LIPID PEROXIDATION AND IT'S PROTECTIVE MECHANISM DURING DEVELOPMENTAL STAGE IN RAT

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Synopsis The concentration of lipoperoxides in liver tissue was extensively high in the fetus and early newborn, but it decreased sharply thereafter. However, the concentrations of lipoperoxides in the blood and lung tissue were low in the fetal period, but increased after birth reaching a peak after about 10 days of life. Then they gradually decreased with development.
The activities of enzymes such as superoxide dismutase, catalase and glutathione peroxidase together with the concentration of α-tocopherol (vitamin E) were extremely low in the fetal and early newborn periods, but increased gradually with development.

Key words: Lipid peroxidation • Superoxide dismutase • Glutathione peroxidase • Catalase • Vitamin E

Introduction
The relation of the peroxidation of membrane structural lipids to the aging has called for more attention recently. The oxygen radicals are also dangerous, and they may lead to the peroxidation of unsaturated fatty acids.
Among the internal organs, the liver responds to most metabolic processes in the body and is intimately involved in the detoxification of drugs and poisonous substances. The lung is exposed to the highest concentration of oxygen throughout life.
We have studied the development-related degree of oxygen toxicity and the defence mechanism for it in rat blood and tissues from birth to adulthood on the basis of determination of lipid peroxidation and antioxidant enzymes.

Materials and Methods
Fetal, newborn and adult Wistar strain rats were used as experimental animals. After decapitation of the rats, their blood was collected, and their tissues were immediately excised and washed well in cold isotonic saline to remove intravascular blood as much as possible.
The concentration of lipoperoxides in the blood was measured by Yagi’s method, and that in the tissues was determined by the methods of Ohkawa et al. and Hunter et al. A Shimadzu Model RF-502 fluorophotometer was used for lipoperoxide analysis.
Protein concentration was determined by the method of Lowry et al.
Superoxide dismutase activity was determined by the method of Beauchamp and Fridovich. Catalase activity was measured as proposed by Cohen et al. Glutathione peroxidase activity was measured using the procedure of Demus-Oole and Swierzewski which is a modification of the method of Paglia and Valentine. A Hitachi Model EPS-3T spectrophotometer was used for enzyme analysis.
The vitamin E (α-tocopherol) concentration in the blood and liver tissue was determined by the method of Abe et al. with a high performance liquid chromatography apparatus (JASCO, FLC-350). The structural fatty acids of total lipids and phospholipids were analyzed by extracting serum lipids by the method of Folch et al., separating each lipid fraction by thin layer chromatography and measuring by gas chromatography (Yanaco Model G-80, Yanako, Japan).

Results
1. Changes in lipoperoxide concentration
As shown in Fig. 1 the change of lipoperoxide concentration in the blood during development was low in the fetal and early newborn periods. It increased with age reaching a peak on about the 15th day of life and gradually diminished thereafter. The concentrations of lipoperoxides in liver homogenates from fetal, newborn and adult rats are shown in Fig. 2. They were measured by Ohkawa's
LIPID PEROXIDATION IN RAT BLOOD AND TISSUES

Fig. 1. Lipoperoxides in the rat blood

Fig. 2. Lipoperoxides in the rat liver tissues

Fig. 3. Lipoperoxides in the rat lung tissues

Especially in the fetuses and up to the 5th day of life, and then a gradual, age-related, decrease approaching the adult level after about 10 days of life. On the other hand, the concentrations, formation and accumulation of lipoperoxides were low in the lungs of fetal and early newborn rats (Fig. 3). They increased with age and reached a peak on about the 10th day of life, gradually diminishing thereafter until the adult level fell below the fetal level.

2. Changes in antioxidant enzyme levels

i) SOD activity: The amounts of superoxide dismutase (SOD) contained in rat liver and lung homogenates are shown in Fig. 4. The SOD activity in fetal liver was measured and found to be 740 units/g wet weight, which is approximately 9% of the adult level of 8,238 units/g wet weight. The newborn level remained low for 7 days after birth, and then it increased to 3,529 units/g wet weight (43% of the adult level) after 10 days and 6,729 units/g wet weight (82% of the adult level) after 30 days. The SOD activity in the liver increased...
sharply with age after birth. The SOD level in the lungs was low as compared with that of liver tissues. The SOD level in the fetal lung was 1,018 units/g wet weight, which is approximately 53% of the adult level of 1,913 units/g wet weight. The SOD enzyme production in the lung tissue persisted throughout the newborn period.

**ii) Catalase activity:** The catalase activity was also low (23 units/mg protein) in the fetal liver, corresponding to approximately 20% of the adult level (120 units/mg protein), but increased rapidly after birth, reaching 50% of the adult level after 5-7 days of life (Fig. 5). In the lung tissues, catalase was extremely low in concentration at all times, and the age-related variations could not be definitely obtained.

**iii) Glutathione peroxidase activity:** Glutathione peroxidase (GSH-Px) activity in the liver was measured and found to be only 7% (0.3μmoles/min/mg protein) of the adult female level (3.3μmoles) in the fetal and early newborn periods, but the level was approximately 20% (0.8μmoles) of the adult level on the 20th day. The difference in GSH-Px activity between sexes after the first 30 days of life became significant, with the male adult level (2.4μmoles) being 61% of the female adult level (Fig. 6). This activity in the lungs was also measured and found to be low (0.1μmoles) in the fetal period and during the first 15 days after birth, but a subsequent gradual rise resulted in the activity in the adult (0.4μmoles) 3 times greater than that in the fetus.

3. Vitamin E concentration

As shown in Fig. 7, the concentration of vitamin E was low in the fetal liver, but increased rapidly after birth, reaching a level 10 times higher within a few days. Thereafter, it decreased gradually. Though we did not measure the vitamin E concentration in lung tissues, Kehrer and Autor7 reported that this concentration in the lungs increased with age. The concentration of vitamin E was 0.8mg/dl in the fetal blood. The blood vitamin E level increased until the 10th day of life and reached a peak (1.9mg/dl) at that time. Thereafter, it decreased...
Fig 7. Vitamin E concentrations in the rat blood and tissues

4. Unsaturated free fatty acid concentration

Since lipoperoxides are produced from unsaturated fatty acids, the levels of fatty acid in the fetus, newborn and adult were measured. The liver level was determined in terms of fatty acid composition in total lipids. As a result, we found that the percentage of some unsaturated fatty acids like linoleic acid (C₁₈:₂) and arachidonic acid (C₂₀:₄) increased with the growth of the rat. But there were no significant changes in the percentage of unsaturated fatty acids during development.

The level in the lungs was determined in terms of fatty acid composition in total phospholipids. We found that the percentage of unsaturated fatty acids of the phospholipid fraction changed little with age as was true with liver tissue (Fig. 8).

Discussion

Radical oxygen species have been accepted to have direct action against unsaturated lipid to form lipoperoxides, causing damage to mammalian cell components. The excess accumulation of lipoperoxides has been accepted to be highly responsible for the development of retinopathy of prematurity and bronchopulmonary dysplasia in newborn infants.

However, enzymes such as SOD, catalase and GSH-Px and also vitamin E act as endogenous protectants against the toxic effects of lipoperoxides.

In the present rat experiments, the lipoperoxide concentrations in the blood and tissues were low in the fetal and early neonatal periods. The concentrations increased with age and reached a peak in the early neonatal period (1-15 days of life) and then gradually decreased.

Based on a review of these results, lipoperoxide concentration may be considered to be relatively low during the fetal period since the intrauterine environment provides lower concentrations of free radicals to the fetus.

The adaptive protective response by enzymes such as SOD, catalase and GSH-Px may not be induced in such an environment, and therefore liver and lung tissue homogenates might be very sensitive to active oxygen and undergo spontaneous lipid peroxidation. The rat fetal condition seems to be similar to the human fetus. In support of this hypothesis, human cord blood is known to contain lower concentrations of lipoperoxides, comparable amounts of SOD and smaller amounts of catalase and GSH-Px than maternal blood collected at delivery. Mino compared the vitamin E concentrations of human fetal and adult liver specimens, and found that the fetal level was only 1/10 the adult level. Our measurements of vitamin E in fetal liver tissue showed much lower levels than that measured in adult liver tissue. In humans, the
concentration in the cord blood is as low as 12–16% of the maternal blood.\textsuperscript{13}

A defense against potential oxygen toxicity develops by adaptive synthesis of SOD, catalase and GSH-Px. However, there is a time lag in such enzyme induction, and this lag may be considered to be the most important contributory factor in the etiology of abnormality in the early stages of newborns. We found these enzymes rapidly increased during 10–15 days after birth to protective levels and provide defense against oxygen toxicity due to lipoperoxide synthesis and subsequent tissue accumulation.

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\section*{References}


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