AGE RELATED CHANGES IN LIPID PEROXIDATION IN RAT BRAIN AND LIVER*

O. P. SHARMA

Division of Biochemistry
Central Drug Research Institute,
Lucknow - 226001, India.

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SUMMARY

Lipid peroxidation in rat liver, unlike in brain shows wide variations with age. In liver, ascorbic acid content also undergoes wide variations and there is negative correlation between ascorbic acid content and lipid peroxidation. Heat-labile antioxidant factors are present in the cytosol fraction. There is inverse relationship between antioxidant activity and lipid peroxidation in liver.

INTRODUCTION

The accumulation of lipopigments is considered to be the evidence for *in vivo* lipid peroxide formation (1). The relevance of lipid peroxidation in the ageing process has been stressed by others (2). The present studies were carried out to investigate the lipid peroxidation system in rat brain and liver as a function of age.

EXPERIMENTAL

Male albino rats of different age groups drawn from the C.D.R.I. stock colony were fasted overnight (with water *ad libitum*) and decapitated. Brain and liver were taken out, washed with chilled 150 mM KCl and homogenates (10% W/V) prepared in 150 mM KCl. Subcellular fractionation was done as described previously (3). 106,000 g supernatant has been designated as the cytosol fraction.

For assay of lipid peroxides 1.0 ml aliquots of tissue homogenates (or other reaction mixtures) were incubated at 37 ± 1°C in a metabolic shaker (120 strokes/min, amplitude 1 Cm) for 3 hours, the amount of lipid peroxides formed was estimated by the thiobarbituric acid reaction and results have been expressed as malonyldialdehyde (MDA) using an extinction coefficient of 1.56×10^5 at 535 nm (4). Protein estimation was done

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Table 1

Phospholipid and iron contents of rat brain and liver with increase in age (Mean ± S.D.)

Age (days)	Lipid Pi (mg/100 gm)		Iron (mg/100 gm)	
	Brain	Liver	Brain	Liver
0	96.8±1.9	131.0±14.0	1.9±0.2	25.2±6.6
15	141.7±13.1	126.2±12.7	1.3±0.2	5.6±1.4
25	136.9±17.6	102.8± 9.3	1.6±0.2	8.3±1.2
30	146.6±21.4	104.9±9.6	1.9±0.1	6.9±1.3
40	162.9±9.4	130.7±13.6	2.1±0.4	8.4±0.8
60	168.1±7.0	149.0±12.6	3.5±1.6	9.4±1.4
240	179.2±15.3	165.6±14.9	2.1±0.4	9.9±2.9

The data are mean of six experiments.

according to Lowry et al. (5), ascorbic acid according to Roe and Kuether (6) and iron according to Fortune and Mellon (7). Lipids were extracted by the method of Folch and Coworkers (8) and lipid phosphorus estimated as described earlier (9).

Ascorbic acid oxidase (EC 1.10.3.3) was prepared from bottle gourd (Lagenaria siceraria) and assayed by the method of Lovett-Janison and Nelson (10). For assaying total antioxidant activity due to antioxidant enzymes like superoxide dismutase (11), glutathione peroxidase (12) and semidehydroascorbate dehydrogenase (13) amount of lipid peroxides formed by unheated and heated (for 5 minutes in boiling water bath) tissue homogenates was estimated and the results have been expressed as percent increase in lipid peroxide formation on inactivating the labile antioxidant enzymes.

RESULTS AND DISCUSSION

Rates of lipid peroxidation and ascorbic acid content are shown in Fig. 1. Phospholipid and iron content in rat brain and liver are shown in Table 1. Amount of lipid peroxides in the brain of 0 day old animals is rather low, it increases with the age of the animal and reaches a maximum in the 20 days old rats,

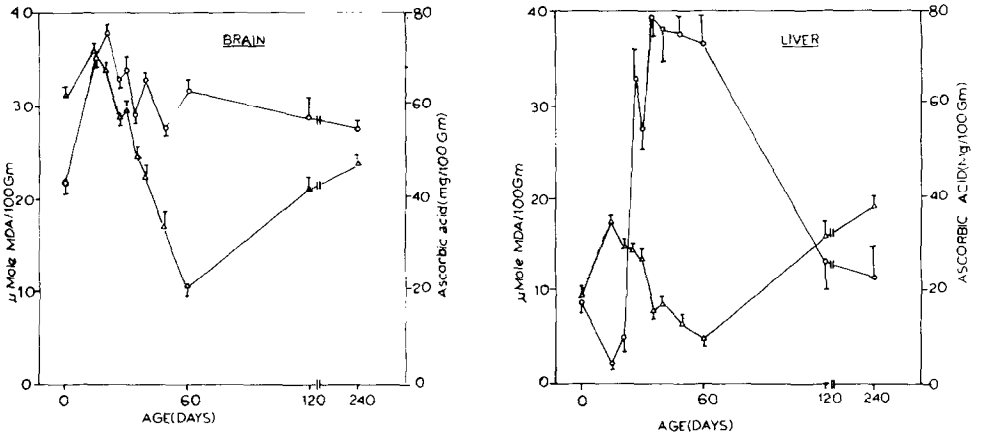

Fig. 1 - Lipid peroxidation and ascorbic acid content of rat brain and liver (Mean ± S.D.) o——o Lipid peroxidation; △——△ Ascorbic acid

thereafter it does not show any marked variation. Phospholipid content in brain is quite low at birth and increases with the age of the animal. Iron content in brain does not undergo marked alterations. On the other hand, lipid peroxide formation in the liver shows wide variations. It is quite low in the suckling rats (0-20 days), after weaning (21 days) it increases and maintains a maximum range during the age of one to two months, thereafter it declines and reaches the level of newborn animals.

On treating tissue homogenates with ascorbic acid oxidase 83.6% and 68.1% inhibition of peroxide formation was noticed in liver and brain respectively. The antioxidant activity of rat brain and liver is apparently due to dismutase reaction which is heat-labile and is shown in Fig. 2 and an inverse relationship between lipid peroxide formation (Fig. 1) and antioxidant activity is evident for liver. Antioxidant activity in brain increases two to three fold within 30 days and remains at this level.

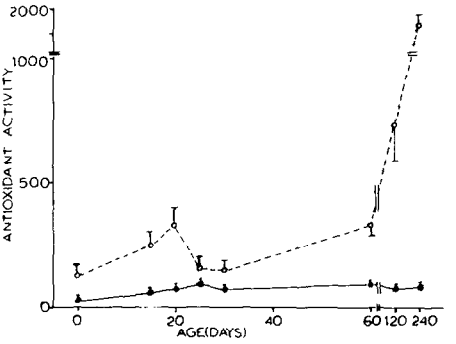

Fig. 2 - Antioxidant activity in rat brain and liver (Mean ± S.D.) ●——● Brain; o----o Liver.

Studies on subcellular localization of the antioxidant activity indicate that it is associated with the particulate free fraction (Table 2). The cytosol fraction from 6 month old rats is less efficient in promoting lipid peroxide formation when reconstituted with microsomes from 6 months or 1 month old rats. On the other hand, microsomes from young as well as old animals do not differ in undergoing lipid peroxidation on addition of cytosol fraction from 1 month old rats. The same observations have been made with mitochondrial suspensions from young and old animals when added to the cytosol fractions.

Statistical analysis of the data presented in Fig. 1 for correlation coefficient show that there is negative correlation between lipid peroxidation and ascorbic acid content in liver. It implies a non-enzymic antioxidant action of ascorbic acid in liver. A similar role has been attributed to ascorbic acid in developing brain (14). However, present studies do not suggest such a role for ascorbic acid in rat brain in the age groups that have been taken for experimentation. There is no correlation between rate of lipid peroxidation, ascorbic acid, phospho-

Table 2

Lipid peroxidation in young and old rats.

Reaction mixture	n mole Malonyl dialdehyde/ml reaction mixture/3hr
Cytosol (1) + Microsomes (1)	14.3
Cytosol (6) + Microsomes (1)	8.6
Cytosol (1) + Microsomes (6)	14.6
Cytosol (6) + Microsomes (6)	7.5

The reaction mixture (0.75 ml) contained cytosol (106,000 g supernatant, 3.22 mg protein) and microsomal suspension in 150 mM KCl (890 ug protein). In parentheses the age of the animal in months is given.

lipid and iron content and enzymatic antioxidant activity in brain. In consonance with the observations of Allison and Stewart (15) brain of young rats had higher amounts of ascorbic acid. Vitamin C has been shown to be the natural mediator of lipid peroxide formation in rat brain and liver (16, 17). Ascorbic acid oxidase brings about the inhibition of lipid peroxidation. The results presented above (Fig. 1) show an antioxidant action of ascorbic acid in liver. These observations, although paradoxical, imply a dual role peroxidant as well as antioxidant that has been ascribed to ascorbic acid by the earlier workers depending upon its concentration in the tissues (14, 18, 19).

Low level of antioxidants in brain may contribute to its special propensity to accumulate lipopigments with ageing (20). Grinna and Barber (21) have also observed lower values of lipid peroxides in older animals. In liver, output of lipid peroxi-

des appears to be subtly regulated by ascorbic acid and enzymatic antioxidants (Fig. 1, 2). The wide variations in lipid peroxidation and antioxidant levels in liver may have relevance to its special role in drug metabolism and disposition (22) in young and old animals. The reason for the reported accumulation of lipofuscin pigments (1) in the old animals inspite of the low levels of lipid peroxides and high antioxidant activity is still obscure. It may also be surmized that lower susceptibility of the liver of old animals to undergo lipid peroxidation is an inbuilt mechanism against the adverse effects of ageing process.

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