Mechanisms of Hemolysis in Liver Disease

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ABSTRACT

Liver disease, particularly alcoholic cirrhosis, is associated with a number of interesting chemical changes which result in structural and metabolic abnormalities of the erythrocyte membrane leading to microscopically observable cell shape changes and hemolytic anemia varying from very mild to potentially lethal.

Increase in unesterified serum cholesterol owing to lecithin cholesterol acyl transferase (LCAT) deficiency in cirrhosis leads to expansion of the lipid bilayer and macrocytosis without megaloblastic changes in precursors. Substitutions of phosphotidyl choline (PC) moieties in the erythrocyte lipid bilayer lead to echinocytes (disaturated PC) or to stomatocytes (dioleate saturated PC).

In some patients, high density lipoprotein (HDL) abnormalities lead to erythrocyte surface changes causing rapid formation of echinocytes. The rapidity and reversibility of these changes suggest blockade of metabolic transport channels critical to the maintenance of erythrocyte membrane shape.

Metabolic changes involving vitamin E deficiency leading to lipid peroxidation and pyruvate kinase instability leading to adenosine triphosphate (ATP) reduction have also been invoked to explain hemolysis associated with acute liver damage. The most severe hemolysis in liver disease is associated with acanthocytes (spur cells) and a marked imbalance in cholesterol-phospholipid ratio. These patients usually have hypersplenism, as well as rigid erythrocyte membrane transformations which are irreversible.

Any of the other erythrocyte membrane shape changes described appear to be reversible if the liver disease abates, but they too may become irreversible if bits of projecting membrane are repeatedly removed by the macrophages of an enlarged spleen.

Introduction

The liver is in large part responsible for the plasmatic environment of the erythrocyte. There is free exchange of unesterified cholesterol and some phospholipids between the plasma and the erythrocyte membrane. The erythro-
ocyte, having no glycogen stores, is dependent upon plasma glucose, the level of which is maintained by the liver. In liver disease, alterations in lecithin cholesterol acyl transferase (LCAT) and in lipoproteins may produce changes in the erythrocyte cholesterol/phospholipid ratio resulting in reduced membrane flexibility and, with processing, by the spleen, irreversible changes in cell shape and hemolysis.

Metabolic functions of the erythrocyte may also be affected by components produced by an abnormal liver. Interference with glucose transport across the membrane or with glycolytic metabolism within the erythrocyte may decrease adenosine triphosphate (ATP) which may result in contractile protein tetany, osmotic imbalance, shape changes, abnormal membrane rigidity and early removal of the erythrocyte in the spleen. Portal hypertension as a result of fibrosis (cirrhosis) in the liver may cause congestive splenomegaly and a form of hypersplenism with sequestration of erythrocytes in the spleen. In addition, recent evidence exists to suggest that the availability of vitamin E may be decreased, particularly in children, with chronic liver disease, possibly accounting for an increased susceptibility of the erythrocytes to intravascular oxidative hemolysis through lipid peroxidation.

This paper will review the existing data that support the various proposed mechanisms of erythrocyte damage in association with liver disease and will attempt to provide a unifying hypothesis for the premature destruction of erythrocytes in patients with abnormalities of liver function.

Review of Data

Lipids account for about 50 percent of the weight of red cell membranes. Mammalian red cell membranes have a molar ratio of cholesterol to phospholipid of about one. Four phospholipids predominate in human erythrocytes: lecithin (phosphatidyl choline), sphingomyelin, phosphatidyl serine, and phosphatidyl ethanolamine. The interactions between cholesterol and phospholipids have important consequences for membrane structure and function.

Cholesterol increases the packing of phospholipids in both layers of the lipid bilayer of the erythrocyte membrane. The close interpositioning of sterols with phospholipids in the lipid bilayers, which acquire excess cholesterol and phospholipids from plasma, causes a degree of immobility to be imposed upon the acylcarbon atoms near the lipid bilayer's inner or outer surface, while increasing the freedom of motion deep within the hydrophobic core of the bilayer creating an intermediate fluid state. The number of saturated double bonds within the phospholipid acyl chains in the bilayer is an important determinant of their fluid qualities.

Cholesterol itself also has an important effect on membrane fluidity. By interposing itself between adjacent phospholipid acyl fatty acid chains, it increases the degree of order within the membrane bilayer. Unesterified cholesterol and phospholipids of plasma lipoproteins readily exchange with their counterparts in the lipid bilayers of the erythrocyte membrane. In patients whose erythrocytes acquire excess cholesterol and phospholipids from plasma lipoproteins in a one to one ratio, the red cells become target cells and the membrane expands in a uniform fashion. This phenomenon is seen in cirrhosis, hepatitis, and obstructive jaundice. In obstructive jaundice, the increase in cholesterol may be as great as 75 percent and that of phospholipid up to 60 percent. A similar abnormality has been described in patients with congenital absence of lecithin-cholesterol acyl transferase (LCAT). This L-CAT deficiency has commonly been found in liver disease.
correlation between serum L-CAT activity and abnormalities of red cell lipids has been difficult to demonstrate.

Target cells do not leave the circulation prematurely in the absence of hypersplenism; however, larger imbalances in the cholesterol/phospholipid ratio often lead to formation of echinocytes (with sea urchin-like projections) and eventually to acanthocytes (spur or thorn cells) which are more rigid and are rapidly removed from the circulation by the spleen.

Recent studies have suggested that echinocytes are more readily found in the blood of patients with liver disease if the cells are examined in wet films or by scanning electron microscope rather than in fixed smears by light microscopy. Owen et al showed that echinocytes can be produced from normal washed erythrocytes when incubated with the high density lipoprotein (HDL) fraction from some jaundiced patients. Other plasma fractions (LDL, VLDL, Alb) are not apparently echinocytogenic. This type of echinocyte change is rapidly reversible if the abnormal cells are incubated in normal HDL. The echinocyte change occurs in minutes and is not accompanied by change in membrane cholesterol content nor by uptake of lysolecithin nor bile acids.

The HDL’s from these jaundiced patients show binding characteristics that were saturable at an estimated 5000 sites per cell. Normal HDL or nonechinocytogenic patient HDL’s show only nonsaturable binding characteristics. Pretreatment of erythrocytes with pronase or trypsin reduces or eliminates binding by abnormal HDL. Thus, it seems likely that echinocytosis in association with liver disease may, in some cases, be associated with membrane changes induced by a rapid shift (in glucose or electrolytes) induced by HDL related binding to specific receptor sites in the erythrocyte surface membrane.

Grahn, as early as 1968, demonstrated that serum from echinocyte-containing blood could induce echinocyte formation in normal cells. Similarly, serum from blood containing acanthocytes also induces formation of echinocytes, but not acanthocytes. Thus, it is argued, an additional factor acting in vivo may be necessary for the production of acanthocytes, probably by excess membrane removal in the spleen.

Subsequent studies by Hui et al show that some low density lipoprotein (LDL) fractions of normal serum produce echinocytes from discocytes. The active constituent appears to be apolipoprotein B (apo B). The HDL of patients with liver disease is enriched with apo E, which competes with apo B of normal LDL for apo B, E receptors on the surface of a number of nucleated cells. In the Netherlands, phosphatidyl choline specific transfer protein from beef liver has been used to replace native phosphatidyl choline (PC) molecules with a variety of PC species differing in fatty acid composition without changing the total phospholipid content of the membrane. No morphological changes were noted when PC was replaced by monosaturated species like 1-palmitoyl, 2 oleoyl PC; 1 palmitoyl, 2 linoleoyl PC, egg PC or PC from rat liver microsomes; however, replacement with disaturated species, 1,2 dimyristoyl PC, 1,2 dipalmitoyl PC, and 1,2-distearoyl PC resulted in formation of echinocytes. It should be noted that PC replacement using C14 labelled compounds is measured in hours (10 to 20 percent in one to two hours, 40 to 60 percent in four to six hours).

Interesting additional observations include the formation of stomatocytes upon replacement with PC species containing two unsaturated fatty acids, e.g., 1,2 dioleoyl PC and 1,2 dilinoleoyl PC. These studies suggest that the discoid shape of the human erythrocyte is opti-
mally stabilized by PC species that contain one saturated and one mono- or di-unsaturated fatty acid, and that the cell tolerates only limited variations in the species composition of its PC. Saturated PC seems to concentrate in the outer monolayer causing outward projection, while unsaturated PC inserts deeper in the lipid bilayer causing inward projection and stomatocytosis.

Dramatic changes in erythrocyte shape are observed when the unsaturation index of PC, defined as the total number of double bonds relative to that of the fatty acids, becomes lower than 0.5 or higher than 1.0. If the erythrocyte PC is replaced by dimyristoyl PC, dipalmitoyl PC or distearoyl PC, the index decreases to 0.5. Echinocytes are formed when about 25 percent of native PC is replaced by dipalmitoyl PC. Stomatocytes form if diunsaturated PC is substituted at 20 to 30 percent and hemolysis occurs at about 40 percent replacement.

Spheroechinocytes can also be found if the original PC is replaced by 1-palmitoyl, 2 arachidonoyl PC. At 20 percent replacement, shape change begins. At 60 percent replacement, when the double bond index becomes greater than 1.0, spheroechinocytes are obvious along with many cells showing typical dimples, which may be due to the loss of internal osmotic pressure, since the cells begin leaking ions at this stage.

Stomatocytes, produced by a bending or folding of the membrane inwards, form after replacement of more than 40 percent of the PC with 1,2 dioleoyl PC. Typical stomatocytes are observed when 20 to 30 percent of the PC is replaced by 1,2 dilinoleoyl PC after a six hour incubation. Further increase causes cell lysis.

Stomatocytosis has been reported in association with alcoholic cirrhosis. Four patients developed hemolysis of varying severity, and one appeared to have an intrinsic intracorpuscular defect because normal cells incubated in his serum did not acquire the shape change, nor did they become stomatocytes after infusion into his blood in vivo. The defect appeared to be related to acute alcoholic binges in these four patients and were not accompanied by lipedema.

Stomatocytosis has also been reported in association with a hereditary red cell anomaly in which there is a remarkable reversal of sodium and potassium concentrations in the erythrocyte. It seems possible that lipid changes in the erythrocyte membrane during acute alcoholic bouts may affect the permeability of erythrocytes to glucose or calcium, thereby decreasing ATP, causing tetany of contractile proteins and directly or indirectly interfering with the sodium, potassium pump.

Hemolytic anemia and susceptibility to H₂O₂ hemolysis have been described in children with vitamin E deficiency and chronic liver disease. Thirty-four children (one to 14 years) with severe chronic liver diseases were studied, 16 of whom were also vitamin E deficient (vitamin E < 5 mg per ml). Vitamin E may be deficient in liver cirrhosis as a consequence of intestinal malabsorption related to cholestasis. Tocopherol (vitamin E) provides a protective effect on lipid peroxidation without which the cells may be susceptible to oxidative lysis and a shortened survival. With respect to controls, cirrhotic children in both vitamin E and non vitamin E deficient groups showed significantly higher values of serum unesterified cholesterol, triglycerides, and total serum phospholipid concentration. There was an intense increase in phosphatidyl choline content in the erythrocyte membrane, as well as in the serum, that was statistically significant in the vitamin E deficient group. The fundamental biochemical lesion in the erythrocyte membrane may be due to accelerated lipid peroxidation inducing formation of malonyldialdehyde.
which promotes polymerization of cytoskeletal proteins. The subsequent decrease in cell flexibility leads to premature removal of the cell from the circulation.

In adult patients, a variety of erythrocyte metabolic defects may be induced including pyruvate kinase instability. There is an interesting acute hemolytic anemia seen in patients with alcohol-induced fatty liver associated with hypertriglyceridemia. There is little in vitro or in vivo evidence that increased triglycerides per se are associated with hemolytic anemia in this syndrome (Zieve's syndrome). Current belief is that hypersplenism accompanying the alcohol-induced fatty liver may be responsible, or that the high density older cells in Zieve's syndrome have decreased stability of the pyruvate kinase enzyme, thus, potentially interfering with ATP production and support of the sodium, potassium pump and the contractile elements of the cell membrane.

The most severe type of hemolytic anemia associated with liver disease is spur cell anemia, an abnormality of erythrocyte membrane cholesterol and phospholipid content found in patients with severe alcoholic cirrhosis, neonatal hepatitis, and Wilson's disease. Jaundice is prominent, splenomegaly is constant. Ascites and encephalopathy are often seen. In many patients, spur cell anemia precedes death by only a few weeks or months. Only a few patients have been observed to recover from spur cell hemolytic anemia if their liver disease abates.

In vitro studies show that phospholipids are present in the erythrocyte membrane in normal amounts, but there is a disproportionate increase in lecithin (PC) and cholesterol is increased 25 to 65 percent. This change results in a C/PL ratio of as much as 1.60 compared to normal of 0.95. The constitution of the erythrocyte membranes in this situation seems to be more dependent on the C/PL ratio of low density lipoprotein (LDL) than on total serum cholesterol.

There appears to be a dynamic process of exchange between the plasma LDL and the erythrocyte membrane since transfused normal erythrocytes will develop the same shape changes as the patients own cells. In vitro, these lipid shifts result in cell membranes with less fluidity. Folding and scalloping of the cell margins are observed suggesting distribution of excess cholesterol within the membrane is uneven.

The morphologic similarity between spur cells of liver disease and acanthocytes of patients with congenital abetalipoproteinemia is striking. Although the C/PL ratio of acanthocytes is normal or only slightly increased, the sphingomyelin/lecithin concentrations are reversed resulting in membrane fluidity changes similar to the spur cell. While the acanthocyte of abetalipoproteinemia has a markedly shortened life span, anemia is not severe, probably because the patients do not have portal hypertension or hypersplenism.

Summary

Liver disease, especially alcohol-induced cirrhosis, has been associated with a variety of erythrocyte membrane lipid changes, (cholesterol, phospholipids, phosphatidyl choline substitutions), enzyme inhibition (L-CAT deficiency, pyruvate kinase instability), electrolyte shifts (echinocytes and stomatocytes), vitamin E deficiency, and with congestive splenomegaly owing to portal hypertension causing hypersplenism.

The abnormalities induced in the erythrocyte membrane by these metabolic alterations lead to rigidity of the membrane and projection of excess membrane into the environment around
the cell. Excess membrane projections expose receptor sites which are removed by the macrophages in the enlarged spleen. This process results in spherocytes, echinocytes, spur cells, stomatocytes and fragmented erythrocytes which have a shortened life span in the circulation.

The resulting hemolytic process can vary from mild to severe depending upon the extent of membrane metabolic changes within the erythrocyte (intrinsic abnormalities) and the degree of splenic enlargement and macrophage activation (extrinsic abnormalities).

Thus, patients with target cells may have little hemolysis and a fairly good prognosis. Patients with echinocytes or stomatocytes may have membrane abnormalities, susceptible to reversal if properly treated, while those with overt spur cell anemia may have irreversible changes associated with severe hemolysis and a poor prognosis.

References


