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Effects of unfractioned heparin and low-molecular-weight heparin on osteoprotegerin and RANKL plasma levels in haemodialysis patients

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Abstract

Background. This randomized crossover study investigated the effects of unfractioned heparin (UFH) and low-molecular-weight heparin (LMWH) on intra- and post-dialytic blood levels of osteoprotegerin (OPG), receptor activator of nuclear factor kappa B ligand (RANKL) and inflammatory cytokines.

Methods. Forty patients on haemodialysis for at least 12 months were selected. UFH or LMWH was randomly assigned and maintained for 1 month, and then, in the following month, each patient was switched to the other form of heparin. In the mid-week session, we determined the changes in anti-Xa activity, OPG, RANKL, IL-1β, IL-6 and TNF-α values before heparin administration and after 15 min, 4, 8 and 24 h (T0, T1, T2, T3 and T4 respectively).

Since these parameters at the various experimental times showed a non-normal distribution, log transformation was applied in order to run parametric ANOVA, with Bonferroni correction for multiple comparisons.

Results. The changes in anti-Xa activity over time were similar but not the same for the UFH and LMWH. A highly significant (P < 0.001) increase in anti-Xa activity was detected at T1, regardless of the type of heparin, as confirmed in the comparison of T0 vs T1 using one-way ANOVA. Moreover, with both heparins, significant differences were found in the comparisons of anti-Xa activity at T1 vs T2 (both P < 0.001) and at T2 vs T3 (P = 0.0003 with UFH; P < 0.0001 with LMWH). Conversely, the difference in anti-Xa activity at T3 vs T4 was still significant with UFH (P = 0.0186) but not significant with LMWH (P = 0.728). When
Comparing anti-Xa activity at T4 vs T0, no significant differences were found either with UFH (P = 0.1996) or with LMWH (P = 0.7470), thus indicating that 24 h after heparin infusion, anti-Xa activity returned back to the pre-infusion values. When we analysed the changes in OPG levels over time, we found that the administration of heparin, regardless of the type, determined an increase in circulating OPG with a zenith at 15 min (T1), with a return back to the baseline levels within the 24th hour post-infusion. One-way ANOVA revealed significant differences in OPG blood levels at T0 vs T1 with both UFH (P = 0.0112) and LMWH (P = 0.0288), whereas no significant difference was observed in the comparisons of OPG levels at T1 vs T2, T2 vs T3, T3 vs T4 and T4 vs T0, either with UFH or with LMWH. The circulating levels of RANKL, IL-1β, IL-6 and TNF-α at the different intra- and post-dialytic times did not show significant variations following heparin administration, either with UFH or with LMWH. One-way ANOVA performed on the log-transformed values of RANKL, IL-1β, IL-6 and TNF-α at the various experimental times (T0 vs T1, T1 vs T2, T2 vs T3, T3 vs T4 and T4 vs T0) revealed no significant intra- and post-dialytic changes in their blood levels, thus confirming that heparin infusion did not affect their blood levels.

Conclusions. These results suggest that heparin-regulated cyclic increases of OPG might play a role in the vascular pathology of haemodialysis patients.

Keywords: end-stage renal disease; haemodialysis; low-molecular-weight heparin; osteoprotegerin; unfractioned heparin

Introduction

It is well known that the long-term use of heparin causes osteoporosis, vertebral collapse and spontaneous fractures [1]. The mechanism by which heparin determines this side effect is still unclear, and it is also debated whether there is actually a difference between unfractioned heparin (UFH) and low-molecular-weight heparin (LMWH). Previous evidence from human and animal studies indicates a lower incidence of heparin-induced osteoporosis associated with the use of LMWH as compared with UFH [2–4].

Some light has been shed in the last few years on the linkage between heparin and the aforementioned bone changes since it has been proven that heparin interferes with osteoprotegerin (OPG) in different ways. OPG is produced by many cell types such as osteoblasts and various vascular cells, including endothelial cells (ECs) and vascular smooth muscle cells (VSMCs). OPG, receptor activator of nuclear factor-κB (RANK) and RANK ligand (RANKL), members of the TNF-related superfamily, is a molecular system with a critical role in bone remodelling, and vascular and immune biology [5,6].

OPG acts as a decoy receptor and prevents RANKL binding to RANK, thus blocking all cellular functions linked with this interaction. Both expression and production of OPG and RANKL are stimulated by several inflammatory cytokines [5–7]. It was recently demonstrated that heparin causes the mobilization of OPG into the circulation by displacement from the endothelial surface [8,9]. On the other hand, heparin inhibits OPG activity by binding it competitively. Vik and co-workers reported that UFH causes a more pronounced mobilization than LMWH in healthy volunteers, indicating that UFH has a higher affinity for OPG than LMWH [10].

Even if UFH is still the most common and effective method of anticoagulation used during haemodialysis, LMWH seems to be at least as safe and effective as UFH [11,12]. Ascertaining whether the intra-dialytic administration of heparin is able to induce a release of OPG is of particular importance in end-stage renal disease (ESRD) population, since these patients who show a high level of bone-related morbidity are subjected to cyclic and long-lasting heparin administration in order to prevent thrombogenesis in the extracorporeal circuit during haemodialysis sessions.

Nevertheless, the increased cardiovascular morbidity and mortality observed among ESRD patients have recently been recognized to be associated with disturbances in both mineral metabolism and bone disease as well as the OPG/RANK/RANKL system [13–15].

The aim of this randomized crossover study is to evaluate, during and after haemodialysis sessions, the effects of administration of UFH or LMWH used as anticoagulant therapy on the blood levels of OPG, RANKL, and inflammatory cytokines IL-1β, IL-6 and TNF-α.

Materials and methods

Subjects

Forty ESRD patients (21 males and 19 females), who had been on chronic haemodialysis for at least 12 months, were selected for the study from a cohort of 352 patients on regular bicarbonate haemodialysis therapy three times a week at our dialysis centre. The inclusion criteria were (i) age >18 years, (ii) clinical stability at least 3 months before the study entry and (iii) a functioning arteriovenous fistula as vascular access. The exclusion criteria were (i) active gastrointestinal bleeding (one or more positive haemoccult tests in the last 8 weeks, melena or haematochezia in the last 3 months), (ii) acute cardiovascular event in the last 3 months (myocardial infarction, angina pectoris, coronary or vascular bypass surgery, or claudicatio intermittens), (iii) malignancy, (iv) acquired or hereditary deficiency of coagulation factors, anti-phospholipid syndrome, (v) deep venous thrombosis, (vi) immunosuppressive therapy, (vii) acute vasculitis, (viii) liver diseases, (ix) active infection, (x) diabetes mellitus and (xi) enrolment in other clinical trials. During the 6-month period prior to the checking phase, none of the selected patients showed any change consistent with high turnover or adynamic bone disease.

The characteristics of the study population are reported in Table 1. All of them were oligaemic (urine volume <200 mL/die). Prior to their inclusion in the study, all the patients had been receiving UFH as anticoagulant during haemodialysis and routinely used polysuphane as dialysis membrane. Kt/Veq was 1.3 ± 0.2. Analysis of the dialysis water showed the absence of bacteria (<100 CFU/mL) or bacteriological contaminant products (endotoxin levels <0.025 endotoxin units/mL).

All the patients received erythropoietin (median dosage 150 IU/kg/week, ranging from 115 to 185 IU/kg/week). Thirty-one patients received antihypertensive medications, but not ACE inhibitors or angiotensin II receptor blockers.

Table 2 describes the treatments for hyperparathyroidism and Ca/P metabolism disorders given to the patients at the time of inclusion; these therapies were kept stable throughout the study period.

Experimental design

All the patients underwent 4-h haemodialysis sessions three times a week with a bicarbonate buffer and a Fresenius F7 HPS dialyser (Fresenius Medical Care, Bad Homburg, Germany).
The anticoagulation schedules tested were (i) standard heparin (sodic heparin, Vister®, Parke-Davis) 50 IU/kg on starting dialysis and 30 IU/kg in continuous intradialytic infusion per dialysis session (stopping the infusion 30 min before the end of the session), and (ii) LMWH (nadroparin calcium, Fraxiparan®, Sanofi Aventis) 64 IU/kg on starting haemodialysis and in the arterial haemodialytic line after a washing phase with 2 L of a heparin-free saline solution 0.9%.

UFH or LMWH was randomly assigned to the patients and maintained for 1 month. Weeks 1 and 2 were considered as a run-in period, and Weeks 3 and 4 as a checking phase. In the following 4 weeks, each patient was switched to the other form of heparin; in this way, each patient was compared against him/herself, receiving one form of heparin in the first month and the other in the following month. Gender distribution of participants was even.

At the checking phase, blood samples were collected during and after the mid-week haemodialysis session for the analysis of anti-factor Xa activity, OPG, RANKL, IL-1β, IL-6, and TNF-α. The time points for specimen collection were as follows: beginning of haemodialysis session (T1), 4 h after (T2), 8 h after (T3), and 24 h after—the day after haemodialysis—(T4). The blood was taken from the antecubital vein using Vacutainer® tubes (Becton Dickinson Vacutainer Systems, Meylan, France), containing trisodium citrate (0.109 mol/L) as an anticoagulant at a ratio of 1:9. Plasma was prepared in a centrifuge for 10 min at 2000 g at room temperature; platelet-poor plasma was harvested, aliquoted in coded plastic tubes, snap-frozen and stored at −70°C until further processing.

The study was approved by our ethical committee, and all patients gave informed consent.

Laboratory assays

The anti-Xa activity was assessed using a chromogenic assay (Hemo-Stat, Heparin; Instrumentation Laboratory, Milan, Italy), and the results were expressed as international unit anti-Xa per millilitre (therapeutic range 0.7–1.2 IU/mL). OPG and RANKL plasma levels were measured using Searchlight® Custom Human 2-plex Array (Pierce Biotechnology, Rockford, IL, USA) according to manufacturer’s recommendations. IL-1β, IL-6 and TNF-α levels were determined using a commercially available multiplex system (FlowCytomix Assay, Bender MedSystems, Wien, Austria) following manufacturer’s instructions.

To validate the results obtained from these analytical assays, the same determinations were carried out in 30 healthy volunteers. In this group, the plasma levels of OPG and RANKL were 3.7 ± 1.8 and 22.0 ± 22.7 pg/mL, respectively; the circulating levels of inflammatory cytokines were 6.3 ± 2.7 pg/mL for IL-1β, 0.89 ± 0.69 pg/mL for IL-6 and 2.8 ± 2.2 pg/mL for TNF-α.

Statistic analysis

The statistical analysis was performed using STATA software for Windows, version 10.0. The changes in anti-Xa activity, OPG, RANKL, IL-1β, IL-6, and TNF-α and values from before to after heparin administration were analysed. The measurements of these parameters at the various experimental times showed a non-normal distribution. Variables were then submitted to natural log transformation in order to normalize the distribution, and the log-transformed data were compared using one-way ANOVA, with Bonferroni correction for multiple comparisons. P-values <0.05 were considered statistically significant.

Results

Between April 2008 and October 2008, 40 patients on chronic haemodialysis at our dialysis centre were enrolled in the study. The clinical and biochemical characteristics of all study patients are listed in Table 1. The data were collected at the time of inclusion because each patient served as control of him/herself, receiving one form of heparin in the first month and the other in the following month. Gender distribution of participants was even—21 were male (52.5%) and 19 were female (47.5%). The age of the patient population ranged from 42 to 72 years (mean age = 63.3 years; standard deviation = 7.2 years).

Table 2 describes the pattern of the therapies for hyperparathyroidism and Ca/P metabolism disorders at the time of inclusion (the therapies given to the patients were kept stable throughout the study period).

<table>
<thead>
<tr>
<th>Treatments</th>
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</tr>
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<tbody>
<tr>
<td>Sevelamer + calcium carbonate</td>
<td>20.0 (8)</td>
</tr>
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<td>Calcium carbonate + oral calcitriol</td>
<td>20.0 (8)</td>
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<tr>
<td>Sevelamer + calcium acetate</td>
<td>12.5 (5)</td>
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<td>Calcium acetate + oral calcitriol</td>
<td>12.5 (5)</td>
</tr>
<tr>
<td>Sevelamer</td>
<td>10.0 (4)</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>10.0 (4)</td>
</tr>
<tr>
<td>Oral calcitriol</td>
<td>5.0 (2)</td>
</tr>
<tr>
<td>Calcium acetate</td>
<td>5.0 (2)</td>
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<td>Sevelamer + oral calcitriol</td>
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and 24 h after—the day after haemodialysis—(T4). The blood was taken from the antecubital vein using Vacutainer® tubes (Becton Dickinson Vacutainer Systems, Meylan, France), containing trisodium citrate (0.109 mol/L) as an anticoagulant at a ratio of 1:9. Plasma was prepared in a centrifuge for 10 min at 2000 g at room temperature; platelet-poor plasma was harvested, aliquoted in coded plastic tubes, snap-frozen and stored at −70°C until further processing.

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</tbody>
</table>
The values of anti-Xa, OPG, RANKL, IL-1β, IL-6 and TNF-α values at the different experimental times T0, T1, T2, T3 and T4 did not follow a normal distribution, so log transformation was applied in order to run parametric ANOVA (Table 3).

The anti-Xa activity log-transformed values at the various experimental times were plotted in Figure 1. Since the anti-Xa activity is expressed in international unit per millilitre, its values ranged between 0 and 1, so the log-transformed data resulted in negative numbers.

The changes in anti-Xa activity over time were similar but not the same for the two forms of heparin. A highly significant (P < 0.001) increase in anti-Xa activity was detected 15 min after heparin administration (T1), regardless of the type of heparin, as confirmed in the comparison T0 vs T1 using two-sample t-test with equal variances (one-way ANOVA). Moreover, either with UFH or with LMWH, significant differences were also found when we compared anti-Xa activity at T1 vs T2 (both P<0.001) and at T2 vs T3 (P = 0.0003 with UFH; P < 0.001 with LMWH). Conversely, the difference in anti-Xa activity at T3 vs T4 was still significant with UFH (P = 0.0186) but not significant with LMWH (P = 0.728). When comparing anti-Xa activity at T4 vs T0, no significant differences were found either with UFH (P = 0.1996) or with LMWH (P = 0.7470), thus indicating that 24 h after heparin infusion, anti-Xa activity returned back to the pre-infusion values.

The changes in OPG levels over time with the two forms of heparin are depicted in Figure 2. The administration of heparin, regardless of the type, resulted in an increase in circulating OPG with a zenith at 15 min (T1), with a return back to the baseline levels within the 24th hour post-infusion. This trend was confirmed by two-sample t-test with equal variances (one-way ANOVA). The comparison of OPG levels found at T0 vs T1 revealed significant differences with both UFH (P = 0.0112) and LMWH (P = 0.0288), whereas no significant difference was observed in the comparisons in OPG levels at T1 vs T2, T2 vs T3, T3 vs T4 and T4 vs T0, either with UFH or with LMWH.

The circulating levels of RANKL, IL-1β, IL-6 and TNF-α at the different times did not show significant variations over time following heparin administration, either with UFH or with LMWH.

### Table 3. Log-transformed mean and standard deviation values of anti-Xa, OPG, RANKL, IL-1β, IL-6 and TNF-α at the different experimental times for UFH and for LMWH

<table>
<thead>
<tr>
<th></th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
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</thead>
<tbody>
<tr>
<td><strong>Anti-Xa (IU/mL)</strong></td>
<td></td>
<td></td>
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<tr>
<td>UFH</td>
<td>-3.63 ± 1.16</td>
<td>-0.20 ± 0.41</td>
<td>-2.04 ± 1.44</td>
<td>-3.28 ± 0.77</td>
<td>-4.14 ± 1.63</td>
</tr>
<tr>
<td>LMWH</td>
<td>-3.41 ± 0.89</td>
<td>-0.30 ± 0.37</td>
<td>-0.90 ± 0.42</td>
<td>-3.21 ± 1.16</td>
<td>-3.23 ± 1.10</td>
</tr>
<tr>
<td><strong>OPG (pmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>UFH</td>
<td>2.96 ± 0.85</td>
<td>3.56 ± 0.81</td>
<td>3.19 ± 0.93</td>
<td>2.92 ± 0.90</td>
<td>2.80 ± 0.91</td>
</tr>
<tr>
<td>LMWH</td>
<td>2.66 ± 0.98</td>
<td>3.23 ± 0.85</td>
<td>3.30 ± 0.99</td>
<td>3.17 ± 0.87</td>
<td>2.85 ± 0.96</td>
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<tr>
<td><strong>RANKL (pg/mL)</strong></td>
<td></td>
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<tr>
<td>UFH</td>
<td>2.17 ± 1.84</td>
<td>1.97 ± 1.37</td>
<td>2.15 ± 1.59</td>
<td>1.42 ± 1.16</td>
<td>1.24 ± 1.00</td>
</tr>
<tr>
<td>LMWH</td>
<td>1.07 ± 1.13</td>
<td>1.28 ± 1.08</td>
<td>1.07 ± 0.69</td>
<td>0.90 ± 0.62</td>
<td>0.83 ± 0.60</td>
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<tr>
<td><strong>IL-1β (pg/mL)</strong></td>
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<tr>
<td>UFH</td>
<td>3.65 ± 1.29</td>
<td>4.18 ± 2.67</td>
<td>4.34 ± 1.96</td>
<td>4.12 ± 3.21</td>
<td>3.76 ± 3.50</td>
</tr>
<tr>
<td>LMWH</td>
<td>4.14 ± 2.98</td>
<td>4.13 ± 3.25</td>
<td>4.32 ± 2.46</td>
<td>3.88 ± 1.87</td>
<td>3.28 ± 2.95</td>
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<tr>
<td><strong>IL-6 (pg/mL)</strong></td>
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</tr>
<tr>
<td>UFH</td>
<td>0.06 ± 2.38</td>
<td>0.51 ± 1.58</td>
<td>0.44 ± 1.99</td>
<td>0.95 ± 1.85</td>
<td>0.12 ± 1.67</td>
</tr>
<tr>
<td>LMWH</td>
<td>1.55 ± 1.31</td>
<td>1.55 ± 1.18</td>
<td>0.96 ± 1.51</td>
<td>2.88 ± 1.23</td>
<td>1.41 ± 1.33</td>
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<tr>
<td><strong>TNF-α (pg/mL)</strong></td>
<td></td>
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</tr>
<tr>
<td>UFH</td>
<td>1.64 ± 1.46</td>
<td>1.03 ± 1.71</td>
<td>1.51 ± 0.81</td>
<td>0.77 ± 1.14</td>
<td>1.55 ± 2.31</td>
</tr>
<tr>
<td>LMWH</td>
<td>1.04 ± 0.72</td>
<td>1.23 ± 0.85</td>
<td>-0.34 ± 1.48</td>
<td>1.35 ± 1.97</td>
<td>0.76 ± 1.28</td>
</tr>
</tbody>
</table>

Anti-Xa, anti-factor Xa; UFH, unfractioned heparin; LMWH, low-molecular-weight heparin.

![Fig. 1. Changes in anti-Xa activity before heparin administration (T0), 15 min after heparin administration (T1), 4 h after—end of the haemodialysis session—(T2), 8 h after (T3) and 24 h after—the day after haemodialysis—(T4). The two forms of heparin used were UFH and LMWH. As the measurements of anti-Xa activity did not follow a normal distribution, the data were submitted to natural log transformation.](image1)

![Fig. 2. Changes in serum levels of OPG before heparin administration (T0), 15 min after heparin administration (T1), 4 h after—end of the haemodialysis session—(T2), 8 h after (T3) and 24 h after—the day after haemodialysis—(T4). The two forms of heparin used were UFH and LMWH. As the measurements of serum OPG did not follow a normal distribution, the data were submitted to natural log transformation.](image2)
One-way ANOVA was performed for the log-transformed values of RANKL and each cytokine serum level found after UFH or LMWH administration. The comparisons of each parameter at the various experimental times (T0 vs T1, T1 vs T2, T2 vs T3 vs T4 and T4 vs T0) revealed no significant intra- and post-dialytic changes of RANKL, IL-1β, IL-6 and TNF-α, thus confirming that heparin infusion did not affect their blood levels.

**Discussion**

Besides regulating bone mass, OPG/RANKL system exerts important effects on the vascular system since it is able to modify the differentiation, morphology and function of vascular cells. Even if VSMCs produce considerably higher amounts of OPG than ECs, both cell types contribute to the production and release of OPG into the circulation [5,15–17]. OPG is also highly expressed in atherosclerotic plaques; moreover, its production and expression are enhanced by several inflammatory cytokines [15]. OPG is bound by its heparin-binding domain to the glucosaminoglycans at the cell surface of VSMCs and ECs; the bond between OPG and glucosaminoglycans is an electrostatic interaction [10]. Heparin seems to cause the mobilization of OPG from the endothelial surface into the circulation [9,10]. Haemodialysis patients are unique in their exposure to heparin, as over the course of a year any patient receives some hundreds of thousands of UFH (UI/mL) or LMWH (anti-Xa/mL). The presence of a possible link between OPG produced by vascular cells and heparin in haemodialysis patients should be evaluated in view of their prolonged and sizeable exposure to heparin. Therefore, in order to verify what recurrently happens in haemodialysis patients, both kinds of heparin were comparatively investigated. The determinations were extended up to 24 h from heparin administration with the aim to evaluate the timing and degree of the release by vascular cells of OPG and RANKL, the changes in their blood levels, and the differences between UFH and LMWH. To our knowledge, this is the first study carried out on haemodialysis patients focused on the effect of UFH, LMWH and inflammatory cytokines on intra- and post-dialytic levels of OPG and RANKL. Our data confirm that, in ESRD patients, OPG basal levels are considerably greater than those reported in the general population [18–20]. This could be explained by several factors: (i) an age-related effect [19], (ii) a reduced OPG removal due to kidney failure [18], (iii) the micro-inflammation typical of uraemic patients resulting in pro-inflammatory cytokine activation which has been associated with high OPG levels [15,21,22], (iv) the correlation of high OPG levels with impaired endothelial function which is very common in uraemic patients [15,23]. All these factors implicated in OPG up-regulation could also promote the release and the consequent increase in OPG levels following heparin administration which, in its turn, might contribute to maintain long-lasting OPG elevation. In agreement with other studies carried out in subjects with apparently normal renal function [10,16], an increase in circulating OPG was observed 15 min after the i.v. administration of either UFH or LMWH. The administration of continuous infusion UFH after starting dialysis did not determine any further rise in OPG blood levels, thus indirectly confirming that the initial peak of OPG is the expression of the displacement of OPG from ECs, as OPG has a higher affinity for heparin than glucosaminoglycans. Subsequently, it is likely that OPG elevation might be also sustained by the secretion of OPG from the intracellular stores in ECs together with the OPG produced by VSMCs and then transported through ECs [24,25].

In our patients, we could not detect significant differences between UFH and LMWH in terms of OPG reduction over time: the return to basal values occurred 24 h after heparin administration, with both forms. However, the plots of OPG levels at the various pre- and post-infusion experimental times shown in Figure 2 seem to indicate a slower decline of OPG levels with LMWH, although this finding did not meet statistical significance. The high molecular weight of OPG (m.w. 120 kD) excludes any intra-dialytic removal. Our data are in contrast with the findings obtained by Vik et al. who noticed a more pronounced increase of OPG with UFH. It is feasible that our results are secondary to the effect of more variables: (i) lower doses of UFH and LMWH than those previously reported were used: higher heparin doses could possibly promote a molecular size-dependent mobilization of OPG, (ii) since a different form of LMWH (nadroparin calcium) was used compared to Vik’s experience, the role played by the different physical–chemical characteristics and molecular weight of the molecule cannot be excluded, and (iii) the intra-dialytic administration of both heparins was intravenous, whereas in Vik’s study, LMWH was administered subcutaneously [10].

Despite the lower doses of UFH and LMWH used, the increase in OPG that we observed was considerable, even if no difference was detected between the two heparins. It is hypothesizable that this finding lies in OPG up regulation by vascular cells in haemodialysis patients. Consistent with a previous study, basal RANKL levels in our population were lower than those found in apparently healthy donors [20]. Moreover, the blood concentrations of RANKL (m.w. 35 kD) did not show any significant change during haemodialysis treatment with both heparins. Considering that the molecular weight of RANKL excludes the hypothesis of any intra-dialytic removal and that heparin has not any direct effect on RANKL release, it is likely that OPG-elevated concentrations (basal as well as intra-dialytic) might reduce the changes in circulating RANKL [20], even if we did not find any relationship between OPG and RANKL. The unbalance between OPG and RANKL constitutes a vascular risk factor either in the general population or in haemodialysis patients [26,27]. Nevertheless, the physiological role of OPG binding to the cell surfaces of ECs and its storage inside VSMCs is still unknown, as well as the consequences for its heparin-induced release. The results of some experimental studies are very intriguing, if not controversial. Malayankar et al. showed that the heparin-mediated delivery of OPG promotes neovascularization in vivo and acts as a survival factor for rat and human endothelial cells [28]. OPG determines opposite effects to those caused by RANKL [9,28]. Although several experimental studies suggest a protective role for OPG in the vasculature [5,29–31], clinical studies, carried out either on subjects
with normal renal function or on haemodialysis patients, have shown a positive association between OPG levels and cardiovascular disease [32–35].

In conclusion, our data demonstrate that the administration of heparin in haemodialysis results in a significant and pulsatile increase in plasma OPG levels with a return to basal values within 24 h later.

In contrast to the observations from healthy volunteers, we have not demonstrated a significant difference between UFH and LMWH. We believe that this result is due to the higher doses of UFH and LMWH used in previous studies compared with those routinely used in haemodialysis anticoagulation. Considering the role that OPG plays in bone remodelling and the progression of vascular calcification, the repeated intra-dialytic increases (three times a week) of OPG levels could play a role in the bone and vascular pathology of haemodialysis patients. Nonetheless, whether OPG is simply a marker of vascular damage, whether it represents a counter regulatory mechanism aimed to limit the vascular disease or actively mediates disease progression is not yet assessed. Heparin may represent a further variable to consider in the pathophysiology of the mineral bone disorders of haemodialysis patients. Further clinical and experimental studies are needed to investigate the correlation between the long-term use of heparin and the OPG–RANKL system, and their clinical impact on bone damage and vascular disease in haemodialysis patients.

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References
Association between time of referral and survival in the first year of dialysis in diabetics and the elderly

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Abstract

Objective. The objective of the study was to estimate the association between time of referral and survival during dialysis in diabetics and patients aged ≥ 70 years.

Design, setting and subjects. This study was a prospective follow-up study in 1438 incident dialysis patients (1996–2004, 62% male, 60 ± 15 years) in The Netherlands.

Main outcome measures. Referral (time between first pre-dialysis visit to a nephrologist and dialysis initiation) was classified as: late (<3 months), early (3–12 months) or very early (≥12 months). All-cause mortality risk within the first year of dialysis was calculated [HR (95% confidence interval, CI), adjusted for age, sex and primary kidney disease (PKD)]. Additive interaction between time of referral and diabetes mellitus (adjusted for age and sex) was assessed by synergy index [S (95% CI)].

Results. Thirty-two percent were late referred, 12% early and 56% very early; 21% had diabetes; and 30% were ≥70 years. Early and late referrals were associated with increased mortality compared with very early referral [HRadjearly: 1.5 (1.0, 2.4), late: 1.8 (1.3, 2.5)]. A similar trend was observed in diabetics and non-dialysis. However, no interaction between time of referral and diabetes was present [Slate 0.8 (0.4, 1.9), Searly 1.2 (0.4, 3.6)]. Likewise, in patients aged <70 and ≥70 years, time of referral was associated with increased mortality, without interaction [Slate 0.9 (0.4, 1.8), Searly 0.8 (0.3, 2.0)].

Conclusion. Late referral is associated with increased mortality in the first year of dialysis. Diabetes or high age does not have an additional worsening effect, implying that timely referral is important in future dialysis patients irrespective of diabetes or high age.

Keywords: diabetes; elderly; late referral; pre-dialysis; survival

Introduction

The incidence and prevalence of chronic kidney disease and the number of patients needing renal replacement therapy increases worldwide [1,2]. This is a consequence of technical developments, improved access to renal replacement therapy, an ageing population and an increase in the incidence of diabetic nephropathy [2–4]. In addition, due to the high prevalence of risk factors like hypertension and diabetes, morbidity and mortality in patients on dialysis is considerably higher compared to the general population [5].

Late referral to a nephrologist, resulting in short pre-dialysis care, is considered as another risk factor for increased morbidity and mortality after initiation of dialysis treatment [6,7]. More precisely, late referral is associated