Protective effects of high-density lipoprotein against oxidative stress are impaired in haemodialysis patients

Marion Morena1, Jean-Paul Cristol1, Thierry Dantoine2, Marie-Annette Carbonneau1, Bernard Descomps1 and Bernard Canaud3

1Department of Biochemistry, Lapeyronie Hospital, University of Montpellier, 2Department of Nephrology, Dupuytren Hospital, University of Limoges and 3Department of Nephrology, Lapeyronie Hospital, University of Montpellier, France

Abstract

Introduction. Cardiovascular diseases represent the major cause of mortality in haemodialysis (HD) patients. Oxidized low-density lipoprotein (Ox-LDL) is a major cardiovascular risk factor, implicated in atherosclerotic plaque formation. It has been suggested that high-density lipoprotein (HD) has the capacity to reduce the oxidative modifications of LDL. The aim of this study is to analyse the protective effects of HDL in HD patients.

Methods. In vitro copper-induced LDL oxidation was evaluated in 12 patients with chronic renal failure (mean age 61.0 ± 12.8 years) and compared to 25 healthy subjects (mean age 57.3 ± 19.2 years). LDL were incubated in oxygen-saturated PBS, LDL oxidation was initiated by Cu (II) in the presence and absence of HDL and assessed by measuring the absorbance (abs) increase at 234 nm due to conjugated diene formation. Duration of lag time, maximum velocity (Vmax) of lipid peroxidation, oxidation slope and half-time of maximum diene formation (T1/2) were obtained by kinetic modelling analysis.

Results. HDL (1.06 ± 0.31 vs 1.23 ± 0.39 mmol/l) and Apo AI (1.17 ± 0.39 vs 1.49 ± 0.20 g/l) levels were decreased in HD patients. In the absence of HDL, LDL obtained from HD patients showed an enhanced susceptibility to oxidation in vitro as demonstrated by the significant decrease in lag time (54.5 ± 22.2 vs 79.4 ± 37.8 min) and a significant increase in Vmax (0.026 ± 0.006 vs 0.017 ± 0.005 abs/min). In all cases, HDL (from 0.1 to 2 μM) prevented LDL oxidation in vitro; however, this effect was significantly reduced in HD patients: increase in lag time 54.2% vs 150.4% in HD vs controls; increase in T1/2 52.2% vs 124.6% in HD vs controls; decrease in Vmax 13.5% vs 38.5% in HD vs controls.

Conclusions. These results suggest that qualitative abnormalities such as an impairment of HDL-associated enzymes are associated with a decrease of HDL levels during HD. Hence, in addition to the known impairment of reverse cholesterol transport, the reduction of HDL protective capacity against oxidative stress could be involved in the development of HD-induced atherosclerosis.

Keywords: high-density lipoprotein; lipid peroxidation; low-density lipoprotein

Introduction

Cardiovascular diseases are recognized as the major cause of mortality and morbidity in haemodialysis (HD) patients [1–3]. Several atherogenic risk factors including dyslipidaemia and oxidative stress have been identified. Chronic renal failure-induced dyslipidaemia substantially contributes to the vascular complications observed in this population [4]. Lipid abnormalities are characterized by a moderate hypertriglyceridaemia and normal total cholesterol. Profound alterations of lipoprotein profile and lipoprotein composition are frequently encountered including elevated apolipoprotein B (Apo B)-containing triglyceride-rich lipoproteins, very low-density lipoprotein (VLDL) and intermediate density lipoprotein IDL) low levels of plasma apolipoprotein AI (Apo AI) and high-density lipoprotein (HDL) affecting the reverse cholesterol transport [5,6]. In addition to these quantitative alterations, an increase in small, dense low-density lipoprotein (LDL) subfractions has been recently been found [7,8]. In non-uraemic patients, these small dense LDL, which are derived from triglyceride-rich lipoproteins, present an increased susceptibility to oxidation [9].

An oxidative stress due to overproduction of reactive oxygen species (ROS) by activated monocytes and impairment in antioxidant defence mechanisms [10–12] has been observed in HD patients [13,14] and may contribute to the accelerated HD-induced atherogenesis by oxidatively modified-proteins and lipids. Indeed, vulnerability of proteins to ROS results in the oxidation of amino acid residues leading to advanced oxidation.
protein products (AOPP) [15,16]. Such compounds have been recently shown to act as mediators of oxidative stress and monocyte activation [17]. Furthermore, it is interesting to note that such phenomena also pre-exists to dialysis treatment [18,19], occurring early in the course of the chronic renal failure and growing with its progression to end-stage renal disease [20]. Oxidized LDL also plays a major role in the development and progression of atherosclerotic lesions [21,22]. In addition to its inducing endothelial dysfunction, oxidized LDL exhibits proinflammatory actions, oxidized LDL exhibits chemotactic effects, expression of macrophage colony-stimulating factors, and adhesive molecules. Finally, oxidized LDL is trapped by the monocyte/macrophage scavenging receptors, leading in turn to monocyte activation [23].

The resulting generation of myeloperoxidase-dependent chlorinated oxidant products is now recognized as a pivotal role in atherogenesis [17]. The presence of oxidized LDL, deriving from lipid abnormalities and oxidative stress, is strongly supported by the increase in anti-oxidized LDL antibodies in plasma of HD patients [24]. However, ex vivo LDL oxidability data are reported as normal in some studies [25–27] or increased in others [24,28].

Recently, HDL have also been demonstrated to potentially reduce oxidative modifications of LDL [29,30]. The prevention of lipoperoxide generation during copper-induced LDL oxidation by HDL could be due to their enzyme content, such as paraoxonase [29] which has been recently reported to be decreased in uraemic patients [31–33]. Clearly, HD-induced LDL oxidative modification is a multifactorial process including (i) qualitative abnormalities of LDL, (ii) oxidative stress and (iii) decrease in HDL protection against LDL oxidation. The present study was designed to investigate the LDL susceptibility to oxidation and the potential antioxidant activity of HDL in HD patients, using copper-induced LDL oxidation as an ex vivo model of lipid peroxidation.

**Subjects and methods**

**Patients**

Twelve chronic HD patients (mean age 61.0 ± 12.8 years) on renal replacement therapy for at least 1 year and 25 normo-

**Table 1.** Characteristics of controls and HD subjects. Twelve chronic HD patients on renal replacement therapy (on line post-dilutional haemodialfiltration) for at least 1 year were compared to 25 normolipidaemic subjects free of lipid-lowering drugs or antioxidant supplementation. Values are expressed as mean ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 25)</th>
<th>HD (n = 12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>16 M/9 F</td>
<td>8 M/4 F</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.3 ± 19.2</td>
<td>61.0 ± 12.8</td>
<td>NS</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>67.7 ± 14.4</td>
<td>63.8 ± 11.6</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.0 ± 5.0</td>
<td>23.8 ± 4.8</td>
<td>NS</td>
</tr>
<tr>
<td>Current smokers (&gt;10 cigarettes/day)</td>
<td>5/25</td>
<td>2/12</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of HD (months)</td>
<td>Not applicable</td>
<td>108.0 ± 129.8</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Plasma lipid profile of controls and HD patients. TG and TC levels were measured by routine enzymatic methods. HDL concentrations were obtained from plasma after precipitation of Apo B-containing lipoproteins by magnesium phosphotungstate. Plasma Apo AI and Apo B were analysed by immunonephelometric assay using a Behring Nephelemaneter BN 100. Plasma vitamin E was determined using HPLC procedure. Values are expressed as mean ± standard error.

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Control</th>
<th>HD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol total (mM)</td>
<td>5.53 ± 0.93</td>
<td>4.53 ± 0.91</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TG (mM)</td>
<td>1.24 ± 0.54</td>
<td>1.48 ± 0.98</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mM)</td>
<td>1.23 ± 0.39</td>
<td>1.06 ± 0.31</td>
<td>NS</td>
</tr>
<tr>
<td>Apo AI (g/l)</td>
<td>1.49 ± 0.20</td>
<td>1.17 ± 0.39</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Apo B (g/l)</td>
<td>1.08 ± 0.20</td>
<td>1.02 ± 0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Vit E (µM)</td>
<td>29.71 ± 6.66</td>
<td>32.30 ± 3.77</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 3. Lipid composition of LDL and HDL in controls and HD patients. LDL and HDL were prepared by sequential ultracentrifugation from plasma. The Apo B and Apo AI content of LDL and HDL respectively was measured by immunonephelometric method using a Behring Nephelemaneter BN 100. TG and TC levels were measured by routine enzymatic methods. Values are expressed as mean ± standard error.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Control</th>
<th>HD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol total (mM)</td>
<td>10.63 ± 3.39</td>
<td>11.9 ± 3.06</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mM)</td>
<td>0.95 ± 0.43</td>
<td>1.96 ± 0.99</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Apo B (g/l)</td>
<td>2.66 ± 0.98</td>
<td>3.23 ± 0.86</td>
<td>NS</td>
</tr>
<tr>
<td>TG/Apo B</td>
<td>0.32 ± 0.07</td>
<td>0.52 ± 0.15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cholesterol/Apo B</td>
<td>1.59 ± 0.16</td>
<td>1.45 ± 0.13</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Ex vivo LDL oxidation in presence or absence of HDL

In order to remove EDTA added during the isolation procedure, LDL and HDL were dialysed thrice for 24 h against a PBS-DTPA buffer (pH 7.4) at 4°C in the dark under continuous agitation.

EDTA-free LDL (0.1 µM Apo B) were incubated in oxygen-saturated PBS (37°C, pH 7.4). LDL oxidation was induced with copper (Cu(II)) (CuCl₂:5 µM) in the presence of increasing concentrations of HDL obtained from the same subject (0; 0.1; 0.5; 0.75; 1; 2 µM Apo AI).

Continuous measurement of the conjugated diene formation

Oxidation of LDL was monitored continuously, during 400 min, according to the method of Esterbauer et al. [37], by measuring the increase in absorbance (abs) at 234 nm due to the conjugated diene formation. Absorbance was automatically recorded at 4-min intervals with a spectrophotometer Uvikon 930 (Kontron Instruments, Montigny le Bretonneux, France). Copper-induced oxidation curves were classically characterized by three different phases: a lag phase corresponding to the consumption of protective endogenous antioxidants, a propagation phase corresponding to the radical-propagated chain reaction, and a decomposition phase [36].

The absorption data were collected in a personal computer (Vectra V.E., Hewlett Packard) and then used to plot the diene curve. Lag time, maximal propagation rate (Vₘₐₓ), of time (dA/dt)), half-time of maximum diene formation (T₁/₂)
and slope of the propagation phase were then determined according to a kinetic modelling (Excel 5, Microsoft) [38].

**Presentation of results and statistical analysis**

Results were expressed as mean values ± standard error. Differences between controls and HD patients were analysed by a Student’s t test. Values were considered statistically significant when \( P < 0.05 \).

**Results**

**Plasma lipid profile**

Plasma lipid profile of controls and HD patients is reported in Table 2. There is a tendency to higher triglyceride levels and lower HDL-cholesterol in HD patients when compared to control subjects, but the difference is not statistically significant, while no difference in the concentration of Apo B is observed. In addition, Apo AI level is significantly decreased in this same population.

**Lipoprotein characteristics**

Lipid composition of LDL and HDL is summarized in Table 3. Triglyceride load in LDL and HDL is higher in HD patients as shown by the significant increase in TG/Apo B and TG/Apo AI ratio respectively.

**Enhanced susceptibility of LDL against copper-induced oxidation in HD patients**

The kinetic parameters of LDL peroxidation, reported in Figure 1 A–D, demonstrate an enhanced susceptibility of HD LDL to *ex vivo* oxidation. All parameters are significantly affected. The lag time and the \( T_1/2 \) of LDL oxidation obtained from HD patients are significantly lower than those observed in control subjects (lag time \( 54.5 \pm 22.2 \) vs \( 79.4 \pm 37.8 \) min and \( T_1/2 \) \( 74.2 \pm 26.0 \) vs \( 111.3 \pm 39.4 \) min). Similarly, slope and maximum velocity (\( V_{\text{max}} \)) of oxidation are significantly higher in HD patients (slope \( 0.025 \pm 0.006 \) abs/min vs \( 0.016 \pm 0.005 \) abs/min and \( V_{\text{max}} \) \( 0.026 \pm 0.006 \) abs/min vs \( 0.017 \pm 0.005 \) abs/min).

**Protective effects of HDL against LDL oxidation are impaired in HD patients**

As shown in Figure 2A, HDL from healthy volunteers protect LDL against copper-induced oxidation in a dose-dependent way (0.1–2 \( \mu \)M). This effect is sensitively observed from 0.5 \( \mu \)M in HDL and leads to a total inhibition at the dose of 2 \( \mu \)M. The protective effect of HDL against LDL oxidation observed in the control group is reduced in HD patients (Figure 2B). This protective effect of HDL virtually disappears in some patients.

In order to quantify the protective effect, results are expressed as relative variations between isolated LDL and LDL plus HDL (1 \( \mu \)M) for each parameter (Figure 3). The presence of 1 \( \mu \)M HDL concentration often leads to an important inhibition against lipid peroxidation in control subjects (>50%). In all cases, HDL prevents LDL against copper-induced oxidation; however, this effect is significantly reduced in HD patients: decrease in \( V_{\text{max}} \), 13.5 in HD vs 38.5% in controls (Figure 3A), increase in lag time, 54.2% in HD vs 124.6% in controls (Figure 3B); increase in \( T_1/2 \) 52.2% in HD vs 124.6% in controls.

**Discussion**

Our study confirms that HD patients are exposed to several atherogenic factors resulting from quantitative and functional lipid abnormalities including triglyceride-rich lipoparticles, increased susceptibility to LDL
HDL effects against oxidative stress impaired in HD patients

Fig. 3. Relative variations of vmax and lag time induced by HDL for HD patients and controls. Results are expressed as relative variations between isolated LDL and LDL plus HDL (1 μM) (mean values ± standard error) in order to quantify the protective effect of HDL.

oxidation and finally impairment of HDL protective effects.

High triglyceride concentration is the main lipid abnormality observed in HD patients. This hypertriglyceridaemia results from a reduced lipolysis of TG-rich VLDL [39,40] that leads to the accumulation of partially metabolized ‘remnant’ lipoproteins (IDL and TG-rich LDL). This lipoprotein catabolism impairment is usually associated with reduced levels of HDL [41] affecting reverse cholesterol transport [6]. It is now well known that such defect in atherogenic lipoproteins catabolism may predispose to the formation of small, dense LDL particles [42,43] which appeared to be more sensitive to ex vivo oxidation. Consequently, a lipid profile containing predominantly small, dense lipoproteins bears a higher risk of coronary artery disease in non-uremic populations [9,42]. The presence of higher content of TG in LDL obtained from HD patients is in agreement with the occurrence of small, dense LDL subfraction in end-stage renal failure as recently reported by O’Neal et al. [8] and Rajman et al. [42]. Despite the occurrence of small, dense LDL subfraction, data concerning oxidability of LDL in HD patients remained still a subject of debate. Sutherland et al. [25] have reported no difference in lag time and a lower maximal oxidation rate, lower concentrations of conjugated dienes being formed in HD patients compared with healthy controls. However, these parameters could be influenced by the LDL content in polyunsaturated fatty acids. Similarly, Loughrey et al. [26] and Westhuysen et al. [27] did not observe any modification of LDL susceptibility to ex vivo oxidation in HD patients. In contrast, our study shows an enhanced susceptibility of LDL to copper-induced oxidation in HD patients. Indeed, the lag time and the $T_{1/2}$ are significantly decreased in this population. Similarly, the $V_{\text{max}}$ and the slope of oxidation are significantly increased. In the absence of reduced vitamin E levels in plasma obtained from HD patients, the weaker resistance to copper oxidation could be due to the occurrence of small, dense LDL rather than in a defect of vitamin E LDL loading [44]. These findings are in total agreement with data from Maggi et al. [24] which showed that uremic LDL appeared more susceptible to ex vivo and in vivo oxidation (as demonstrated by a higher anti-oxidized LDL antibody/oxidized LDL ratio) while vitamin E content of LDL did not differ between the two groups. Thus, lipid abnormalities including hypertriglyceridaemia, presence of small, dense LDL and enhanced LDL susceptibility to oxidation contribute synergistically to the atherogenic profile of HD patients.

Several arguments have suggested a protective role of HDL against LDL oxidative modifications. Klimov et al. [45] have reported a protective effect of HDL to be more sensitive to ex vivo oxidation. Consequently, a lipid profile containing predominantly small, dense LDL subfraction, data concerning oxidability of LDL in HD patients remained still a subject of debate. Sutherland et al. [25] have reported no difference in lag time and a lower maximal oxidation rate, lower concentrations of conjugated dienes being formed in HD patients compared with healthy controls. However, these parameters could be influenced by the LDL content in polyunsaturated fatty acids. Similarly, Loughrey et al. [26] and Westhuysen et al. [27] did not observe any modification of LDL susceptibility to ex vivo oxidation in HD patients. In contrast, our study shows an enhanced susceptibility of LDL to copper-induced oxidation in HD patients. Indeed, the lag time and the $T_{1/2}$ are significantly decreased in this population. Similarly, the $V_{\text{max}}$ and the slope of oxidation are significantly increased. In the absence of reduced vitamin E levels in plasma obtained from HD patients, the weaker resistance to copper oxidation could be due to the occurrence of small, dense LDL rather than in a defect of vitamin E LDL loading [44]. These findings are in total agreement with data from Maggi et al. [24] which showed that uremic LDL appeared more susceptible to ex vivo and in vivo oxidation (as demonstrated by a higher anti-oxidized LDL antibody/oxidized LDL ratio) while vitamin E content of LDL did not differ between the two groups. Thus, lipid abnormalities including hypertriglyceridaemia, presence of small, dense LDL and enhanced LDL susceptibility to oxidation contribute synergistically to the atherogenic profile of HD patients.

Several arguments have suggested a protective role of HDL against LDL oxidative modifications. Klimov et al. [45] have reported a protective effect of HDL dependent on incubation time and HDL concentration. In the present work, this protective and dose-dependent action of HDL against LDL oxidability by transition metals was also observed in normal subjects. However, the lack of HDL effect in preventing conjugated diene formation observed in HD patients suggests that HDL are both quantitatively modified and functionally impaired in HD patients. Several enzymes such as lecithin cholesterol acyltransferase (LCAT), protease, phospholipase, platelet-activating factor acetylhydrolase, and paraoxonase are associated in the HDL lipoprotein complex [46]. Mackness et al. demonstrated that HDL-associated paraoxonase, a component of HDL, might contribute to its protective action against LDL oxidation [47] and prevent accumulation of lipoperoxides in LDL [29]. Interestingly, reduced serum paraoxonase activity was also reported in a population with high risk of coronary heart disease such as diabetes and hereditary hypercholesterolaemia [48]. Recent reports strongly support an impairment of paraoxonase activity [6,31–33] but not of platelet-activating factor acetylhydrolase activity [6] in HDL of HD patients. Accordingly, the absence of protective activity of HDL against LDL oxidation might be related to the impairment of HDL-associated enzymes and especially paraoxonase. This hypothesis deserves further study.
In conclusion, this study shows a decreased HDL antioxidant capacity of HD patients that may contribute to the accelerated development of atherosclerosis in HD patients. This antioxidant defect could work in concert with the known impairment of reverse cholesterol transport stressing the implication of quantitative and functional HDL abnormalities in HD-related atherosclerosis. Impairment of HDL metabolism necessitates further analysis with respect to the recognized Apo B-containing lipoprotein abnormalities including occurrence of small, dense LDL and enhanced susceptibility to ex vivo oxidation. LDL and HDL quantitative and functional abnormalities might be taken into account for future prophylactic approaches against HD-induced atherosclerosis.

References


47. Mackness MI, Arrol S, Abbott C, Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis* 1993; 104: 129–135


Note added in proof

Since submission of this report, Hasselwander et al. (March 1999) compared the HDL inhibitory effect on copper-induced LDL oxidation in HD and control groups. In their work, HDL isolated from patients on HD have the same potential to inhibit copper-induced LDL oxidation as healthy control subjects. The differences observed between the two studies about HDL antioxidant capacity could be related either to lipoproteins purification or Apo AI/Apo B ratio which is 70 vs 1–20 in our study.


Received for publication: 8.3.99
Accepted in revised form: 11.10.99