Effect of Atherogenesis on Membrane Lipid Constituents of Rabbit Erythrocytes

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SUMMARY

Lipids from erythrocyte membranes of normal and cholesterol fed animals were isolated and the various fractions were analyzed quantitatively. Erythrocyte membrane lipid composition of animals fed with an atherogenic diet for 2 months revealed marked elevations in cholesterol followed by significant alterations in phospholipids, free fatty acids and triglycerides. These findings were discussed from the point of view of the role of membrane lipids in the maintenance of cell integrity, shape, and homeostasis.

Additional Indexing Words:

Cell integrity Cell shape Permeability

CHANGES in lipid composition and metabolic activity of erythrocyte membranes have been reported in a variety of pathological conditions like spur cell anemia,¹⁾⁻³⁾ hypothyroidism,^{4),5)} diabetes mellitus,⁶⁾ liver diseases,⁷⁾⁻¹⁰⁾ lecithin-cholesterol acyl transferase deficiency,¹¹⁾ and Zieve's syndrome.¹²⁾ In most of the above conditions an altered plasma lipid profile has been attributed to red cell lipid changes.

In atherosclerosis, the biochemical composition of blood is greatly altered.¹³⁾⁻¹⁸⁾ This completely changes the environment for the circulating erythrocytes. Very few attempts have been made to study the effect of hypercholesterolemia on the composition of erythrocyte membranes.¹⁵⁾ In the present study, we aim to analyze the changes in lipid composition of erythrocyte membranes isolated from normal and experimental atherogenic animals with a view to evaluate their role in membrane structure, function and integrity.

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MATERIALS AND METHODS

White albino rabbits of the same age and sex weighing about 800–1,000 Gm were purchased from King Institute, Guindy and were fed with a normal laboratory diet (Hind Lever Rabbit Feed). A batch of 20 animals were divided into 2 groups of 10 each. Group I served as normal controls. Group II animals were fed with an atherogenic diet (normal diet +1% cholesterol). Serum cholesterol levels were monitored every week. Animals with serum cholesterol levels between 1,400–1,600 mg% were taken up and their serum cholesterol levels were maintained within the above range by dietary adjustment of cholesterol. At the end of 2 months, blood was drawn from control and experimental animals into heparinized tubes by ear vein puncture. Plasma was separated by centrifugation at 3,000 g for 10 min. Packed red cells were washed thrice with physiological saline. A known volume of cells with a known hematocrit were used for the isolation of red cell membrane. The method employed was essentially the same as described by Dodge et al.¹⁹⁾ The Hb-free red cell ghosts were suspended in minimum quantity of physiological saline. The lipids from the membranes were extracted using chloroform-methanol mixture (2:1) by the method described by Folch et al.²⁰⁾ The lipids were dried at 37°C to constant weight and were redissolved in the above chloroform-methanol mixture. Aliquots of this extract were used for the estimation of cholesterol,²¹⁾ phospholipids,²²⁾ free fatty acids,²³⁾ and triglycerides.²⁴⁾ Membrane proteins were estimated by the method of Lowry et al.²⁵⁾

RESULTS

Table I gives the levels of membrane lipids from erythrocytes of normal and cholesterol fed animals. The total lipid content of erythrocyte membranes from experimental animals was increased by 150% when compared to normal controls ($p < 0.005$).

Analysis of individual lipid components, namely cholesterol, phospholipids, free fatty acids and triglycerides, also revealed marked variations in experimental animal membranes. The levels of cholesterol were found to be

Table I. Effect of Atherogenesis on Lipid Composition of Erythrocyte Membranes Compared to Normal Controls

Parameter	Control	Experimental	p value
Total lipids	243.2 ± 16.20	600.00 ± 55.50	<0.005
Cholesterol (Total)	143.1 ± 10.30	425.00 ± 40.10	<0.005
Phospholipids	9.90 ± 1.25	15.05 ± 1.50	<0.05
Free fatty acids	37.80 ± 10.50	75.90 ± 21.30	<0.005
Triglycerides	44.60 ± 11.50	78.40 ± 16.40	0.05

p is the statistical significance at 5% level.

Values were the average of 6 individual experiments in duplicate and expressed as mg/Gm membrane proteins ± S.D. Details were given under Materials and Methods.

elevated by 200%, while other lipid components, namely phospholipids, free fatty acids and triglycerides, showed 50%, 100%, and 60% elevations, respectively.

DISCUSSION

The architecture of red cell membranes is mainly regulated by the composition of lipids and proteins as suggested by Singer and Nicolson.²⁶⁾ The results presented above show that dietary induced hyperlipidemia is accompanied by concomitant changes in erythrocyte membrane lipids.

The elevations observed in total lipid fractions of erythrocyte membrane may be a mere reflection of dietary induced hyperlipidemia. Thus, Neerhout²⁷⁾ has shown that changes in red cell membrane lipids are acquired in the circulation through interaction with altered plasma lipids. Hence, it is conceivable that the elevations seen in the membrane lipids are due to increased molecular interactions which in turn is due to increase in plasma lipid level in cholesterol-fed animals.

The elevations observed in membrane cholesterol can be explained by the contention that red cell cholesterol is in free equilibrium with unesterified plasma cholesterol, which in turn is regulated by the enzyme lecithin-cholesterol acyl transferase (LCAT).²⁸⁾ Hence, in dietary induced hypercholesterolemia, the acylation capacity of the above enzyme seems to be insufficient when compared to the excess unesterified circulating cholesterol, resulting in elevation of membrane cholesterol and hence an altered membrane surface area, which results in altered red cell shape.²⁹⁾ An alternative explanation is, an abnormality in the usual equilibrium of plasma lipoprotein cholesterol with that of membrane cholesterol. Increased cholesterol content of the membrane along with decreased unsaturation has been observed in aging.³⁰⁾

These changes in membrane lipids are also accompanied by permeability changes and abnormal hematology.³¹⁾ Here, it is interesting to observe that cholesterol regulates the permeability of biological membranes by affecting the internal viscosity.³⁰⁾ From these observations, it is tempting to speculate that the change in internal viscosity subsequent to altered membrane lipid composition may be responsible for enhancing the atherogenic process in the aorta along with other factors such as shear rate and turbulent flow.³²⁾⁻³⁴⁾

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