Original Article

Disturbed LDL and scavenger receptor functions in monocytes from chronic haemodialysed patients

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Abstract

Background. The most frequent complication in patients with end-stage renal failure on chronic haemodialysis (HD) treatment is atherosclerosis, i.e. the different forms of heart and vascular diseases. The complete disorder of serum lipid and lipoprotein patterns is well demonstrated, whereas our knowledge about the low-density lipoprotein (LDL) and scavenger receptor expression and function are poorly understood.

Methods. In our current work, LDL and scavenger receptor expression and functions were simultaneously studied in monocytes obtained from 15 healthy male control subjects and from 11 chronic HD male patients applied with 125I-labelled LDL, isolated from healthy volunteers. To study the scavenger LDL receptors, labelled acetylated LDL (acLDL) was used.

Results. LDL binding to the monocytes of the HD-group was found to be decreased in comparison to that of the controls. As a result, the 50 µg LDL protein-induced inhibition of endogenous cholesterol synthesis was also diminished. In contrast, acLDL binding was greatly increased, though it could trigger only a low apoE synthesis. Consequently the number of cholesterol inclusions in monocytes was increased.

Conclusions. The disturbed expression and function of LDL and scavenger receptors both may play significant role in pathogenesis of cardiovascular complications in chronic HD patients. Based on our current results, it can be assumed that dysfunction of scavenger receptors is at the centre of cardiovascular complications of HD patients with renal failure.

Key words: cholesterol synthesis; foam cell; haemodialysis; LDL receptor; monocyte; scavenger receptor

Introduction

Chronic haemodialysis (HD) patients have marked cardiovascular morbidity and mortality. Furthermore, lipid and lipoprotein disorders are undoubtedly associated with cardiovascular morbidity. It is well known that many parameters of lipid and lipoprotein pattern are changed in these patients [1,2]. It is well demonstrated that the serum levels of triglyceride (TG), cholesterol, and Lp(a) are significantly increased in chronic HD patients. Nevertheless, our knowledge about the role of low-density lipoprotein (LDL) and scavenger receptors is relatively poor [3,4]. However, not only the enhanced expression of scavenger receptors has been described, but also the diminished expression of LDL receptors in lymphocytes of uraemic patients [5].

In healthy subjects, the monocytes with specific LDL receptors are able to bind LDL, thus enabling its internalization and degradation [6,7]. The most significant biological effect of LDL receptor activation, with subsequent internalization of LDL particles, is the suppression of endogenous cholesterol synthesis in cells via the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase [7]. Specific LDL receptors are present on the surface of many cells (e.g. fibroblasts, hepatocytes, lymphocytes, monocytes etc.), while scavenger receptors are expressed on macrophages or macrophage-like cells. The latter cells, via one of these scavenger receptors, through low- or high-affinity and capacity receptor pathways, are capable of incorporating modified LDL [8]. It should also be noted that LDL, under in vitro circumstances in sera of HD patients has an enhanced susceptibility to form oxidized LDL [9]. Cholesterol is then released from the incorporated and partially degraded modified LDL, which, in turn exists intracellularly in the form of cholesterol inclusions, resulting in the formation of foam cell. The effect of modified LDL in macrophages is that it stimulates the synthesis and secretion of apoE, which can enhance the cholesterol efflux from macrophages. High-density lipoprotein (HDL) molecules and
l-IDL receptors on macrophages play a significant role in the excretion of excess cholesterol [8].

In our previous study we found that monocytes obtained from healthy control subjects after a 72 h incubation in RPMI 1640 medium produced a significantly increased LDL and scavenger LDL expression [10]. However in the present experiment it was surprising to find that after just 12 h of incubation not only LDL, but also scavenger LDL receptors were present in a well-determinable density on the surface of monocytes obtained from HD patients. The aim of the present study was to examine simultaneously the expression of LDL and scavenger receptors on the monocytes obtained from control subjects and HD patients after a 12-h incubation. We also investigated the LDL-induced inhibition of HMG-Coenzyme A reductase and the acLDL-induced apoE secretion and foam-cell formation.

Subjects and methods

Patients

The study was performed on 15 healthy male subjects and 11 male patients with end-stage renal disease on maintenance HD. The patients were placed on HD therapy of 4 h sessions thrice weekly, using 1.3 m² polysulphone capillary dialyser (Fresenius 126). The Kt/V ratio was 1.10 ± 0.20. The investigated patients had no other enzyme elevation, proteinuria, diabetes mellitus, or other endocrinological disorders, were non-smokers, and took no lipid-lowering medications or antihypertensive agents that could alter lipoprotein metabolism. Each patient was involved in our long-term dialysis programme and their uraemic state developed from tubulo-interstitial nephritis because we wanted to exclude those with immunological and metabolic disorders that are known to alter lipid metabolism. The mean HD time was 60 ± 12 months. The venous fasting blood samples were always taken in the mornings. (Table 1).

Cell culture

Monocytes were isolated from the venous blood of controls and from HD patients before HD treatment. Monocytes were isolated according to the method of Boyum [11]. The mononuclear cell suspensions were placed on Nunclon Petri dishes (90 mm diameter) pre-treated with fetal calf serum, and further separated by the method of Kumagai et al. [12]. The cell suspensions were 93–96% pure for monocytes and the proportion of viable cells was 90–95%. For cell culture, RPMI 1640 (Gibco) medium containing 10% fetal calf serum was used in an ASSAB CO₂ incubator at 37 °C (CO₂ 5%, air 95%, humidity 95%). Monocyte monolayers were incubated under sterile conditions, and were washed vigorously before the experiments.

LDL isolation

LDL was obtained from the pooled sera of healthy male volunteers by KBr density gradient ultracentrifugation according to the method of Cornwell et al. [13], as modified by Szondi et al. [14]. LDL concentrations were expressed in micrograms LDL protein/ml. The acetylation of LDL was performed according to the method of Basa et al. [15].

Specific and scavenger LDL receptor activity

Both receptor activities were measured by the method of Goldstein and Brown [6]. Labelling of non-acetylated and acetylated LDL was performed according to the method of Shepherd et al. [16]. The specific activities of the preparations were 300–400 c.p.m./µg. The monocyte–monolayer containing 10⁶ cells per well (Nunclon plates with 16 wells) were incubated with 50 µg [¹²⁵I]LDL or [¹²⁵I]acetylated LDL (acLDL) in 1 ml serum free RPMI 1640 medium either with or without 300 µg non-labelled LDL or acLDL. The cell-bound radioactivities were determined after a 60-min incubation at 4 °C. The amount of intracellular degraded LDL was also measured according to the method of Brown and Goldstein [9]. Briefly, monolayers were incubated with either 50 µg [¹²⁵I]LDL or [¹²⁵I]acLDL in the presence or absence of non-labelled ligands at 37 °C for 4 h. The amount of degraded native or acLDL was quantified from the trichloroacetic acid-soluble fraction of the supernatants. This fraction was used to extract the free iodine by applying chloroform after hydrogen peroxide treatment. Values were then expressed as nanograms of LDL or acLDL protein/10⁶ monocytes. To clarify the available concentration for experiments, in all cases the [¹²⁵I]LDL was added to 10⁶ monocyte monolayers after 12-h incubation in 0, 10, 20, 30, 40, 50, 75 and 100 µg protein/ml RPMI 1640 final concentrations also in excess of cold LDL (700 µg LDL protein). After 12-h incubation in a parallel investigation [¹²⁵I]acLDL was added to the monolayers in 0, 10, 20, 30, 40, 50, 75, 100, 150 and 200 µg protein concentration to determine the available concentration. Based on these preliminary investigations the Vmax and Kₘ values were calculated. Based on the data for specific binding of [¹²⁵I]LDL and [¹²⁵I]acLDL, 50 µg/ml LDL protein concentration was used for the experiments.

In all of our experiments the monocytes were incubated for 5 days and the binding of 50 µg/ml [¹²⁵I]LDL and [¹²⁵I]acLDL was observed daily. Furthermore, the 12-h incubation period appeared to be the most optimal duration for experiments.

Endogenous cholesterol synthesis

The method was performed as described by McNamara et al. [17]. Monocytes (10 cells) in 0.6 ml Hanks’ balanced salt solution (HBSS) containing 2.5 mmol/l 2-[¹⁴C]acetate (Isotope Institute of the Hungarian Academy of Science, Budapest, Hungary) were incubated with or without 50 µg LDL for 4 h at 37 °C. At the end of the incubation, the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>HD patients</th>
</tr>
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<tbody>
<tr>
<td>Number of subjects</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4 ± 2.8</td>
<td>23.7 ± 3.4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.8 ± 5.5</td>
<td>54.2 ± 6.4</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.5 ± 0.51</td>
<td>5.68 ± 1.2</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.7 ± 0.25</td>
<td>2.9 ± 0.42</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.32 ± 0.10</td>
<td>1.05 ± 0.12</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.12 ± 0.33</td>
<td>3.50 ± 0.65</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD.
reaction was terminated with 0.5 ml 1.0 M KOH and the samples were saponified for 90 min at 70 °C. As an inner standard, 1.23-[H]cholesterol having a radioactivity of 3 × 10^4 d.p.m. was used. The unsonapified lipids were extracted with hexane, the extract was placed on aluminium oxide columns, and the steroid fraction eluted with a 1:1 mixture of acetone/diethylether. After drying, the radioactivities were counted and the values expressed as pmol [14C]acetate/h/10^6 monocytes.

**ApoE secretion**

The monocytes were vigorously washed and 1.0 ml RPMI 1640 containing 5% heat-inactivated mixed human serum plus 50 μg acLDL protein was added to the cells. After 12-h incubation at 37 °C, the supernatants were removed and concentrated using an Amicon 25 filter. The apoE concentration of the aliquots was determined using laser nephelometer (Hyland) as previously described [10,18]. Values were expressed as μg apoE/12 h/10^6 cells.

**Foam-cell formation**

The apoE producing monocyte monolayers were washed with 1-HBSS buffer. After removing the supernatant, the cells were fixed at room temperature and then washed again. The monocytes were then stained with Oil Red 0 (BDII Chemicals) for 20 min. To differentiate and remove the nonspecific Oil Red 0 stainings, 50% alcohol was used followed by three rinses with distilled water. The cholesterol inclusions were then enumerated under a light microscope by counting at least 100 to 150 cells per 400 × magnification. Data was expressed as the number of inclusions/monocyte [19].

**Statistical analysis**

For mathematical analysis the unpaired t-test with Welch’s correction, the ANOVA Student–Newman–Keuls SAS 6.12 program, and Graphpad Software Inc. Prism V. 2.0 One Side Binding (Hyperbola) program were applied.

**Results**

Figure 1 shows that the binding of labelled LDL to monocytes of healthy control subjects increased during the first 48 h of incubation, and after 48 h the binding slightly decreased until the 5th day. In contrast, in the HD group the LDL binding decreased significantly. The binding of [125I]LDL was moderately lower on the first day than in control group and that declined more markedly also from the 2nd day. The scavenger receptor expression on control cells was very low and a reasonable increase was found only from the 3rd day (Figure 2). The binding of [125I]acLDL to the HD monocytes was increased significantly on the first day of culturing and a further increase in binding was found after the 3rd day of culturing. By using the mathematical analysis of the two curves, differences between the control and HD groups were significant in the case of both LDL and scavenger receptors. The [125I]LDL and [125I]acLDL binding, intracellular degradation, and their biological consequences (cholesterol synthesis inhibition, apoE secretion, foam-cell formation) in control and HD-monocytes after 12 h of incubation are presented on Table 2. According to our data the binding and intracellular degradation of labelled LDL was decreased by HD monocytes (P < 0.001). Based on the Kd and Vmax values of LDL receptors it was found that on HD monocytes the Vmax was decreased compared to the control value, whereas
their affinity ($K_d$) to LDL was not changed. In addition, in the HD group a significant decrease in the 50 μg LDL protein/ml-induced inhibition of [14C]acetate incorporation into the cholesterol fraction of cells was detected, suggesting that either decreased LDL receptor density or functional receptor impairment are present on HD monocytes. In contrast, the acLDL binding and intracellular degradation increased extensively in monocytes from HD patients compared to the control cells. In addition, either $K_d$ or $V_{max}$ of scavenger receptors on HD-monocytes were enhanced compared to the control values. The 50 μg acLDL protein-triggered apoE release was decreased in HD monocytes compared to the release from the control cells. These findings were supported by the increase in cholesterol inclusions, i.e. foam-cell formation in the HD group.

**Discussion**

The role of LDL and scavenger receptors in disturbed lipid metabolism of uraemic and HD-treated patients is multifactorial. In the circulation of these patients the unchanged LDL level is associated with low HDL level, making the LDL/HDL ratio high [1,20]. According to the experiments of Portman et al. [5] it is known that in lymphocytes of uraemic patients not only the LDL receptor function, but also the mRNA level is decreased. In contrast, the number of scavenger receptors on monocyte–macrophages increased significantly as described by Ando et al. [21,22]. According to the authors the sera of uraemic patients can enhance scavenger receptor expression *in vitro* on U 937 cell line. However, in our experiments the monocytes were obtained from patients with HD, while the LDL and its acetylated form originated from healthy volunteers. It is known from the earlier publications of Brown et al. [6], that the LDL receptor expression increases after 12–24 h *in vitro* incubation, while at this early period of incubation, the scavenger receptor expression is low because the expression of this receptor depends on the *in vitro* monocyte–macrophage maturation. It should be noted that the CD36 (class B) scavenger receptor expression increases simultaneously within the HD group a significant decrease in the 50 μg LDL protein/ml-induced inhibition of [14C]acetate incorporation into the cholesterol fraction of cells was detected, suggesting that either decreased LDL receptor density or functional receptor impairment are present on HD monocytes. In contrast, the acLDL binding and intracellular degradation increased extensively in monocytes from HD patients compared to the control cells. In addition, either $K_d$ or $V_{max}$ of scavenger receptors on HD-monocytes were enhanced compared to the control values. The 50 μg acLDL protein-triggered apoE release was decreased in HD monocytes compared to the release from the control cells. These findings were supported by the increase in cholesterol inclusions, i.e. foam-cell formation in the HD group.

Table 2. Different effects of 50 μm/mL LDL or acLDL protein on monocytes of controls and HD patients after 12 h incubation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control ($n=15$)</th>
<th>HD ($n=11$)</th>
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</thead>
<tbody>
<tr>
<td>[125I]LDL protein binding (ng/10⁶ cells)</td>
<td>101.9 ± 12.3</td>
<td>74.3 ± 7.1*</td>
</tr>
<tr>
<td>[125I]LDL binding $V_{max}$ (ng/10⁶ cells)</td>
<td>209.7 ± 53.5</td>
<td>107.8 ± 13.6*</td>
</tr>
<tr>
<td>[125I]LDL binding $K_d$ (μg/ml)</td>
<td>58.9 ± 29.3</td>
<td>45.5 ± 12.5</td>
</tr>
<tr>
<td>[125I]LDL protein degradation (ng/10⁶ cells)</td>
<td>145.2 ± 16.8</td>
<td>98.7 ± 12.1*</td>
</tr>
<tr>
<td>[125I]acLDL protein binding (ng/10⁶ cells)</td>
<td>18.7 ± 2.2</td>
<td>96.3 ± 11.8*</td>
</tr>
<tr>
<td>[125I]acLDL binding, $V_{max}$ (ng/10⁶ cells)</td>
<td>41.9 ± 5.5</td>
<td>131.3 ± 59.6*</td>
</tr>
<tr>
<td>[125I]acLDL binding, $K_d$ (μg/ml)</td>
<td>35.7 ± 13.8</td>
<td>469.4 ± 211.2*</td>
</tr>
<tr>
<td>[125I]acLDL protein degradation (ng/10⁶ cells)</td>
<td>51.6 ± 6.7</td>
<td>187.7 ± 22.3*</td>
</tr>
<tr>
<td>Incorporation [14C] acetate pmol/h/10⁶ cells in resting monocytes</td>
<td>11.8 ± 2.1</td>
<td>12.5 ± 2.4</td>
</tr>
<tr>
<td>A Incorporation [14C]acetate (pmol/h/10⁶ cells)</td>
<td>5.8 ± 0.47</td>
<td>3.1 ± 0.38*</td>
</tr>
<tr>
<td>ApoE secretion (μg/12 h/10⁶ cells)</td>
<td>9.4 ± 1.2</td>
<td>2.8 ± 0.4*</td>
</tr>
<tr>
<td>Cholesterol inclusions/cell</td>
<td>2.8 ± 0.30</td>
<td>11.2 ± 2.3*</td>
</tr>
</tbody>
</table>

Table demonstrates the $V_{max}$ and $K_d$ values of LDL and acLDL binding calculated with Graphab Software Inc. Prism Ver 2.0. One site binding (hyperbola program) *P<0.001 (Calculated by unpaired $t$-test with Welch’s correction).
LDL had only a weak inhibiting effect on the regulation of endogenous cholesterol synthesis, which may be involved in the development of atherosclerosis. In contrast, the applied ligand for scavenger receptors \((13^{135}\text{I})\text{acLDL}\) after 12-h incubation, bound to monocytes of HD patients more intensively than to the control cells, while the apoE synthesis was decreased, together with an elevated foam-cell formation.

Finally, we assume that the impaired LDL receptor function cannot by itself be responsible for the serious atherosclerotic complications in end-stage renal failure patients on maintenance dialysis. The high degree of scavenger receptor expression supported by our data, the lowered apoE secretion, and the increased foam-cell formation can contribute significantly to the serious atherosclerotic plaque formation in patients treated with HD.

Acknowledgements. This work was supported by a grant from Hungarian OTKA (T-6098).

References


Received for publication: 19.11.98
Accepted in revised form: 31.5.99

I. Kárpáti et al.