Regulation of cholesterol metabolism in the liver in vivo and in vitro

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At serum cholesterol levels higher than 5 mm the risk for coronary heart disease increases progressively with serum cholesterol concentration (Martin et al., 1986). There is good evidence now that reduction of serum cholesterol in hypercholesterolaemic men can reduce the incidence of coronary heart disease (Lipid Research Clinics Program, 1984). More insight into the regulation of cholesterol metabolism in men may offer possibilities for a sensible intervention in order to achieve such a reduction.

Because of its central role in the metabolism of cholesterol, the human liver is our main study object. Here, the major part of the cholesterol synthesis, cholesterol conversion to bile acids and the delivery of cholesterol to the plasma in the form of very-low-density lipoprotein particles, take place. Also, the liver contributes an important part in the uptake of cholesterol from dietary source in the form of chylomicron remnants, from very-low-density lipoprotein remnants as intermediate density lipoproteins and low-density lipoproteins (LDL) and from peripheral cells via high-density lipoproteins (HDL). Studies in vitro with human hepatocytes face the major problem of the limited and infrequent availability of fresh human liver. Therefore most of our studies have been performed with the human hepatoma cell line Hep G2. We showed (Havekes et al., 1983; Cohen et al., 1984, 1985) that these cells provide us with a good model for the investigation of regulation of cholesterol and lipoprotein metabolism in human hepatocytes.

LDL receptor

LDL-receptor activity was shown to be present on the Hep G2 cells (Havekes et al., 1983) and was decreased by incubation with LDL (Cohen et al., 1984). On the other hand, a heavy HDL fraction stimulated receptor activity. This increase was only partially prevented by simultaneous incubation with LDL (Havekes et al., 1986a). Similar results were obtained with primary cultures of human hepatocytes (Havekes et al., 1986b) and are in contrast with the commonly observed effects of LDL and HDL on extrahepatic LDL-receptor activity (Goldstein & Brown, 1977). If this regulation of LDL-receptor activity by serum lipoproteins also occurs in the human liver in vivo, these observations suggest that the presence of heavy HDL and possibly other apolipoprotein A-I-containing particles in plasma, could be responsible for maintaining a receptor-mediated catabolism of LDL by the liver, notwithstanding the exposure of the liver cells to physiological concentrations of LDL.

Some insight into the mechanism of the downregulation of the LDL-receptor activity by LDL was obtained by using compactin, an inhibitor of the cholesterol synthesis at the site of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase). Blocking the cholesterol synthesis in Hep G2 cells with compactin resulted in an increase of LDL-receptor activity, which could be prevented by simultaneous addition of either mevalonate or LDL (Cohen et al., 1984). It was concluded that LDL influx could compensate for a mevalonate-derived suppressor of the LDL-receptor activity, which is lacking in the presence of compactin and is probably cholesterol or a cholesterol metabolite.

Abbreviations used: LDL, low-density lipoproteins; HDL, high-density lipoproteins; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase.

HMGC reductase

Also the HMG-CoA reductase activity, the major regulation site of the cholesterol synthesis, was increased after incubation of Hep G2 cells with compactin, as measured in the cell homogenate after removal of the inhibitor. Mevalonate could completely abolish the compactin effect, indicating a feedback inhibition of a mevalonate-derived metabolite(s) on the HMG-CoA reductase activity (Cohen et al., 1984). As is shown in Fig. 1 (cf. lanes c and a) the induction of the reductase activity by compactin was accompanied by an increase in the amount of reductase mRNA, suggesting that the mevalonate-derived effector(s) suppresses the expression of the reductase gene. LDL, which decreased the reductase activity by lowering the quantity of mRNA coding for the reductase (see Fig. 1, lane f), by itself could only partially prevent the compactin-induced increase in reductase activity, but together with a low concentration of mevalonate in the incubation medium, LDL completely abolished the compactin effect (Cohen et al., 1985). It was concluded that the effectors derived from mevalonate, which play a role in the feedback regulation of HMG-CoA reductase, can be divided into at least two components: one part which can be compensated for by LDL influx, and another part, which is not related to LDL uptake. Lane b in Fig.1 shows that incubation of Hep G2 cells with LDL together with mevalonate resulted in a strong reduction of the reductase mRNA, much stronger than by LDL alone (lane f), indicating that both classes of effectors play a role in the level of reductase-mRNA synthesis and/or degradation.

In order to obtain more information about the nature of the mevalonate-derived effectors the effects on HMG-CoA reductase activity of drugs which inhibited the cholesterol synthesis at other sites were tested in Hep G2 cells (Boogaard et al., 1987). In experiments using U18666A, an inhibitor of squalene-2,3-epoxide cycloase, a biphasic effect on reductase

Fig. 1. Northern blot analysis of HMG-CoA reductase mRNA levels in Hep G2 cells

Hep G2 RNA was fractionated by electrophoresis on a 1% agarose gel and transferred to a nitrocellulose membrane. The blot was hybridized with cloned cDNA's coding for human HMG-CoA reductase (pRed 102; Luskey & Stevens, 1985) and human serum albumin. Lanes (a–f) represent RNA from Hep G2 cells, incubated for 18 h in Dulbecco's modified Eagle's medium supplemented with 1% human serum albumin and (a) no addition, (b) mevalonate (3 mM) and LDL (200 μg/ml), (c) compactin (2 μM), (d) U18666A (20 μM), (e) U18666A (20 μM) and LDL (200 μg/ml), (f) LDL (200 μg/ml). RNA preparations from duplicate incubations were loaded. Approximately the same amount of total RNA (10 μg) was applied per lane.
activity was observed. At concentrations of the drug up to 0.5 μM a decrease in reductase activity was found. However, at concentrations higher than 3 μM, HMG-CoA reductase activity was increased in a concentration-dependent manner. Analysis by t.l.c. of 14C-labelled non-saponifiable lipids formed from [14C]acetate during the cell incubations showed that at the concentration of U18666A that decreased the reductase activity polar sterols were formed, suggesting that these polar sterols play a role in suppressing the reductase activity. [14C]Sterols were not detectable at high U18666A concentrations, suggesting that the absence of sterol effector(s) resulted in an increase in reductase activity. That this effector influenced the level of reductase mRNA, as expected, is confirmed by the experiment shown in lane d of Fig. 1, where it can be seen that incubation of Hep G2 cells with a high concentration of U18666A resulted in an increase in the amount of mRNA. Hep G2 cells were incubated with LDL and U18666A simultaneously in order to answer the question of whether LDL could compensate for the missing effector in the presence of high concentrations of U18666A. However, the reductase activity remained elevated, notwithstanding the almost doubling of the cholesterol content of the LDL pool (Boogaard et al., 1987). This is also the case at the reductase-mRNA level (Fig. 1, lane e). These observations indicate that LDL-mediated reduction of reductase mRNA is not merely the result of introducing cholesterol to the cells.

In the presence of a high concentration of U18666A, when no sterol formation took place, the additional increase in reductase activity, produced by simultaneously added compactin, could be prevented by addition of mevalonate (Boogaard et al., 1987). This indicates the existence of a non-sterol mevalonate-derived effector in addition to the sterol-dependent regulation, sustaining that of mevalonate (Boogaard et al., 1987). It is based on a treatment resulting in an increased hepatic LDL uptake by means of a maximal exposure of LDL receptors and a concomitant drainage of cholesterol, via bile acids, from the body. Proposed is a treatment with cholestyramine, interrupting the enterohepatic circulation of bile acids, which results in an increase in LDL-receptor activity and cholesterol synthesis, combined with an inhibitor of bile cholesterol synthesis, which in its turn will additionally induce the number of LDL receptors. Specific inhibitors of HMG-CoA reductase, related to compactin, such as mevinolin, simvastatin and CS514, seem to be good candidates for such a therapy. The results of limited trials with compactin and cholestyramine (Mabuchi et al., 1983) and with simvastatin (Mo et al., 1986) were promising. If these drugs are shown to be non-toxic in the long run, they will have an important role in the therapy of hypercholesterolaemic individuals.

Squalene synthetase

Amongst the other enzymes of the cholesterol biosynthetic pathway, squalene synthetase was mentioned to be a common to the activities mentioned above. The regulation mechanisms, determining the LDL-receptor and HMG-CoA reductase activities, seem to have at least the sterol component in common.

Bile acids

Studies concerning the regulation of bile acid synthesis in rate and focused on the activity of the rate-limiting enzyme cholesterol 7α-hydroxylase. However, it is not clear whether this enzyme is also rate-determining in other species or at all circumstances. In different species the major bile acids synthesized are different, as we were able to confirm in primary cultures of hepatocytes isolated from rat, pig and human liver and in Hep G2 cells. The major bile acids synthesized in cultured human hepatocytes and Hep G2 cells were chenodeoxycholate and cholate (the primary bile salts in man), whereas in pig hepatocytes heoholate (J. Kwekkeboom, unpublished work) and in rat hepatocytes cholate and β-muricholate (Princen et al., 1986) were the major bile acids. Although the bile acid synthesis rate in neonatal pig hepatocytes was comparable with that in rat hepatocytes, cholesterol 7α-hydroxylase activity was hardly detectable in microsomal preparations from neonatal pig liver, while this enzyme had a considerable activity in microsomes from adult pig and rat liver. On the other hand, the conversion of cholesterol to 26-hydroxycholesterol was very pronounced in the homogenates of the neonatal pig hepatocytes, suggesting that an alternative pathway, in which cholesterol 26-hydroxylase is the first step in bile acid formation, is the major route in this case.

Now that we have some knowledge about the effect of a number of compounds on several activities involved in cholesterol metabolism, a rational therapy for reducing serum cholesterol levels in hypercholesterolaemic patients has been developed (Brown & Goldstein, 1986). It is based on a treatment resulting in an increased hepatic LDL uptake by means of a maximal exposure of LDL receptors and a concomitant drainage of cholesterol, via bile acids, from the body. Proposed is a treatment with cholestyramine, interrupting the enterohepatic circulation of bile acids, which results in an increase in LDL-receptor activity and cholesterol synthesis, combined with an inhibitor of bile cholesterol synthesis, which in its turn will additionally induce the number of LDL receptors. Specific inhibitors of HMG-CoA reductase, related to compactin, such as mevinolin, simvastatin and CS514, seem to be good candidates for such a therapy. The results of limited trials with compactin and cholestyramine (Mabuchi et al., 1983) and with simvastatin (Mo et al., 1986) were promising. If these drugs are shown to be non-toxic in the long run, they will have an important role in the therapy of hypercholesterolaemic individuals.