Preliminary Communication

Influence of low molecular weight heparin compared to conventional heparin for anticoagulation during haemodialysis on low density lipoprotein subclasses

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Abstract

Background. In haemodialysis (HD) patients, low density lipoprotein (LDL) particle distribution is characterized by a higher proportion of more atherogenic dense LDL. Though clinical studies showed favourable effects of low molecular weight (LMW) heparin compared to standard heparin on triglycerides (TG) and cholesterol (CH) in HD patients with hypertriglyceridaemia, it is not known if LMW heparin influences LDL subfraction pattern. Thus, the aim of this pilot study was to investigate if a switch to LMW heparin influences LDL subfractions and apolipoproteins.

Methods. Ten outpatients with fasting TG >230 mg/dl in the chronic HD programme on heparin for anticoagulation (AC) were switched to dalteparin (80 IU/kg body weight as a bolus). Blood samples were drawn for CH, TG, LDL-CH, HDL-CH, apolipoproteins (apo), very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), and LDL subclasses at the beginning and after 12 months of therapy. Lipoproteins were isolated by preparative ultracentrifugation. Total LDL were fractionated into six density classes by equilibrium density gradient ultracentrifugation [(density in kg/l): LDL-1 1.019–1.031, LDL-2 1.031–1.034, LDL-3 1.034–1.037, LDL-4 1.037–1.040, LDL-5 1.040–1.044, LDL-6 1.045–1.063]. CH and TG were determined enzymatically, apolipoproteins by turbidimetry.

Results. In eight patients suitable for evaluation cholesterol decreased from 241 to 202 (P <0.05) and TG from 557 to 278 mg/dl (P <0.01), whereas LDL-CH and HDL-CH did not change significantly. A 28.2% decrease of VLDL (P <0.01) and a 19.3% decrease of IDL (P <0.05) paralleled by a significant drop of apoB were observed. Buoyant LDL subclasses increased (LDL-2, +34.3% and LDL-3, +20.3%) whereas dense LDL (LDL-5, −13.4% and LDL-6, −33.1%) decreased (P <0.05 for LDL-6). The ratio of buoyant LDL to dense LDL increased from 0.46±0.28 to 0.72±0.33 (P <0.05).

Conclusion. In hypertriglyceridaemic HD patients, dalteparin improved metabolism of TG-rich lipoproteins, increased buoyant LDL and decreased potentially atherogenic dense LDL. Preservation of lipoprotein lipase by LMW heparin may be a possible mechanism to explain our findings.

Keywords: atherogenic lipoprotein-phenotype; haemodialysis; LDL-subfractions; low molecular weight heparin

Introduction

The annual cardiovascular disease mortality in end-stage renal disease (ESRD)-patients on haemodialysis (HD) is 9.5%, which is 35 times higher than in the general population in the US [1]. The characteristic perturbations in lipoprotein metabolism are considered

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>53.3 ± 9.8</td>
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<tr>
<td>HD time/week (h)</td>
<td>12.3 ± 2.2</td>
</tr>
<tr>
<td>Previous HD-therapy (months)</td>
<td>37.6 ± 27.5</td>
</tr>
<tr>
<td>Dry body weight (kg)</td>
<td>81.5 ± 13.0</td>
</tr>
<tr>
<td>Serum-creatinine (mg/dl)</td>
<td>10.0 ± 2.9</td>
</tr>
<tr>
<td>Serum-phosphate (mg/dl)</td>
<td>4.7 ± 1.2</td>
</tr>
<tr>
<td>Total heparin dose/HD before switch to dalteparin (U)</td>
<td>5500 ± 1066.2</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>3/5</td>
</tr>
</tbody>
</table>

Values are means ± SD.
as a risk factor for high cardiovascular mortality rates in these patients [2]. Common dyslipidaemic changes are increases in triglycerides, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL) and remnant lipoproteins, increases in lipoprotein-(a) and decreased high density lipoprotein (HDL) cholesterol [3]. Plasma low density lipoprotein (LDL) cholesterol is usually not elevated in HD patients [4]. However, like other lipoproteins LDL are not homogeneous, but represent a mixture of particles varying in terms of size, density and composition. Using density-gradient ultracentrifugation LDL can be separated

Fig. 1. Intraindividual changes of (A) triglycerides, (B) cholesterol, (C) LDL-cholesterol (LDL-CH), (D) ratio LDL1 + 2/5+6, and (E) HDL-cholesterol (HDL-CH) before and during 12 months of therapy with dalteparin.
into six specific LDL subfractions. An altered LDL particle size distribution with a shift towards smaller LDL with higher density (so-called dense LDL, density range 1.044–1.063 g/ml) was shown in ESRD patients treated with HD or peritoneal dialysis [5,6]. Dense LDL exhibit a lower affinity to the LDL receptor [7], have a longer plasma half-life, show an increased susceptibility to oxidative modification [8], and have a higher ability to penetrate the vascular intima [9]. Patients who reveal a lipoprotein pattern with a predominance of dense LDL are at increased risk for coronary artery disease [10]. The association between elevated plasma triglycerides, dense LDL and decreased HDL cholesterol is referred to as atherogenic lipoprotein phenotype (ALP) and is a common feature of dyslipidaemia in ESRD patients.

The primary cause for the accumulation of triglyceride-enriched lipoproteins in patients on HD is a defective catabolism of triglyceride-rich lipoproteins by lipoprotein lipase (LPL) and hepatic lipase (HL). LPL activity in post-heparin plasma of uraemic patients was found to be lower than in healthy individuals [11]. Reasons may be a reduced enzyme synthesis by the parenchymal cell caused by peripheral insulin resistance and secondary hyperparathyroidism, which both down-regulate synthesis of LPL, or a circulating inhibitor of the enzyme [11]. Although to date the exact mechanisms are not clear in detail, in ESRD patients, decreased LPL activity may largely contribute to the generation of atherogenic dense LDL [12]. This is underlined by the fact that LPL deficiency is a genetic risk factor for the development of coronary heart disease [13]. Activity of HL, however, is inversely correlated to LDL particle size [14].

Heparin, which HD patients receive for anticoagulation of the extracorporeal circuit, releases LPL attached to the heparan sulphate coating of the endothelial surface. Repeated administration of heparin for anticoagulation during HD causes depletion of LPL and may exhaust lipolytic capacity. Low molecular weight (LMW) heparin releases LPL from the vessel wall to a lesser extent [11]. As a result, lipolytic potential increases gradually over months, if patients are switched from unfractionated to LMW heparin [15].

Though low LPL is a risk factor for the accumulation of triglycerides and dense LDL, to date no data exist as to whether LMW heparin influences the profile of LDL subfractions in HD patients. Thus, the primary aim of our study was to clarify whether a switch from conventional heparin to LMW heparin in HD patients may beneficially change the distribution of LDL subclasses.

### Patients and methods

#### Patient characteristics

Ten hyperlipidaemic patients (6 men, 4 women) with fasting triglycerides > 230 mg/dl on chronic haemodialysis, who received standard heparin were switched to the LMW dalteparin as anticoagulant during haemodialysis. All patients were outpatients and in a stable condition. Subjects were free of acute medical illness at the time when blood samples were drawn. One patient had refused to give informed consent and one patient had received a statin during the course of the study. The remaining eight patients were considered suitable for further evaluation. Two patients suffered from diabetes mellitus, one was being treated with insulin. One patient had overt coronary heart disease, two patients had a history of peripheral artery disease, two had a history of stroke, and none had overt heart failure. All participants were prescribed a normocaloric diet and low water and potassium intake. Patients received medication including erythropoietin, calcium-containing phosphate binders, multivitamins, ACE inhibitors, angiotensin II receptor antagonists and/or calcium antagonists. Concomitant lipid-modifying therapy (one patient under statin treatment) was not changed during the duration of the study. Patients received no anabolic steroids, thyroid hormones, testosterone, oestrogen, progestogen therapy or phosphate-binding drugs with lipid-lowering effects such as sevelamer (Renagel). This study was approved by the local ethics review committee. Informed consent was obtained from all subjects before the beginning of the study.

#### Table 3. Apolipoproteins in haemodialysis patients treated with standard heparin 1 year after switch to dalteparin therapy

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>Begin (mg/dl)</th>
<th>End (mg/dl)</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoA-I</td>
<td>122.1 ± 37.1</td>
<td>118.4 ± 19.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>40.4 ± 8.7</td>
<td>32.7 ± 5.2</td>
<td>0.016</td>
</tr>
<tr>
<td>ApoB</td>
<td>129.3 ± 23.9</td>
<td>114.9 ± 23.7</td>
<td>0.039</td>
</tr>
<tr>
<td>ApoC-II</td>
<td>9.3 ± 5.0</td>
<td>5.7 ± 2.9</td>
<td>0.023</td>
</tr>
<tr>
<td>ApoC-III</td>
<td>34.5 ± 11.1</td>
<td>25.3 ± 8.9</td>
<td>0.023</td>
</tr>
<tr>
<td>ApoC-II/III</td>
<td>0.26 ± 0.06</td>
<td>0.21 ± 0.04</td>
<td>n.s.</td>
</tr>
<tr>
<td>ApoE</td>
<td>13.0 ± 5.3</td>
<td>9.0 ± 2.3</td>
<td>0.008</td>
</tr>
</tbody>
</table>

<sup>a</sup>P values vs beginning.

#### Table 2. Lipid profile of haemodialysis patients treated with standard heparin and 1 year after switch to dalteparin therapy

<table>
<thead>
<tr>
<th></th>
<th>CH (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>Ratio LDL 1+2dLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Begin</td>
<td>241 ± 53</td>
<td>557 ± 385</td>
<td>79.5 ± 31.3</td>
<td>30.3 ± 12.5</td>
<td>0.46 ± 0.28</td>
</tr>
<tr>
<td>End</td>
<td>202 ± 39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>278 ± 167&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.4 ± 29.1</td>
<td>31.0 ± 7.8</td>
<td>0.72 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>P<0.05 vs beginning; <sup>b</sup>P<0.01 vs beginning.
Fig. 2. Concentrations of apoB in VLDL, IDL, and the LDL subfractions before and during twelve months of therapy with dalteparin. Solid squares, before dalteparin therapy; open circles, after 12 months of dalteparin therapy. *$P<0.05$ vs beginning; **$P<0.01$ vs beginning.

Fig. 3.
Haemodialysis

Patients underwent 3.5–5.0 h of chronic maintenance haemodialysis three times (n = 7) or twice (n = 1) per week using polysulphone high-flux dialysers (Fresenius F 60 S; Fresenius, Bad Homburg, Germany) during the entire study. Patients had been on HD for 8–89 months. Water produced by reverse osmosis, and concentrate were distributed by distinct loops. The dialysate routinely used contained bicarbonate (32.5 mmol/l) and blood flow ranged from 200 to 300 ml/min. Before the change to dalteparin, anticoagulation was performed using a heparin bolus (1500–2500 IE) followed by continuous heparin administration (500–1000 IE/h). After the change to dalteparin, patients received 80 anti-Xa U/kg body weight as a bolus at the beginning of the dialysis procedure.

Blood samples and lipoprotein separation

After an overnight fast, 20 ml of blood was drawn for lipoprotein analysis before and after 12 months after the switch to dalteparin. Lipoproteins were isolated by sequential preparative ultracentrifugation using the following densities: density < 1.006 kg/l for VLDL, density between 1.006 and 1.019 for IDL, density between 1.019 and 1.063 kg/l for LDL, and density between 1.063 and 1.21 for HDL [16]. LDL subfractions were separated according to the method of Baumstark et al. [17]. Total LDL (density 1.019–1.063 kg/l) were fractionated into six density classes by equilibrium density gradient centrifugation. Density ranges of the subfractions were: LDL-1, < 1.031 kg/l; LDL-2, 1.031–1.034 kg/l; LDL-3, 1.034–1.037 kg/l; LDL-4, 1.037–1.040 kg/l; LDL-5, 1.040–1.044 kg/l; LDL-6, > 1.044 kg/l. All centrifugation steps were carried out at 18°C, using partly filled polycarbonate bottles (6 ml) in a 50-Ti rotor. Variability in LDL subfractions with respect to nutritional status had been assessed in five probands sampled after an overnight fast, 3 h after breakfast, and 3 h after lunch [18]. The average of the intra-individual coefficients of variance (CVs) of each individual for apolipoprotein B (apoB) were 9.6 and 10.2% for VLDL and IDL, and 4.4, 5.0, 4.2, 2.5, 3.6, and 3.2 for LDL 1 to LDL 6 respectively.

Lipoprotein chemistry

Cholesterol (CH), free cholesterol, triglycerides (TG), and phospholipids (PL) were determined enzymatically with the CHOD-PAP, GPO-PAP, and PLD-PAP methods (Roche Diagnostics, Mannheim, Germany) respectively. The concentration of esterified cholesterol (CE) was calculated as the difference between total cholesterol and free cholesterol.

Fig. 3. Intrainsdividual changes of apoB in (A) VLDL, (B) IDL, and (C–H) LDL subclasses before and during 12 months of dalteparin therapy.
Concentrations of apo were determined by turbidimetry on a Wako 30R analyser (Wako Chemicals, Tokyo, Japan) using specific polyclonal antisera (Rolf Greiner Biochemica, Frickenhausen, Germany) for the respective antigen. The inter-assay CVs ranged between 1.0 and 6.6% for the lipid measurements and between 2.4 and 8.0% for the apolipoprotein measurements respectively.

Statistical analysis

Results were expressed as mean ± SD for each value. For statistical analysis the mathematical software BIAS v. 7.04 (Dr Hanns Ackermann, Department of Biomathematics, University of Frankfurt/Main, Germany) was used. Differences between different parameters were tested for significance using the non-parametric, Wilcoxon matched pairs test. Differences between groups were considered significant at \( P < 0.05 \).

Results

Patient characteristics at baseline are summarized in Table 1. At the end of the study, body weight was 79.85 ± 15.36 kg (n.s. vs beginning), creatinine was 9.63 ± 2.29 mg/dl (n.s. vs beginning), and phosphate was 6.0 ± 1.58 mg/dl (n.s. vs beginning).

Significant decreases in triglycerides and total cholesterol were observed 1 year after switch of anticoagulation for extracorporeal circuit with dalteparin (Figure 1A and B). A slight, but non-significant increase, was seen for LDL cholesterol (Figure 1C). However, the ratio of buoyant LDL (LDL-1 + LDL-2) to the dense LDL fractions LDL-5 + LDL-6 (dLDL) shifted in favour of the large buoyant LDL subfraction after therapy with dalteparin (Figure 1D). HDL cholesterol did not change significantly (Figure 1E) (Table 2).

Decreases in total cholesterol and triglycerides paralleled a significant drop in apoB concentrations, the major apolipoprotein constituent of VLDL, IDL and LDL. ApoA-I and apoA-II concentrations decreased, but only the decrease of apoA-II was significant. Both apoC-II and apoC-III concentrations decreased significantly, whereas no significant change in the apoC-II/apoC-III ratio was observed. ApoE concentration was reduced 1 year after therapy with dalteparin (Table 3). Lipoprotein (a) rose non-significantly from 21.8 ± 19.1 to 32.6 ± 26.1 mg/dl.

The density distribution of apoB-100-containing lipoproteins with standard heparin treatment at the beginning of the study compared with that after 1 year of therapy with dalteparin is shown in Figure 2. Each apoB-100-containing lipoprotein has only one apoB-100 molecule. Thus, the concentration of apoB-100 in each density fraction reflects the number of lipoprotein particles present in this particular fraction. Compared to the therapy with conventional heparin, after anticoagulation with dalteparin a 28.2% decrease of VLDL (\( P < 0.01 \)), and a 19.3% decrease of IDL (\( P < 0.05 \)) was observed. LDL subclasses with lower density were significantly raised (LDL-2, +34.3% and LDL-3, +20.3%, \( P < 0.05 \) and \( P < 0.01 \) respectively) whereas dense LDL decreased (LDL-5, −13.4% and LDL-6, −33.1%, \( P < 0.05 \) for LDL-6). Intraindividual changes of VLDL, IDL and LDL-subclasses are shown in Figure 3.

Discussion

In this study, we found significant decreases in the mean concentrations of triglycerides and total cholesterol 1 year after switching anticoagulation of extracorporeal circuit in haemodialysis patients from conventional heparin to the LMW-heparin dalteparin. This is consistent with other clinical studies showing a favourable effect of LMW heparin compared to standard heparin on triglycerides and cholesterol in hypertriglyceridaemic HD subjects [19,20]. However, it should be noted that this effect did not occur with all preparations of LMW heparin that were used, nor was it seen in normolipidaemic patients on HD. Notably, heparin preparations with a higher percentage of relatively large heparin molecules do not appear to have this effect [21,22].

Beyond the previously published data on the effects of different heparins on lipid metabolism, this study for the first time explores the changes in the distribution of LDL subclasses after therapy with dalteparin. Though total LDL cholesterol concentration was not significantly changed by dalteparin therapy, VLDL and IDL were decreased, and the more buoyant LDL subfractions LDL-2 and LDL-3 were raised, whereas dense LDL particles decreased (Figure 2). The decrease of VLDL was paralleled by lower concentrations of the major apolipoprotein constituent of VLDL, apoB, and by decreasing apoE concentrations.

What might be the mechanism for the observed changes of LDL subclass distribution after a switch from standard heparin to dalteparin therapy? Lipoprotein and hepatic lipase have structural homologies, and are probably evolved from a common ancestor [23]. Both enzymes are complementarily involved in the degradation of triglyceride-rich lipoproteins. As the conversion of VLDL to LDL is completely inhibited if lipoprotein lipase activity is blocked in the monkey [24], IDL and LDL-1 may be products of a lipoprotein lipase-mediated catalysis of triglyceride-rich precursors. HL, in contrast, appears to be involved in the conversion of triglyceride-enriched particles to smaller LDL with higher density [11], as buoyant, triglyceride-rich LDL particles accumulate in patients with hepatic lipase deficiency [25] and after inhibition of HL [26]. Therefore, higher LPL activities result in a more effective degradation of triglyceride-rich lipoproteins to buoyant and medium-dense LDL, and reduce the substrate for lipolysis by HL, resulting in a decrease of dense LDL (Figure 4).

It is of interest that the distribution of LDL...
In conclusion we showed for the first time that in HD patients with marked hypertriglyceridaemia, not only triglycerides and cholesterol but also dense LDL as well as HDL cholesterol was not seen after therapy with dalteparin instead of standard heparin. It may be hypothesized that the decrease in apoC-III, which, in contrast to apoC-II, is an inhibitor of LPL, increases LPL activity. However, as the ratio apoC-II/III did not rise significantly, this is not very likely. Rather the increase in lipoprotein lipase activity by 'endothelial preservation' of LPL after dalteparin therapy appears to be responsible for the favourable shift of the LDL subfraction profile towards buoyant LDL and less dense LDL.

In conclusion we showed for the first time that in HD patients with marked hypertriglyceridaemia, not only triglycerides and cholesterol but also dense LDL decrease after a switch from standard heparin to dalteparin. As patients with elevated dense LDL have a significant higher risk of atherosclerotic disease, our findings may indicate beneficial clinical effects of LMW heparin with regard to cardiovascular disease in haemodialysis patients. Limitations of this pilot study may be the relatively small study population, including also patients with diabetes mellitus, as well as the lack of a control group. As all patients included had markedly elevated triglycerides a statistical regression-towards-the-mean cannot be completely ruled out. Furthermore, it is not clear whether, in patients with common dyslipidaemic changes in renal failure, LMW heparin affects the distribution of LDL subclasses similar to hypertriglyceridaemic HD patients.

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References


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