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Short Communication

HIGH-DENSITY LIPOPROTEIN AND CEREBRAL ENDOTHELIAL CELLS *IN VITRO*: INTERACTIONS AND TRANSPORT

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Abstract—Primary cultures of bovine cerebral endothelial cells were used as an *in vitro* model for the blood–brain barrier to study the transport and interactions of high-density lipoprotein (HDL) across monolayers of these cells. Transport of ^{125}I-apoE free HDL across a monolayer of bovine cerebral endothelial cells occurred in a linear fashion up to a concentration of 70 μg/mL, suggesting paracellular transport of HDL. Bovine cerebral endothelial cells possess a high affinity binding site for HDL with a mean dissociation constant (K_D) of 10.8 ± 2.6 μg/mL (N = 4). Maximal binding of apoE free HDL to cerebral endothelial cells proved to be temperature-dependent: at 4° a B_{max} value of 42 ± 9.3 ng/mg cell protein was found, while at 37° this value was 177 ± 70.4 ng/mg cell protein. Cell association of ^{125}I-HDL could be effectively displaced by HDL, not by low-density lipoprotein or acetylated low-density lipoprotein, and association was not coupled to degradation. The *in vitro* blood–brain barrier cell system possesses high affinity binding sites for HDL, which are probably not involved in the transport of HDL across cerebral endothelial cells.

Key words: blood–brain barrier; cerebral endothelial cells; high-density lipoprotein; lipoprotein receptor; apolipoprotein; transport

The transport of large or hydrophilic molecules, such as drugs, into the central nervous system is impeded by the BBB§. The functional and anatomic basis of this barrier resides in the cerebral endothelial cells [1]. The entry of lipoproteins into the brain is also hindered by the BBB [2]. The presence of several kinds of apolipoproteins, such as apolipoprotein E, A-I. and their respective receptors in the brain, indicates that the central nervous system has similar mechanisms for lipid transport as peripheral tissues. A component of the HDL, apoA-I, was found in the cerebrospinal fluid in concentrations amounting to 0.4% of the original plasma levels, though the mRNA encoding for apoA-I was not detected in brain tissue, suggesting the need for transport of the proteins from the blood into the brain [3].

In this study we show that HDL is transported across a monolayer of cultured cerebral endothelial cells and that HDL binds selectively with high affinity to primary cultures of bovine cerebral endothelial cells.

Materials and methods

BCEC were isolated and cultured according to De Vries *et al.* [4]. Lipoproteins were isolated and radiolabelled according to Schouten *et al.* [5]. The specific radioactivity of the radiolabelled compound ranged from 200 to 400 cpm/ng protein. Transport of ^{125}I-apoE free HDL across a monolayer of cerebral endothelial cells was studied essentially according to Van Bree *et al.* [6], and cells were cultured on a 6.5 mm^2 polycarbonate Transwell insert (pore size 0.3 μm) for this purpose. BCEC were cultured for 10 days and the TEER ranged from 50 to 60 Ω/cm^2. Cells were preincubated with MEM containing 2% BSA for 2 hr, while cell monolayers were subsequently incubated with 3.5, 7, 35, and 70 μg/mL ^{125}I-apoE free HDL in MEM with 2% BSA. Samples of 50 μL were taken from the acceptor compartment for 2 hr every 15 min, mixed with 80 μL 35% TCA, incubated for 15 min at 37°, centrifuged for 2 min at 15,000 rpm, and the pellets counted for radioactivity to determine whether ^{125}I-HDL was intact after transport.

Association and degradation of ^{125}I-apoE free HDL with BCEC were performed as described for AcLDL [4]. Total binding of ^{125}I-apoE free HDL was measured after cells had been incubated for 2 hr at 4° with various amounts of ^{125}I-apoE free HDL ranging from 0.6 μg/mL to 60 μg/mL. Non-specific binding was determined in parallel by incubation of ^{125}I-apoE free HDL in the presence of a 10-fold excess of unlabelled apoE free HDL with a minimum of 60 μg/mL. To determine the receptor binding kinetics, BCEC were incubated with 5 μg/mL ^{125}I-apoE free HDL for 10, 30, 60, 120, and 180 min, respectively, at 37° in the absence or presence of the inhibitor of lysosomal enzyme activity, chloroquine (100 μM). Degradation of apoE free ^{125}I-HDL was determined in the presence or absence of chloroquine as described by Van Berkel *et al.* [7]. As a control, degradation of 5 μg/mL ^{125}I-HDL in medium at 37° was determined at indicated time intervals.

Results and discussion

Maximal binding (B_{max}) of ^{125}I-apoE free HDL to BCEC was 42 ± 9.3 ng/mg cell protein with an apparent affinity coefficient (K_D) of 10.8 ± 2.6 μg of ^{125}I-HDL/mL (N = 4)

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§ Abbreviations: BBB, blood–brain barrier; apoA-I, apoprotein A-I; BCEC, bovine brain endothelial cells; TEER, transendothelial electrical resistance; MEM, minimal essential medium; AcLDL, acetylated low density lipoprotein.

Table 1. The apparent affinities and maximal binding of ^{125}I-HDL to bovine cerebral endothelial cells after a 2 hr incubation at 4° and 37°, respectively*

Temperature	K_m (μg/mL)	B_{max} (ng/mg)
4° (N = 4)	10.8 ± 2.6	42 ± 9.3
37° (N = 3)	8.7 ± 3.0	177 ± 70.4

* Data are expressed as the mean ± SEM.

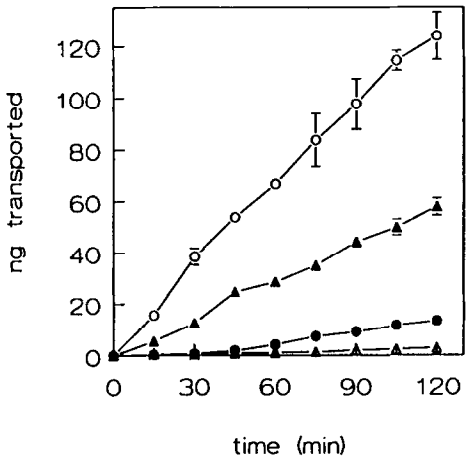

Fig. 1. Transport of various concentrations of ^{125}I-HDL across a monolayer of BCEC. Transport of 3.5 (△), 7 (●), 35 (▲), and 70 (○) μg/mL ^{125}I-HDL across monolayers of BCEC in time at 37° (N = 4).

(Table 1), which is in the same range as reported for passaged cerebral endothelial cells [8]. The mean dissociation constant (K_M) (N = 3) and the mean B_{max} after 2 hr at 37° is shown in Table 1. The difference in values of maximal binding of HDL to BCEC obtained after a 2 hr incubation at 37° and at 4° suggests that maximal binding is dependent on temperature. Similar effects of temperature on the maximal binding of apoE free HDL to cells were described by Kagami *et al.* [9]. It was suggested that the tertiary structure of HDL, important for an optimal interaction with its receptor, is temperature-dependent.

The *in vitro* cell association of apoE free HDL with bovine cerebral endothelial cells increased in a monophasic manner (r = 0.991) with time up to 45 ng/mg of cell protein after 3 hr. Cell association was not affected by the presence of chloroquine (Fig. 1). Degradation products of ^{125}I-apoE free HDL of BCEC at 37° were measured, a very low level of degradation being determined after 3 hr (Fig. 2). Degradation of ^{125}I-HDL in medium after 3 hr served as a control, with very low levels being determined. Degradation of ^{125}I-HDL by BCEC proved to occur at the same level as control incubations, even up to a concentration of ^{125}I-HDL of 70 μg/mL (data not shown). The degradation of ^{125}I-HDL by BCEC in the presence of chloroquine was not different from control level, indicating that the association of HDL with BCEC is not coupled to apoprotein degradation. In addition, BCEC incubated with fluorescent (DiI)-labelled HDL showed no uptake of the fluorescent-labelled compound, since the fluorescence appeared to be

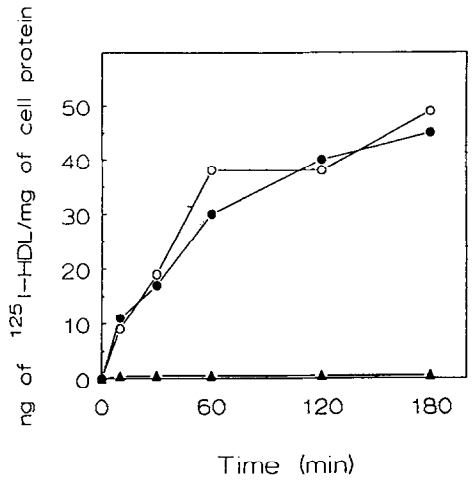

Fig. 2. Time course of the cell association of ^{125}I-HDL. Cells were incubated for indicated times with 5 μg/mL of ^{125}I-HDL and cell association determined in the absence (○) or presence (●) of chloroquine (100 μM). Control levels of degradation were determined (▲). Data are from one typical experiment out of three.

located at the endothelial cell membranes during a 2 hr incubation at 37° (data not shown).

Transport of ^{125}I-apoE free HDL across a monolayer of BCEC revealed that transport occurred in a linear fashion at the concentrations studied (r = 0.995; N = 4). Two hours after the administration of 70 μg/mL ^{125}I-HDL, 124 ng of ^{125}I-HDL (0.177% of the dose) had passed the cell monolayer (Fig. 1). *In vitro* clearance values ranged from 0.00392 ± 0.0007 μL/min (mean ± SEM) to 0.0084 ± 0.00095 μL/min (mean ± SEM). A concentration-dependent increase in the transport of ^{125}I-HDL was observed but no saturation of transport could be determined, indicating a non-receptor mediated transport. Transport of ^{125}I-apoE free HDL across a confluent monolayer of BCEC showed in time linear transport. The amounts of transported HDL were two- to three-fold higher than values obtained in studies performed in our laboratory using particles of equal size to HDL, e.g. the fluorescein dextran of 150 kDa, with a size of approximately 8 nm [6]. This difference found in transport may be explained by the more lipophilic composition of the apoE free HDL particle as compared to the more hydrophilic particle FD150. Since transport was linear with increasing concentrations of HDL, it may be assumed that HDL transport across a monolayer of BCEC *in vitro* occurs via the paracellular route and is not receptor-mediated. The transport values of HDL found in this study are comparable to those found in the *in vivo* situation, indicating that our *in vitro* model resembles the transport of HDL across the BBB *in vivo* [3]. Recently, apoA-I expression has been shown in porcine brain endothelial cell cultures [10].

Thus, HDL is transported across the BBB *in vitro* without degradation. The interaction of HDL with cerebral endothelial cells may indicate the need of the neuronal tissue for HDL, suggesting that HDL is important for cholesterol metabolism in the central nervous system.

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