Plasma lipids and lipoproteins in liver disease

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SUMMARY There are many changes in the plasma, lipids, and lipoproteins in patients with liver disease. They have proved difficult to study but our understanding of these changes has increased greatly during recent years. In obstructive jaundice hyperlipidaemia is a fairly constant finding and this appears to be due to the regurgitation of phospholipid from the obstructed biliary tree. The plasma lipids tend to fall with parenchymal liver disease. The composition of the lipoproteins depends on the activity of the plasma enzyme lecithin: cholesterol acyl transferase. When LCAT activity is high the individual lipoprotein fractions are normal. When it is reduced all of the lipoprotein fractions are affected but the pattern found with obstruction is quite different from that found with parenchymal disease. The changes in plasma lipoproteins appear to be associated with change in the lipid composition of cellular membranes and this may have important functional implications.

In 1955 Sheila Sherlock produced the first edition of her now classic monograph Diseases of the Liver and Biliary System. She wrote little about plasma lipids in liver disease and did not mention lipoproteins. This was not neglect of the subject but simply a reflection of knowledge at that time. The study of lipoproteins was in its infancy; plasma lipids were difficult to measure and only one plasma lipid estimation was done routinely—the total cholesterol. There was no good description of the plasma lipid changes with liver disease, let alone an understanding of the way in which they were produced.

Early studies

Austin Flint (1862) had suggested almost 100 years before that the blood cholesterol level was affected by diseases of the liver. He found it raised in three patients with parenchymal liver disease and attributed this ‘cholesterolaemia’ to the failure of the diseased liver to remove cholesterol from the blood. A more important observation was made by Widal et al. (1912) who studied two patients with extra-hepatic obstruction and found hypercholesterolaemia due predominantly to a rise of free sterol. Epstein (1932) reviewed the early work in this field. He confirmed that an increase of free cholesterol was characteristic of obstructive jaundice. Ester cholesterol was raised in most of his ‘obstructed’ patients but, in the remainder, ester cholesterol fell even if the total cholesterol was grossly raised. A low ester was also seen in severe parenchymal disease. The fall in ester in both types of liver disease was attributed to liver cell damage. Epstein’s results have been confirmed by later workers.

There have been fewer studies on other plasma lipids. Man and his colleagues (1945) noted that serum phospholipid changes in liver disease tended to follow those of cholesterol; above a certain level the relationship between the two compounds was virtually linear. Subsequently, they demonstrated that this relationship depended on the association between free cholesterol and phospholipid and postulated that lecithin was the phospholipid involved (Allbrink et al., 1950). Until recently plasma triglycerides were difficult to measure; ‘neutral fat’ levels were in the high normal range in most patients with liver disease but in some patients with biliary obstruction and infective hepatitis they were markedly raised and subsided as jaundice decreased (Man et al., 1945).

But studies on the lipid composition of whole plasma provide an incomplete picture of the plasma lipid changes which occur with liver disease. Lipids are not simply dissolved in plasma but are present in a variety of lipid-protein complexes called lipoproteins (Macheboef, 1929). Blix and his colleagues (1945) noted that normal lipoproteins migrated in an electrical field either with A1-globulins or with B-globulins. Soon afterwards Gofman and his colleagues (1954) employed the ultracentrifuge for the study of plasma lipoproteins. On the basis of their behaviour in the ultracentrifuge
lipoproteins of the β-globulin fraction gradually became known as low density lipoproteins (LDL) and α-lipoproteins as high density lipoproteins (HDL). A separate fraction, very low density lipoproteins (VLDL) was also identified; these have pre-β mobility on lipoprotein electrophoresis (Fredrickson et al., 1967).

Unfortunately, ultracentrifugal separation of plasma lipoproteins was, and still is, a costly and time-consuming procedure and progress in the field of liver disease was slow for many years.

**Obstructive jaundice**

Early plasma lipoprotein studies were concentrated on patients with obstructive jaundice because their plasma lipid levels are often very high. Gofman and his colleagues (1954) found a massive increase in LDL with chronic biliary obstruction, and a marked reduction in HDLs for which they used the term 'wipe out'. They hinted that LDL might have a different composition from those found in other hyperlipidaemic states and this was confirmed by Eder and his colleagues (1955). Using Cohn fractionation they found large amounts of LDL in a fraction which normally contained HDL; almost all of its cholesterol was unesterified. Russ and her colleagues (1956) extended these studies in a patient with primary biliary cirrhosis. The unusual LDL was shown to have a low cholesterol:phospholipid ratio and a low protein content; it did not react with antisera to normal LDL. LDL reacting with these antisera was found in appropriate Cohn fractions but its composition was abnormal. Russ et al. believed that several abnormal LDL were present, and heterogeneity of LDL with obstructive jaundice was also postulated by Furman and Conrad (1957).

Switzer (1967) removed ‘normal’ LDL from the low-density fraction of patients with primary biliary cirrhosis by using antibodies to normal LDL. An abnormal lipoprotein remained which was rich in phospholipid and with 96-100% of its cholesterol unesterified. Triglyceride was virtually absent and there was little protein. The amino acid pattern of this lipoprotein resembled that of VLDL and antiserum to it reacted with VLDL but not with normal LDL or HDL.

Switzer’s results were confirmed and extended by Seidel and his colleagues (1969). They called the abnormal lipoprotein LP-X, believing that it contained a specific apolipoprotein, Apo-X, with an extraordinary lipid-binding capacity. After delipidation, however, Apo-X appeared by both immunoprecipitation and polyacrylamide gel electrophoresis to be identical with Apo-C, a group of at least three peptides, which can be isolated from VLDL (Seidel et al., 1970).

LP-X has been studied by electron microscopy (Hamilton et al., 1971). In whole serum abnormal particles were seen which looked like coins or discs and tended to form rosettes. Larger structures resembling myelin figures were also observed and were often continuous with the rosettes. The major axis of the discs was about 400-600 Å and their thickness approximately 100 Å. Chemically these particles were composed of approximately equimolar amounts of lecithin and free cholesterol with small amounts of albumin and apolipoproteins. Hamilton and his colleagues suggested that the discs were partially flattened vesicles and that their walls were a continuous lipid bilayer. The origin of LP-X remains a matter of dispute.

The observation that LP-X occurs in plasma of patients with obstructive jaundice has been well publicised. Other lipoprotein abnormalities found in this condition are less widely recognised. Müller et al. (1974) found a large (30-70 nm), triglyceride rich LDL and suggested that this might account for the high plasma triglyceride seen in many patients with obstructive jaundice. There are also abnormalities in the HDL fraction. Its composition is abnormal and in some patients electron microscopy reveals disc shaped particles (4 nm thick and 15-20 nm in diameter) which have a tendency to form rouleaux (Forte et al., 1974). It is thought that they represent ‘nascent’ HDL—the form in which HDL is released from the liver (Hamilton et al., 1976).

Other developments have helped us to understand the lipoprotein changes described above. Glomset (1968) and his colleagues demonstrated the key role in lipoprotein metabolism of the plasma enzyme lecithin-cholesterol acyl transferase (LCAT). LCAT catalyses the transfer of fatty acid from the β-position of lecithin to the 3β-OH group of cholesterol with the production of cholesteryl ester and lysolecithin; it is responsible for the production of most of the cholesteryl ester in normal plasma. If LCAT activity were reduced one might expect free cholesterol and lecithin to accumulate in plasma and cholesteryl ester and lysolecithin levels to fall. Such changes occur in the rare disease familial LCAT deficiency (Norum et al., 1972) and also with biliary obstruction (although in the latter condition cholesteryl ester may be normal, raised, or reduced). There are other similarities between the lipid and lipoprotein abnormalities in these two conditions. In both HDL are reduced in quantity and show similar changes on chemical analysis, electron microscopy, and analytical ultracentrifugation: the LDL fraction in LCAT deficiency contains LP-X and large triglyceride rich particles.
similar to those found with biliary obstruction: in both the composition of VLDL is abnormal and it migrates with $\beta$ and not pre-$\beta$ mobility: lipid rich target erythrocytes are found in both conditions. These similarities suggest that low LCAT levels play an important part in the genesis of the lipoprotein changes of obstructive jaundice. In early studies we found low levels of LCAT activity in patients with obstructive jaundice and in those with parenchymal liver disease (Calandra et al., 1971). But, subsequently, several workers showed that LCAT levels in obstructive jaundice may be normal or even high (Simon, 1974) and this cast doubt on the extent to which LCAT deficiency could account for the lipoprotein changes of obstructive jaundice.

Recent work in our laboratory has helped to clarify the situation (Agorastos et al., 1978). We studied patients with obstructive jaundice with a wide range of plasma LCAT activity. Patients with low LCAT activity showed lipoprotein changes resembling those of familial LCAT deficiency. Plasma lipid levels were high and abnormalities described above were found in all three lipoprotein fractions (VLDL, LDL, and HDL); they correlated with the degree to which LCAT activity was depressed. In the LDL fraction we found LP-X and the large triglyceride rich particles described by Müller et al. (1974). We also found LDL of normal size (approx 20 nm): its composition was abnormal as it contained more triglyceride than usual and less cholesteryl ester; these compositional changes were similar to, but more striking than, those found by Kostner et al. (1976) in the lipoprotein they called Lp-Bc.

"Obstructed" patients with normal or high LCAT activity had high plasma lipid levels and we believe that the hyperlipidaemia which occurs regardless of LCAT activity is due to the regurgitation of biliary phospholipid from the obstructed biliary tree (McIntyre et al., 1975; Agorastos et al., 1978). The individual lipoprotein fractions in patients with normal or high LCAT were normal in composition, structure, and electrophoretic mobility and to our surprise we did not find LP-X in these patients.

Parenchymal liver disease

Our results suggested that the lipoprotein changes in obstructive jaundice are the consequence of both hyperlipidaemia and of low plasma LCAT activity. Low LCAT levels are also found in patients with parenchymal liver disease (Calandra et al., 1971) who tend to have low plasma lipids. The plasma lipid and lipoprotein changes in parenchymal disease have received much less attention than those of obstructive jaundice but some abnormalities have been recognised for many years. Cholesteryl ester levels fall, often to very low levels, while free cholesterol levels are normal or even high (Epstein, 1932). Man et al. (1945) and Phillips (1961) claimed that parenchymal liver damage depressed serum phospholipids and cholesteryl ester but found that neutral fat or triglyceride levels were high in some patients with hepatitis. There have been few studies on the individual lipoprotein fractions. Pierce and Gofman (1951) found normal or even high levels of the $S_\tau$ 10-20 subclass of LDL in patients with cirrhosis, (most normal LDL floating in the $S_\tau$ 0-10 subclass), while Pierre et al. (1954) found high LDL levels ($S_\tau$ 0-20) in patients with hepatitis.

We have recently studied plasma lipoproteins in 16 patients with parenchymal liver disease of varying degrees of severity and with a wide range of plasma LCAT activity (Day et al., 1978). Patients with normal or high LCAT activity had normal plasma lipid levels and lipoprotein fractions of normal structure, electrophoretic mobility and composition. Patients with low LCAT activity showed a number of lipoprotein abnormalities. Their HDL were similar to those found with familial LCAT deficiency and in those patients with obstructive jaundice who had low LCAT levels; this suggests that the HDL changes with obstructive or parenchymal liver disease relate to LCAT deficiency per se, presumably because the enzyme does not act on nascent HDL released from the liver. VLDL levels were strikingly depressed in patients with parenchymal liver disease and low LCAT and were much lower than in either of the other two conditions mentioned. Presumably these low levels were due to failure of VLDL synthesis and release, either because of malnutrition or because of damage to the parenchymal cells responsible for the manufacture of VLDL.

Our most interesting findings were in the LDL fraction. In contrast to the patients with obstructive jaundice only one peak was found on column chromatography regardless of the plasma LCAT activity. When LCAT was normal or high the composition of this peak was normal, but when LCAT activity was low the particles in this peak, which were of normal size, had an abnormal composition. They were deficient in cholesteryl ester and rich in triglyceride like the "normal-sized" LDL fractions found with obstructive jaundice (and low LCAT) and with familial LCAT deficiency. Their presence in plasma also seems to be a direct consequence of LCAT deficiency perhaps because less cholesteryl ester is produced to take the place of triglyceride during the catabolism of VLDL to normal LDL. Patients with parenchymal liver disease and low LCAT levels had low total plasma lipids because...
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VLDL and HDL levels were greatly reduced; surprisingly, the LDL concentration was not reduced. As these patients had a very low VLDL, which is thought to be the precursor of LDL, it seems likely that their LDL catabolism was greatly reduced but this requires further study.

Conclusion

The lipoprotein changes of liver disease may be of practical as well as theoretical interest. We know that the plasma lipoprotein abnormalities of liver disease cause changes in the lipid composition of erythrocyte membranes (Cooper, 1970) and these changes are associated with an abnormal permeability of the membrane to sodium (Owen and McIntyre, 1978). There are similar changes in platelets (Owen, J. S., personal communication) and possibly in the membranes of other cells. Such changes could affect cellular function and be responsible, at least in part, for the general cellular disturbance found in patients with severe liver disease. As we should be able to correct the abnormal composition of plasma and thus of cell membranes, at least on a temporary basis, we are faced with an exciting therapeutic possibility. Perhaps the rest of the story will be told in coming editions of Diseases of the Liver and Biliary System.

Several of the studies mentioned in this paper have been carried out in Professor Sherlock’s own department and I am grateful for her continued encouragement and support. I should also like to thank the many colleagues involved in our studies on the lipid and lipoprotein changes in liver disease: they include John Agorastos, Sebastian Calandra, Marco Dini, Richard Day, David Harry, Jim Owen, Tony Pearson, and John Pyrovolakis.

References

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