 278, 105-108.
4. Kosugi, H., Kato, T. and Kikugawa, K. (1987) Formation of yellow, orange, and red pigment in the reactions of alk-2enals with 2-thiobarbituric acid. *Anal. Biochem.* 165, 456-464.
5. Naganuma, T., Uzuka, Y. and Tanaka, K. (1982) Quantitative estimation of intracellular neutral lipids of the yeast, *Lipomyces starkeyi*. *Agric.Biol.Chem.* 46, 1213-1217.
6. Liu, J. and Mori, A. (1993) Age-associated changes in superoxide dismutase activity, thiobarbituric acid reactivity and reduced glutathione level in the brain and liver in senescence accelerated mice (SAM): a comparison with ddY mice. *Mech. Aging Dev.* 71, 23-30.
7. Sato, E., et al. (1996) Early and transient increase in oxidative stress in the cerebral cortex of senescence-accelerated mouse. *Mech. Aging Dev.* 86, 105-114.
8. Nomura, Y., et al. (1989) Biochemical changes related to aging in the senescence-accelerated mouse. *Exp. Gerontol.* 24, 49-55.