ATHERO-SCLEROSIS VI

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Effect on LP-X on Hepatic Cholesterol Synthesis

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Cholestasis causes changes in the pattern of plasma lipoproteins which reflect abnormal liver function. Cholestatic hypercholesterolemia is one of the oldest known forms of hypercholesterolemia in humans. The associated increase in the content of unesterified cholesterol is found in lipoprotein-X, a unique lipoprotein characteristic of obstructive jaundice. It floats at a density of 1.063 g/ml during ultracentrifugation and is characterized by its high content of phospholipid and unesterified cholesterol and the absence of apo-B, the major apoprotein of LDL. It exists in the form of a vesicle with an albumin core, its precursors are synthesized in liver and normally excreted in bile. During biliary obstruction this lipid material refluxes into plasma to form LP-X. (1) Even though LP-X is rich in cholesterol, it is surprising that cholesterol circulating in the form of LP-X does not exert feedback control through HMG-CoA reductase on hepatic cholesterol synthesis during cholestasis. (2)

Liver plays an important role in the regulation of cholesterol metabolism and some of the factors controlling hepatic cholesterol biosynthesis can be summarized as follows: (I) Composition and size of the bile pool (II) Hormonal regulation via cyclic AMP (III) Fluidity of the microsomal membrane and its influence on synthesis or activation of microsomal enzymes (IV) Circadian rhythm (V) Dietary status (VI) Biological age (VII) Enterohepatic circulation of bile salts and cholesterol (VIII) Intracellular enzymes and metabolic status (IX) Exchange of membrane cholesterol of the tissue with plasma lipoproteins (X) Negative feed-back due to dietary cholesterol regulated by the receptors for apo-B or apo-E or both.

During cholestasis factors I, VII and X are disturbed. Various conflicting explanations have been offered to explain the increased hepatic cholesterogenesis in cholestasis. Kattermann and Creutzfeldt (3) suggested that cholestasis alters hepatic metabolism in such a way that cholesterol feed-back is impaired at the cellular level. Weis and Dietschy (4) showed that diminished supply of lymph lipoprotein due to interruption of the enterolymphatic circulation during cholestasis causes increased hepatic cholesterol synthesis. They were able to correct this defect by infusion of lymph lipoproteins. However Cooper and Ockner (5) did not observe the complete suppression of enhanced hepatic cholesterol synthesis in biliary obstruction by infusion of lymph lipoproteins. The possibility that other factors such as LP-X may exert an influence on hepatic cholesterol metabolism remains open. Since the appearance of LP-X is a consistent feature of cholestasis, studies on its site of uptake, its effect on the activity of HMG-CoA reductase and on the uptake of chylomicron remnants in the liver were undertaken, in order to clarify the hypercholesterolemia of cholestasis.

Methods

Isolation and Labelling of LP-X

In order to establish the site of LP-X uptake, LP-X was isolated from human bile by a combination of ultracentrifugation and Cohn fractionation (6). After checking its purity by chemical, electrophoretic and immunochemical analysis, it was iodinated in the albumin moiety with ^{125}I-Iodine by the standard iodination technique. It was then dialyzed and recentrifugated for 20 hours at d 1.065 g/ml to remove any traces of free iodine and albumin. Before use the preparation was again dialyzed and checked for purity.

Liver Perfusion Technique

Livers were perfused after isolation with Krebs-Ringer-bicarbonate buffer containing 2.5% albumin and 10% hemoglobin in the form of human erythrocytes in a recirculating system.

Isolated Hepatocytes

Hepatocytes were prepared by the technique of collagenase perfusion. They were incubated in Krebs-Ringer-bicarbonate buffer containing 4% albumin at 37°C.

Isolated Lymphocytes

Lymphocytes were isolated from heparinized blood over lymphoprep gradient. They were then incubated in RPMI medium containing 10% foetal calf serum. After 2 hours of incubation unattached cells were incubated in the fresh medium.

Fibroblasts

Fibroblasts derived from normal subjects were maintained in Dulbecco's minimal essential medium with 25 mM $NaHCO_3$, 20 mM Hepes buffer, pH 7.4 and 10% foetal calf serum. Cultures between 5 to 10 passages were used for experiments.

Results and Discussion

Clearance of LP-X from Plasma

For in vivo experiments rats were anaesthatized with Evipan natrium and 1 ml ^{125} I LP-X (about 2-3 mg free cholesterol) was injected into the saphenous vein. As seen in Fig. 1 LP-X disappears very rapidly from the circulation. The disappearance of ^{125} I LP-X is similar to the long term decay curve obtained in rats which have been injected with unlabelled LP-X. (1)

Uptake of LP-X in vivo, in Isolated Perfused Livers, Isolated Hepatocytes, Lymphocytes and Monolayer Cultures of Fibroblasts

Measurement of ^{125} I-activity in various organs after the administration of ^{125}I LP-X shows that most of the radioactivity is found in the spleen. When rats were injected with ^{125} I LP-X, the amount of radioactivity found in the spleen was 7-fold greater on a g wet weight basis than that found in liver at 60 min. after injection (Fig. 2). Measurement of the radioactivity at various time intervals shows that this high activity in spleen was maintained even 24 h after injection of ^{125} I LP-X. A small amount of radioactivity was found in other organs, such as pan-

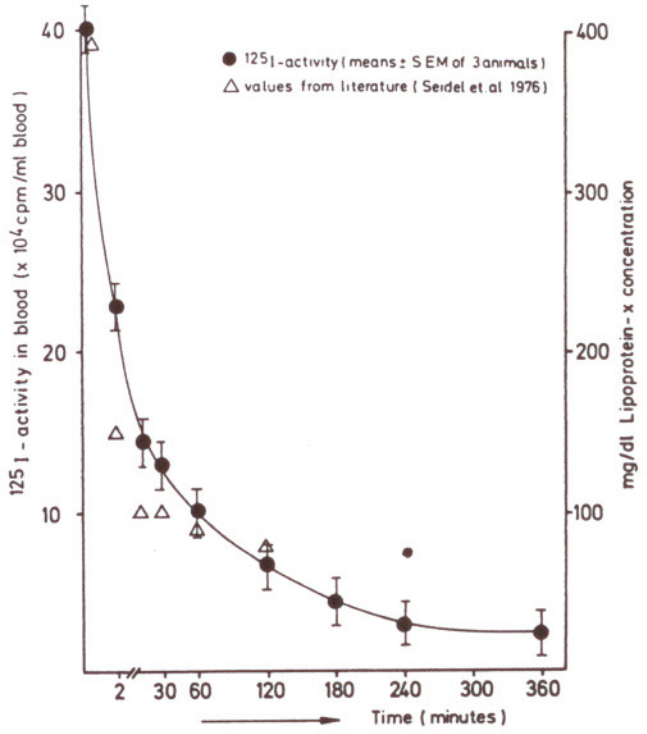

Fig. 1.
Kinetics of removal of ^{125}I LP-X from blood.
Rats were injected with 1 ml of ^{125}I LP-X (2-3 mg cholesterol) in saphenous vein and blood was removed at various time intervals to measure ^{125}I-radioactivity

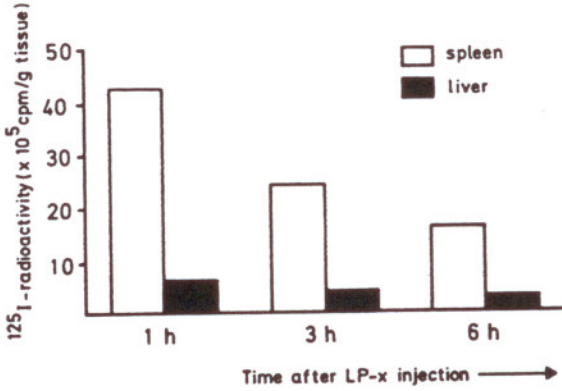

Fig. 2.
Distribution of ^{125}I-radioactivity in liver and spleen after administration of ^{125}I LP-X (2-3 mg cholesterol)

creas, kidney, lung, heart and muscles. In contrast, after injection of ^{125}I-albumin a small but equal amount of radioactivity was found in the liver and spleen. These results clearly show that in rats LP-X is mainly taken up the spleen and that the amount of LP-X removed from the circulation by the liver is only a small fraction of that removed by the spleen. Experiments with isolated perfused livers showed that when ^{125}I LP-X is added to the perfusion medium, it is removed by the liver to only a small extent, but within minutes. However when hepatocytes were isolated from these perfused livers, it was observed that only a small percentage of the ^{125}I activity found in the liver was present in parenchymal cells. Instead it was mainly present in the non-parenchymal cells. These in vitro experiments substantiate the results of the in vivo experiments, showing that the spleen and non-parenchymal liver cells remove LP-X from the circulation.

The binding and uptake of ^{125}I LP-X by lymphocytes, fibroblasts and hepatocytes was concentration dependent but did not reach saturation kinetics (Fig. 3). Hepatocytes and fibroblasts bound only a small fraction of the amount bound by lymphocytes. This again stresses the importance of non-hepatic cells such as lymphocytes in the removal of LP-X.

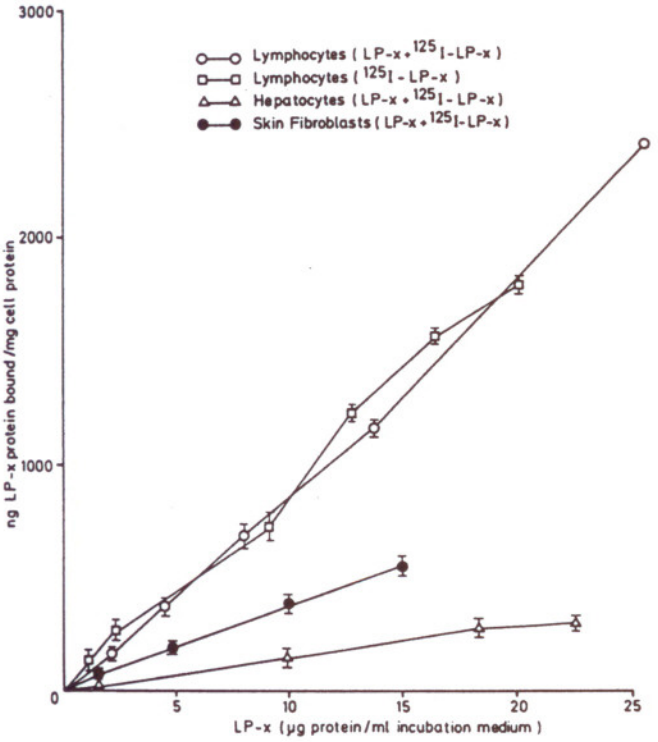

Fig. 3.
Binding and uptake of LP-X by isolated hepacytes, lymphocytes and monolayer cultures of fibroblasts. Cells were incubated with ^{125}I LP-X (1.48×10^4 cpm/µg LP-X protein) and unlabelled LP-X. Incubations were carried out at 37°C for 120 min

LP-X and HMG-CoA reductase activity

Parallel to the binding or uptake of LP-X by lymphocytes, suppression of the activity of HMG-CoA reductase similar to that with LDL was noted in these cells. Lymphocytes isolated from the blood of cholestatic patients also contained very low activity of HMG-CoA reductase as compared to controls (0-11.5 pmol as compared to 55 pmol/mg protein/h). However addition of LP-X to the perfusion medium caused a 5-fold increase in the activity of HMG-CoA reductase in microsomes of these livers (Fig. 4). An increase in the activity of the enzyme was also noted in microsomes of hepatocytes incubated with LP-X. In vitro incubation of isolated hepatic microsomes with LP-X resulted in the suppression of the enzyme activity in a concentration dependent manner. This increase in the activity of HMG-CoA reductase by LP-X is not specific for hepatic tissue. Inclusion of LP-X in media of monolayer cultures of fibroblasts, either in the presence of foetal calf serum or lipoprotein deficient serum, increases the activity of the reductase. These data suggest that in those cells which poorly bind or take up LP-X, a leaching of cellular cholesterol might occur.

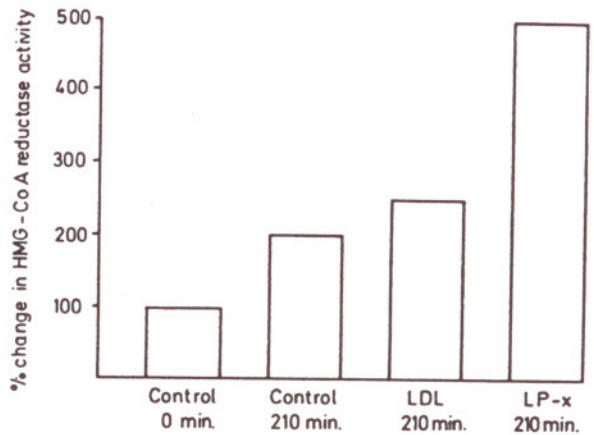

Fig. 4.
Effects of LP-X or LDL on the activity of HMG-CoA reductase in isolated perfused rat livers. LDL or LP-X was added to the medium after 30 min equilibration period (37 mg cholesterol/dl medium)

LP-X and Uptake of Chylomicron Remnants by Liver

It is well established that hepatic tissue almost completely removes chylomicron remnants from the circulation. In the presence of LP-X in the perfusion medium the removal of ^{14}C cholesteryl oleate labelled remnants was significantly reduced (about 50% of control). The liver tissue also had about 50% of the ^{14}C-activity as compared to controls. Similarly isolated hepatocytes bound about 50% of the ^{14}C-labelled remnants, as compared to those without LP-X. Thus both the reduced uptake of remnants and probably leaching of cellular cholesterol due to the high phospholipid content of LP-X may play a major role in enhanced cholesterol synthesis in cholestatic liver and result in hypercholesterolemia.

The pathobiochemical features of cholestatic hypercholesterolemia may be summarized as follows:

1. Physicochemical conversion of biliary lipid micelles into the LP-X vesicle after reflux of bile into the plasma.
2. Disturbed triglyceride hydrolysis of plasma lipoproteins through unphysiological apoprotein binding on the LP-X vesicle.
3. Disturbed enterohepatic circulation of bile acids and cholesterol.
4. Lack of inhibition of hepatic cholesterol biosynthesis by chylomicron remnants in the presence of LP-X.
5. Inability of liver to take up cholesterol in the form of LP-X.
6. Leaching of hepatic cellular cholesterol by LP-X, possibly because of its high ratio of phospholipid to cholesterol.

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References

1. Seidel D, Buff HU, Fauser U, Bleyl U (1976) On the metabolism of lipoprotein-X. Clin.Chim.Acta 66:195-207
2. Liersch M, Baggio G, Heuck CC, Seidel D (1977) Effect of lipoprotein-X on hepatic cholesterol synthesis. Atherosclerosis 26: 505-514
3. Kattermann R, Creutzfeldt W (1970) The effect of experimental cholestasis on the negative feed-back regulation of cholesterol synthesis in rat liver. Scand J Gastroenterol 5: 337-342
4. Weis JH, Dietschy JM (1971) Presence of an intact cholesterol feed back mechanism in the liver in biliary stasis. Gastroenterology 61: 77-84
5. Cooper AD, Ockner RK (1974) Studies of hepatic cholesterol synthesis in experimental acute biliary obstruction. Gastroenterology 66: 586-595
6. Manzato E, Fellin R, Baggio G, Walch S, Neubeck W, Seidel D (1976) Formation of lipoprotein-X, its relation to bile compounds. J. clin. Invest. 57: 1248-1260