

Altered Fatty Acid, Cholesterol and Na⁺/K⁺ ATPase Activity in Erythrocyte Membrane of Rheumatoid Arthritis Patients

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Rheumatoid arthritis (RA) is a chronic inflammatory disease whose cause remains obscure. Blood from 15 RA patients and controls was taken and their ghosts separated. The ghosts were analysed for cholesterol content, Na⁺/K⁺ ATPase activity and eicosapentaenoic acid. The cholesterol content in the ghosts of RA patients was significantly lower as compared with the set of controls. There was a major difference in the activity of Na⁺/K⁺ ATPase between the two groups with RA patients showing significantly elevated activity. The ghosts of the RA patients exhibited major abnormality in the polyunsaturated fatty acids of phospholipids with the level of eicosapentaenoic acid (ω -3, 20:5) being significantly reduced.

Introduction

Biological membranes have a high content of cholesterol which plays an important structural role in the lipid core of biological membranes and exerts an influence on membrane structure and function (Spector and Yorek, 1985). Normally the molar ratio of cholesterol to phospholipids in the erythrocyte membrane is about 0.8–0.9. In an *in-vitro* study this ratio has been reduced to as low as 0.4, by incubating erythrocytes with cholesterol free phosphatidylcholine vesicles (Cooper *et al.*, 1978). Experimental evidence is also gathered suggesting that there is not always a linkage between membrane fatty acid composition and cholesterol content (Spector *et al.*, 1979).

An increase in membrane cholesterol level leads to an extensive folding of the plasma membrane (Lindblom *et al.*, 1981), thereby modulating the activity of certain membrane bound enzymes. Modulation of the cholesterol level on the activity of membrane bound Na⁺/K⁺ ATPase is established, with high levels of cholesterol inhibiting the enzyme while low levels activating the enzyme relative to normal, unmodified membranes (Yeagle, 1983).

Although many cellular functions and responses are affected when the membrane lipid composition is altered, the reports are somehow conflicting and it is very difficult to draw any general conclusion (Cullis *et al.*, 1983). The purpose of this study was to find the status of eicosapentaenoic acid (ω -3, 20:5) and cholesterol in the erythrocyte membrane of rheumatoid arthritis patients and look at the effect on the activity of Na⁺/K⁺ ATPase. In this study significantly low levels of cholesterol and eicosapentaenoic acid in the erythrocyte membrane of rheumatoid arthritis patients as compared to normals were found. On the other hand high activity of Na⁺/K⁺ ATPase was observed in the erythrocyte membranes of patients.

Materials and Methods

Eicosapentaenoic acid, Cholesterol, Cholesterol oxidase and esterase were obtained from Sigma USA. All the Organic solvents of high grade (purity 99.8%) were used (E.Merck, Darmstadt, Germany).

Sample collection and erythrocytes isolation

Patients affected by rheumatoid arthritis, classical or defined according to the American Rheumatism Association (Ropes *et al.*, 1959) were admitted to the present study. Patients with a history of myocardial infarction, neoplastic diseases, simultaneous use of steroidal/hypolipidemic drugs

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were not included in the study. Controls included were of same sex, same age ± 5 yrs. Exclusion criteria was the same as that for patients.

Preparation of erythrocyte ghosts

Best preparation of human erythrocyte ghosts were obtained as follows; Erythrocytes were given osmotic shock in cold using 20 mOsm phosphate buffer (NaH_2PO_4 , 10 mM, Na_2HPO_4 , 6.65 mM, pH 7.4). After freeze/thaw, the suspension was centrifuged at 500 x g and the supernatant discarded. The pellet was suspended in 20 mOsm phosphate buffer (pH 7.4) and agitated gently for 5 min with a rod. After centrifugation at 20,000 x g for 40 min, the supernatant was discarded. The process was repeated twice. At this stage the dry weight of the pellet was determined. For obtaining the ghosts suspension, 5 ml of the phosphate buffer (0.01 M, pH 7.0) was added to the pellet of the last centrifugation and the suspension stored at -20°C until used for analysis.

Analysis of the fatty acids

Total lipids were extracted with chloroform:methanol (2:1, v/v) and the phospholipid class separated by TLC. The phospholipid band was scraped off the plates (1.5 mg approx.) and then subjected to analysis of fatty acids by gas chromatography as follows: Lipids were treated overnight with 2.0 ml of 0.3N NaOH in methanol / water (9:1, v/v). The methanolic solution extracted twice with 1.0 ml petroleum ether. The residual methanolic solution acidified with aqueous HCl and extracted again with 1.0 ml petroleum ether. The ether extract containing the free fatty acids was evaporated and the residue dissolved in 2 ml of 3% H_2SO_4 in anhydrous methanol. The solution was heated at 70°C for 2 hrs in a stoppered tube under nitrogen. After methylation 2 ml of water was added to the methanolic solution. The aqueous methanolic solution was extracted 3 times with 1.0 ml of petroleum ether and the volume reduced. Aliquots of this solution were injected into the column of Shimadzu Gas Chromatograph, using FID detector, GP 10% SP-2330 on 100/120 Chromosorb WAW column; Injector/detector and column temperatures of 250°C and 180°C respectively. The elution pattern was stored on a semi-

logarithmic diagram using 18:0 as reference fatty acid.

Aliquots of the extract from the membrane suspension were used to quantitate the cholesterol enzymatically (Masoom and Townshend, 1985).

Assay procedure of Na^+/K^+ ATPase

The enzyme assay was based on the release of inorganic phosphate by ATP which was measured colorimetrically. One unit of Na^+/K^+ ATPase is defined as the amount of the enzyme which produced 1.0 nmol of Pi / min per mg of erythrocyte ghosts protein under specified conditions. To 1.0 ml of the reaction medium consisting of 3 mM MgCl_2 , 3 mM ATP, 130 mM NaCl, 20 mM KCl, pH 7.5 (adjusted with 30 mM Histidine), was added 25 μl of the membrane suspension (650 μg protein / ml suspension). After an incubation for 10 min at 37°C , the reaction was terminated by the addition of 0.1 ml of 50% trichloroacetic acid. The inorganic phosphate released was estimated by diluting the incubated mixture with 1.0 ml of ice cold water and adding to it 1.0 ml each of the molybdate- H_2SO_4 solution and the reducing agent (ascorbic acid) solution. The absorbance at 660 nm was read after 20 minutes. The molar absorptivity of the molybdophosphoric acid blue solution in aqueous medium after ascorbic acid reduction observed is 1.04×10^4 at $\lambda_{\text{max}} = 660\text{nm}$.

Results and Discussion

Fig. 1 shows the cumulative results obtained for the cholesterol content and ATPase activity in

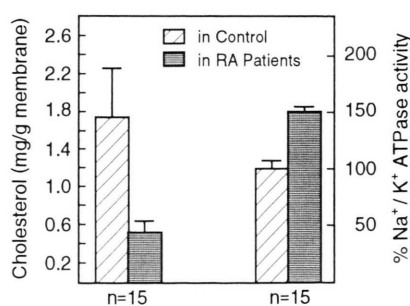


Fig. 1. Cholesterol content and Na^+/K^+ ATPase activity in human erythrocyte membrane of normal individuals and rheumatoid arthritis patients. The activity of the enzyme is presented as a percentage of the normal. The Na^+/K^+ ATPase activity value of 550 nmole Pi / min per mg erythrocyte ghosts protein was taken as 100%.

erythrocyte membranes of fifteen each of normal individuals and the rheumatoid arthritis patients. There is a significant decrease in the cholesterol content coupled with over 50% increase in the activity of ATPase. The level of eicosapentaenoic acid (ω -3, 20:5, mg/100 mg phospholipid) in the rheumatoid arthritis patients is significantly reduced (0.70 ± 0.50 , $n=15$) as compared with the controls (1.10 ± 0.80 , $n=15$).

Ghosts which are readily available, robust and studied in detail provided the best model for this study. The data obtained reveal two interesting points, the effect of membrane cholesterol on Na^+/K^+ ATPase activity and the reduced level of eicosapentaenoic acid in the membrane. Na^+/K^+ ATPase is an integral membrane protein which is involved in ATP utilization. An alteration in the activity of this enzyme in general might have important consequences for the cellular energy metabolism. In rheumatoid arthritis an increase in the activity of Na^+/K^+ ATPase coupled with a decrease in the cholesterol content of the membranes is interesting. Whether it is due to a direct interaction between cholesterol and the enzyme or there is a resultant increase in the number of molecules of the enzyme in the membrane needs to be studied experimentally.

The results of the fatty acid analysis of the red cell membranes indicate that the metabolism of eicosapentaenoic acid is abnormal in rheumatoid arthritis. Although the precise nature of this ab-

normality needs further investigation, it is clear that the biophysical characteristics of the red cell membranes are altered. Dietary manipulation can affect the lipid composition of biological membranes which in turn leads to changes in the biophysical characteristics of the membranes and the activities of the membrane associated enzymes (Stenson *et al.*, 1989). Fish oil is known to be rich in eicosapentaenoic acid. It has been suggested that ω -3, 20:5, competes with ω -6, 20:4 at the level of the cyclooxygenase, inhibiting the formation of thromboxane- A_2 and increased synthesis of thromboxane- A_3 resulting in decreased platelet aggregability. ω -3, 20:5 is also converted to prostacyclin of the 3-series, having anti-aggregatory properties (Fisher and Weber, 1984). These findings may make it possible in man to change the spectrum of biologically, highly active prostanoids by nutritional means and alter it in a favourable direction. In the 2nd phase of the study supplements of fish oil will be added to the diet of these patients and the degree of modification of clinical situation coupled with changes in the membrane lipid composition of erythrocyte membranes will be studied.

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