Characteristics of High-Density Lipoprotein Binding Sites in Cultured Parenchymal, Endothelial, and Kupffer's cells from Rat Liver

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Liver endothelial cells possess surface high-affinity binding sites for HDL_3, whose affinity is 4 times higher than that of the sites on hepatocytes and Kupffer's cells. The maximal number of high-affinity binding sites on endothelial cells is 437 ng/mg protein, which surpasses this parameter on the hepatocyte surface several times.

Key Words: *high-density lipoproteins; hepatocyte culture; liver endothelium culture; Kupffer's cell culture; Scatchard analysis*

Binding of apolipoprotein A of high-density lipoproteins (apoA HDL) with various cells meets all the criteria for receptor interaction: it is specific, saturable, high-affinity, reversible, and accompanied by biological effects [5,6,15].

The binding parameters for apoA-binding sites on extrahepatic tissues are well studied and indicate the similarity of binding sites for apoA-containing HDL on the surface of various cells [3,6,15].

On the other hand, the reported parameters of apoA-containing HDL with the liver are somewhat contradictory. The majority of investigators report a dissociation constant (K_d) of 20 μg/ml, which is close to that for peripheral tissues [6,7,10,11,15], while Morrison *et al.* found a two-component binding, the first K_d (36 μg/ml) being close to that obtained by others, while the second (0.94 μg/ml) suggests a considerably higher affinity binding.

Studies of apoA-binding sites in the liver have been primarily performed on parenchymal cells, which account for up to 95% of the liver mass [6,9-11,15], whereas the role of nonparenchymal liver cells, which make up about 40% of the cell population and also possess apoA-binding sites, has been little studied. The nonparenchymal cells are represented by at least two cell populations (Kupffer's and endothelial cells) and from a direct barrier between the parenchymal compartment of the liver and the circulatory bed.

In the present study we investigated the binding of HDL_3 (apoE-free) with primary cultures of hepatocytes, endotheliocytes, and Kupffer's cells isolated from rat liver.

MATERIALS AND METHODS

Mature male Wistar rats weighing 150-200 g were used in the experiments. The animals were maintained on a standard laboratory ration.

HDL_3 (ρ =1.125-1.216) were isolated by preparative ultracentrifugation in NaBr solution [13] from plasma obtained from healthy subjects and hypercholesterolemic patients after plasmapheresis. The protein composition of the HDL_3 samples was analyzed in 10% PAGE under denaturing conditions followed by Coomassie blue staining. The protein composition of the HDL_3 isolated from the

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plasma of healthy subjects was represented mainly (98%) by apoAI, while the HDL_3 samples of hypercholesterolemic patients contained 60% apoAI and 40% apoAII. All HDL_3 contained no more than 0.1% apoE. NaBr was removed by dialysis (1:1000 v/v) against two changes of solution containing 0.145 M NaCl and 0.3 mM EDTA (Sigma). HDL_3 were labeled as described earlier [8]. The labeled HDL contained 2% of radioactivity in the trichloroacetic acid-soluble fraction, the specific radioactivity being 400-600 cpm/ng.

A suspension of hepatocytes was obtained by *in situ* enzymatic perfusion of the liver with a collagenase solution, as described elsewhere [1]. Cell viability determined by trypan blue exclusion was no less than 85%. Hepatocytes were seeded on collagen-precoated plastic dishes.

Endothelial and Kupffer's cells were isolated as follows: in order to prevent cell aggregation, 1 ml of 1% protamine sulfate (Serva) was added to the supernatant obtained after centrifugation of the initial suspension of liver cells containing nonparenchymal liver cells (NLC). For removal of destroyed cells the suspension of NLC was twice centrifuged in Hanks solution (Flow Lab.) supplemented with 5 mM HEPES at 250 g for 2 min. The NLC-containing pellet was resuspended in Hanks solution supplemented with 5 mM HEPES and recentrifuged in a metrizamide (Sigma) density gradient (final concentration 17.5%) at 400 g for 20 min. The NLC-containing fraction was then collected and washed free of metrizamide by centrifugation at 250 g for 2 min. The cells were counted using a hemocytometer. Cell viability was determined by trypan blue exclusion. The yield of cells was about 250 mln. Endothelial and Kupffer's cells were separated from the NLC suspension using the method of selective adhesion of macrophagal cells to plastic dishes [12]. To this end, the obtained cell suspension in Williams's E medium (Flow Lab.) was placed on culture dishes not coated with collagen. Twenty minutes later, the attached and plated Kupffer's cells were vigorously washed free of the endothelial cells. The liver endothelial cells were then centrifuged at 250 g for 2 min and seeded in William's E medium on collagen-coated plastic culture dishes.

The liver cells were cultured in William's E medium supplemented with 10 μg/ml insulin (Sigma), 100 μg/ml kanamycin (Sigma), and 20 mM HEPES (Flow Lab., UK) in the presence or absence of dexamethasone (Sigma). The medium was replaced every day. The cells retained 98% viability during 5 days of culturing, as determined by trypan blue exclusion.

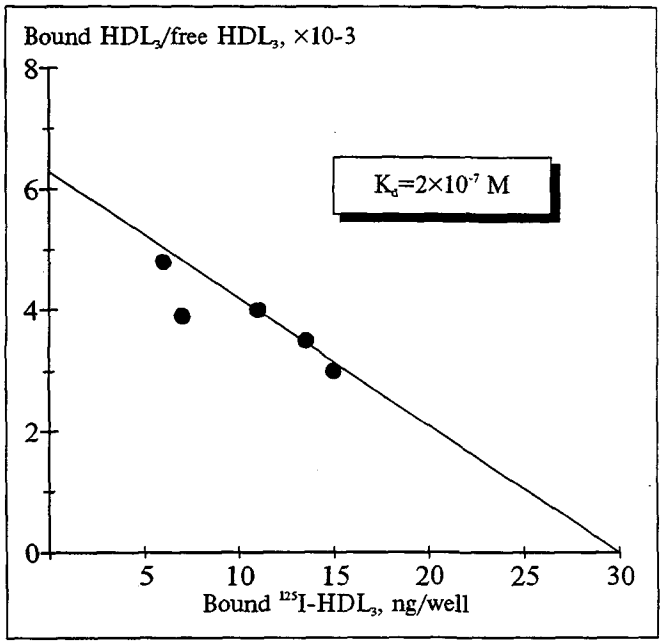

Fig. 1. Scatchard plot of dose–dependent binding of ^{125}I–HDL_3 with rat hepatocytes. Hepatocytes were cultured during 48 hours in the presence of 10^{-5} M dexamethasone. The curve is representative of 3 independent experiments.

For the study of dose-dependent ^{125}I-HDL_3 binding with primary cultures of liver cells, these were incubated with various concentrations of ^{125}I-HDL_3 (1-50 μg/ml) and BSA (20 mg/ml) at 37°C during 2 hours in the presence or absence of a 20-fold excess of unlabeled HDL_3. After completion of the incubation the plates with cells were placed on ice, washed three times with ice-cold Hanks solu-

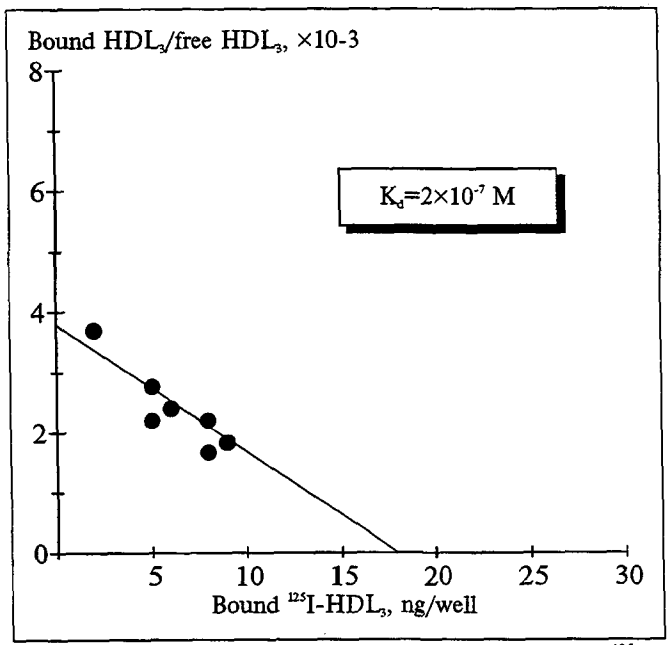

Fig. 2. Scatchard plot of dose–dependent binding of ^{125}I–HDL_3 with Kupffer's cells. Kupffer's cells were cultured during 24 hours without dexamethasone.

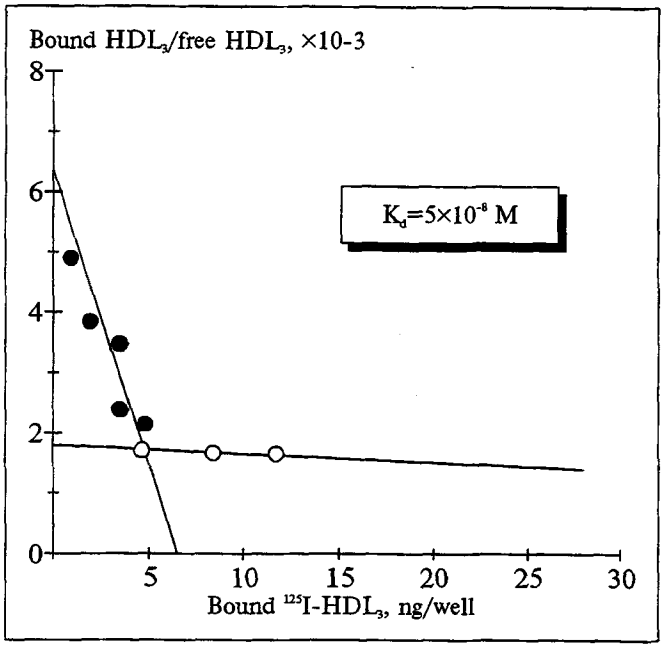

Fig. 3. Scatchard plot of dose–dependent binding of ^{125}I–HDL_3 with endothelial cells. Endothelial cells were cultured during 24 hours without dexamethasone.

tion, and incubated in Hanks solution containing dispase and protease (100 μg/ml each, Sigma) at 4°C for 30 min for determination of surface ^{125}I-HDL_3 binding. Under these conditions the cells remained attached to the plastic and retained no less than 95% viability as determined by the trypan blue test. After completion of the incubation the solution was transferred to counting vials and assessed for radioactivity with a Compugamma γ-counter (LKB). The specific binding was calculated as the difference of radioactivity in the presence or absence of a 20-fold excess of unlabeled ligand.

The obtained data are representative of two or three experiments, and each value was determined in duplicate or triplicate measurements.

The dose-binding dependences were analyzed after Scatchard [14]. The K_d in a two-component Scatchard curve was determined after Rosenthal [2]. The protein content in the samples was measured after Bradford [4].

RESULTS

Scatchard analysis of ^{125}I-HDL_3 binding with hepatocytes and Kupffer's cells is illustrated on Figs. 1 and 2. The curves are linear, which indicates the presence of binding sites with similar parameters. For hepatocytes and Kupffer's cells K_d were 20 (2×10^{-7} M) and 23 μg/ml ($2\text{з}10^{-7}$ M), respectively, assuming the molecular weight of the ligand to be equal to 100 kD. This suggests the similarity of binding sites in these cells. The maximal number of ^{125}I-HDL_3 binding sites is 120 ng/mg protein on hepatocytes and 850 ng/mg proteins on Kupffer's cells.

The values of K_d for hepatocytes and Kupffer's cells are consistent with the results obtained by others on hepatocytes and cells from extrahepatic tissues of various animal species and man [6,7,10, 11,15].

Figure 3 represents a Scatchard plot of the dose-dependent binding of the ligand with endothelial cells. The nonlinear character of the curve suggests a two-component binding, the high-affinity and low-affinity parts being characterized by K_d values of 5 μg/ml (5×10^{-8} M) and 250 μg/ml (22×10^{-7} M), respectively. The latter is characteristic for a nonspecific interaction.

Thus, as follows from our data, endothelial liver cells possess high-affinity binding sites for HDL_3 with a 4-fold higher affinity in comparison with hepatocytes and Kupffer's cells. The maximal number of high-affinity binding sites on the surface of endothelial cells is 437 ng/mg protein, which surpasses that on the surface of hepatocytes several times.

The existence of two different classes of binding sites discovered by us - with a higher affinity on endothelial cells and a lower affinity on other liver cells - probably explains the results of Morrison *et al.* [9], who, using a membrane fraction of rat liver homogenate, observed a two-component binding with a high-affinity component. This high-affinity component is apparently related to the presence of high-affinity receptors on endothelial liver cells.

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