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THE RELATIONSHIP BETWEEN LIPID COMPOSITION OF RED BLOOD CELLS AND THEIR SUSCEPTIBILITY TO LIPID PEROXIDATION

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Red blood cells from 31 healthy donors were examined for the cholesterol content, the fatty acid composition, and the susceptibility to lipid peroxidation induced by either hydrogen peroxide or phenylhydrazine. Lipid peroxidation was monitored by the release of pentane and ethane. In addition, plasma fatty acids were measured in order to find out, whether plasma and red cell fatty acids were correlated. In experiments with hydrogen peroxide, a significant positive correlation was found between the proportion of arachidonic acid (C 20:4n – 6; $r = 0.57$, $p < 0.01$) and docosahexaenoic acid (C 22:6n – 3; $r = +0.71$, $p < 0.01$), and the release of pentane and ethane, respectively. A significant negative correlation was found between the membrane cholesterol content and the pentane release ($r = -0.44$, $p < 0.05$). In experiments performed with phenylhydrazine, red cell membrane lipid composition did not influence the susceptibility of red cells to lipid peroxidation. A close correlation was found between plasma and red cell fatty acids (palmitic acid, $r = +0.46$, $p < 0.01$; linoleic acid, $r = +0.41$, $p < 0.05$; arachidonic acid, $r = +0.59$, $p < 0.01$; docosahexaenoic acid, $r = +0.67$, $p < 0.01$). The results demonstrated that the degree of peroxide-induced oxidation of erythrocyte lipids depends on the content of polyunsaturated fatty acids in the membrane, which on the other hand, is determined by plasma fatty acids. It is suggested that dietary variations may influence the susceptibility of red cells to lipid peroxidation.

KEY WORDS: Lipid peroxidation, erythrocytes, red blood cells, alkanes, lipids.

INTRODUCTION

The main conditions which favour rancidification of biological structures are a high degree of saturation in lipids, a rich supply of oxygen, and the presence of transition metal catalyst.[1] No living system meets these requirements as suitable as red cells. In fact, several studies have shown that normal red blood cells are exceptionally resistant to oxidative changes.[2,3] Protection can be partly ascribed to cellular antioxidants, but it mainly reflects effective structural compartmentalisation of cellular constituents.[4]

When red cells are incubated with high concentrations of hydrogen peroxide in the presence of a catalase inhibitor, membrane lipids are slowly and partly oxidized.[2,5] Phenylhydrazine, long known as a haemolytic agent, induces lipid peroxidation of red cell membrane fatty acids as well.[6] Recent studies show that haem iron is an effective catalyst for red cell membrane lipid peroxidation only in its reduced ligand state in experiments performed with hydrogen peroxide.[7] However, phenylhydrazine-induced lipid peroxidation of red cells was not dependent on the presence of haem-II-iron.[8]

A variety of experiments performed with red cells from patients with various diseases indicated that mainly the proportion of arachidonic acid influences the

susceptibility of red cells to lipid peroxidation.[9-11] In the present study, we examined the degree of lipid peroxidation in red cells from healthy donors in relationship to the lipid composition. Lipid peroxidation induced by hydrogen peroxide or phenylhydrazine, has been assessed after oxidant stress by measuring the pentane and ethane release known to be a reliable index of lipid peroxidation of $n - 6$ and $n - 3$ polyunsaturated fatty acids, respectively.[12]

MATERIALS AND METHODS

All chemicals used were analytical grade. Celluloses were purchased from Sigma (Munich, F.R.G.), sodium citrate from Braun (Melsungen, F.R.G.) and all other chemicals from Merck (Darmstadt, F.R.G.).

Blood donors comprised 31 healthy individuals (age range 22–40 years). Clinical, haematological and biochemical examinations revealed no abnormalities in any of these donors.

For red cell analysis blood was mixed with sodium citrate (3.13%) as an anticoagulant (1:5, v/v). Red cells were separated from plasma by the method of Beutler *et al.*[13] with microcristalline and alpha-cellulose (1:1, w/w). Red cell membranes were isolated as described by Burton *et al.*[14]

Lipid Peroxidation

The susceptibility to lipid peroxidation was estimated by the incubation of red cells (haematocrit 2.5%) with 10 mmol/l hydrogen peroxide and 1 mmol/l sodium azide, or phenylhydrazine 5 mmol/l (final concentrations), in a phosphate-buffered solution of pH 7.4 for 2 hours at 37°C, as previously described.[3-6] The analysis of hydrocarbons in the head space vials was performed as described elsewhere.[5,6]

Lipid Analysis

Cholesterol in red cell membranes was estimated as described by Ott *et al.*[18] For the analysis of fatty acids, lipid extraction was performed by the method of Folch,[15] as modified by Jaeger *et al.*[16] A solution of chloroform/methanol 2:1 (v/v) was added to membranes in a ratio of 20:1. The sample was filtered and non-lipid material removed by the addition of 2.0 g Sephadex G-25. Transmethylation of fatty acids was performed with 0.5 ml BF_3 in methanol as described by Jaeger.[16] Fatty acid methylesters were extracted in hexane and analyzed by gas chromatography (Carlo Erba Fractovap 2150 with FID) using a capillary column (SP 2330, Supelco, 15 m × 0.2 μm) and hydrogen as carrier gas (flow 0.8 ml/min). Fatty acids were identified and calculated as previously described.[17]

Statistics

The linear correlation and the correlation coefficient was calculated according to Pearson.

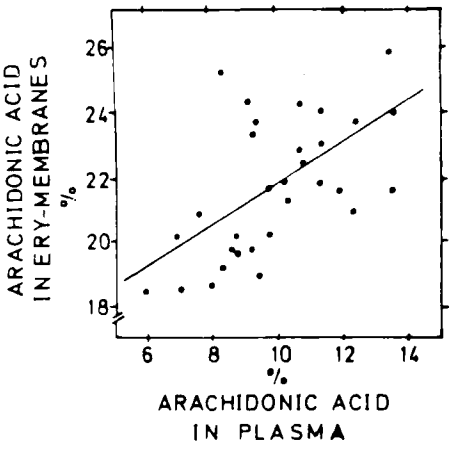

FIGURE 1 Relationship between the proportion of arachidonic acid in plasma and red cell membranes. $r = +0.59$, $y = 0.62x + 15.5$, $p < 0.01$. Arachidonic acid expressed as % of all fatty acids evaluated. $n = 31$.

RESULTS

Lipid Analysis

Fatty acid analysis of red cell membranes showed that arachidonic acid (C 20:4 $n - 6$) is the major polyunsaturated fatty acid amounting to about 20% (mean ± S.D.: 21.5 ± 2.1%; range 18.4 to 25.8%) of all fatty acids evaluated and to almost 40% of unsaturated fatty acids, respectively. Docosahexaenoic acid amounted to about 7% of all fatty acids evaluated (7.4 ± 1.3, range 4.9 to 9.7%) and to about 13% of unsaturated fatty acids. Red cell membranes contained 815 ± 174 nmol cholesterol/mg protein. A close correlation was found between plasma and red cell fatty acids (palmitic acid, $r = +0.46$, $y = 0.37x + 16.5$, $p < 0.01$; linoleic acid, $r = +0.41$, $y = 0.18x + 5.4$, $p < 0.05$; arachidonic acid, Fig. 1, and docosahexaenoic acid, Fig. 2).

Lipid Peroxidation

Hydrocarbons released during red cell lipid peroxidation are pentane and ethane. In experiments with hydrogen peroxide, 1.34 ± 0.38 nmol pentane/gHb/ 2 h and 0.35 ± 0.13 nmol ethane/gHb/2 h was produced. A significant positive correlation was found between the proportion of arachidonic acid (Fig. 3) and docosahexaenoic acid (Fig. 4), and the production of pentane and ethane, respectively. Significant negative correlations were found between further fatty acids and hydrocarbons (Table I). The remaining fatty acids were not significantly correlated to any hydrocarbon.

However, there was no relationship between the fatty acid composition of red cell membranes and the susceptibility of red cells to phenylhydrazine-induced lipid peroxidation.

An evident negative correlation exist between the cholesterol content of red cell membranes and the peroxide-induced formation of pentane (Fig. 5).

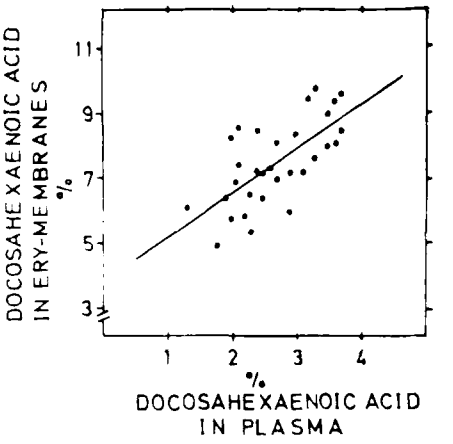

FIGURE 2 Relationship between the proportion of docosahexaenoic acid in plasma and red cell membranes. $r = +0.67$, $y = 1.35x + 3.8$, $p < 0.01$. Docosahexaenoic acid expressed as % of all fatty acids evaluated. $n = 31$.

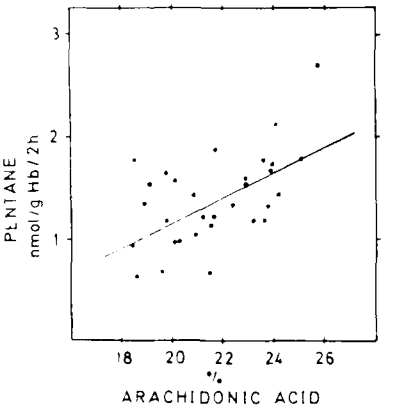

FIGURE 3 Relationship between the proportion of arachidonic acid in red cell membranes and pentane formation during hydrogen peroxide-induced lipid peroxidation. Arachidonic acid expressed as % of all fatty acids evaluated. $r = +0.57$, $y = 0.12x - 1.26$, $p < 0.01$, $n = 31$.

DISCUSSION

Pentane and ethane have been demonstrated to be the major hydrocarbon gases evolved during decomposition of methylated $n - 6$ and $n - 3$ fatty acids, respectively. Our experiments, performed with hydrogen peroxide as oxidizing agent, pointed out a close relationship between the release of pentane and ethane, and the amount of arachidonic acid ($n - 6$ fatty acid) and docosahexaenoic acid ($n - 3$ fatty acid) in red cell membranes, respectively, whereas no correlation existed between the evolution of pentane and linoleic acid ($n - 6$ fatty acid). This could be attributed to

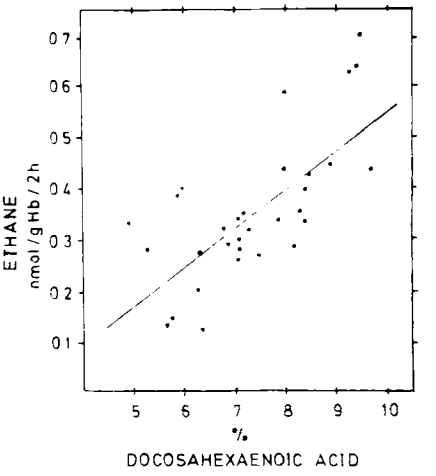

FIGURE 4 Relationship between the proportion of docosahexaenoic acid in red cell membranes and the ethane formation during hydrogen peroxide-induced lipid peroxidation. Docosahexaenoic acid expressed as % of all fatty acids evaluated. $r = +0.71$, $y = 0.76x - 0.21$, $p < 0.01$, $n = 31$.

the fact that arachidonic acid and docosahexaenoic acid are mainly located in the inner leaflet of the membrane (in phosphatidylethanolamine and phosphatidylserine), whereas linoleic acid is the major unsaturated fatty acid of the outer leaflet (phosphatidylcholine), and on the other hand, oxygen radicals, involved in the initiation of membrane lipid peroxidation, are thought to be produced during the oxidation of cytoplasmatic haem-II-iron.[7]

This is in good agreement with our previous finding that mainly arachidonic and docosahexaenoic acid are substrates for red cell membrane[20] or microsomal lipid peroxidation,[17] whereas linoleic acid is a minor substrate for fatty acid breakdown in red cell membranes.[21]

Our results showing that the amount of polyunsaturated fatty acids affects the sensitivity of membranes to lipid peroxidation is consistent with recent observations of Szebeni *et al.*,[22] which showed that rapid haemoglobin oxidation and lipid peroxidations occur in experiments with unsaturated liposomes but not with saturated phospholipids. Mowri *et al.*[23] studied the effect of lipid composition of liposomes on their sensitivity to peroxidation and showed that the content of polyunsaturated fatty acids is responsible for the degree of lipid peroxidation. The complex interrelationship between the qualitative lipid composition of the membrane, the

TABLE I

Correlation coefficients between fatty acids and hydrocarbons. $n = 31$

Correlation Fatty acid/Hydrocarbon	r	y	$p <$
Palmitic acid/Pentane	−0.65	−0.19 x + 6.23	0.01
Oleic acid/Pentane	−0.47	−0.12 x + 3.32	0.01
Palmitic acid/Ethane	−0.48	−0.04 x + 1.44	0.01
Oleic acid/Ethane	−0.42	−0.03 x + 0.85	0.05

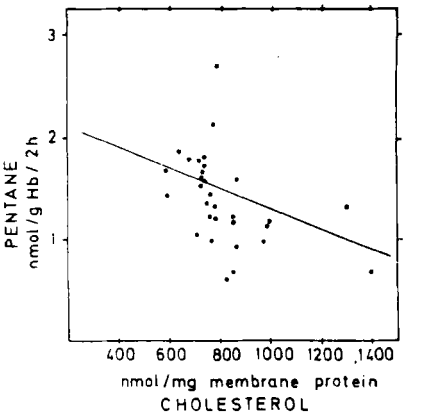

FIGURE 5 Relationship between the cholesterol content of red cell membranes and the pentane formation during hydrogen peroxide-induced lipid peroxidation. $r = -0.44$, $y = -0.001\,x + 2.3$, $p < 0.05$. $n = 31$.

pro-oxidant catalyst and the presence of calcium or other active ions was reported by Gutteridge.[24]

In contrast to our results obtained with hydrogen peroxide as oxidizing agent, proportions of fatty acids of red cell membranes did not influence the degree of phenylhydrazine-induced lipid peroxidation. The cause of the latter fact remains to be investigated.

Our results obtained with red cells from healthy subjects demonstrated that a higher red cell membrane cholesterol concentration may result in a protection against hydrogen peroxide-induced lipid peroxidation. This confirms recent observations of Bereza *et al.*[19] who performed experiments with rats fed high amounts of cholesterol leading to elevated concentrations of cholesterol in their red cell membranes. In addition, experiments performed by Szebeni and Toth[25] suggest a possible role for a high cholesterol content and preferential localization of phosphatidylserine in the inner bilayer leaflet of erythrocyte membrane in protecting against haemoglobin-induced lipid peroxidation in the membrane. In summary, our results suggest that variations in the concentrations of cholesterol and fatty acids in red cell membranes due to diet and diseases may influence the susceptibility of red cells to lipid peroxidation.

Acknowledgements

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