EFFECT OF EOSINOPHILIA ON THE HETEROGENEITY OF THE ANTICOAGULANT RESPONSE TO HEPARIN IN HAEMODIALYSIS PATIENTS

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Summary

The anticoagulant response to heparin was determined, during haemodialysis, in a group of seven patients with eosinophilia and in a control group. The heparin half-life was similar in the two groups, but the heparin effect index was lower in patients with eosinophilia. The dose-response curve showed a reduced sensitivity to heparin in patients with eosinophilia. In patients with eosinophilia a significant reduction in eosinophil count was observed during cuprophan dialysis, but not during polyacrylonitrile dialysis. The hyposensitivity to heparin might be related to eosinophil degranulation, during cuprophan dialysis, with release of a major basic protein that neutralises heparin.

Introduction

It has been shown that the heparin anticoagulant effect, in both normal subjects and in uraemic patients, does not only depend on the heparin concentration but also on other factors: concentration of the antithrombin III, platelet factor 4 and α1-acid glycoprotein [1,2]. During haemodialysis an additional factor could be the aggregation and degranulation of granulocytes in the early phase of dialysis [3]. Eosinophils, which are frequently increased in dialysis patients [4], contain granule products that can neutralise the heparin anticoagulant activity [5]. The present study was undertaken to determine whether the presence of eosinophilia in dialysis patients was accompanied by modifications in the anticoagulant response to heparin during conventional dialysis.

Patients and methods

Two groups of haemodialysis patients entered the study. Group I consisted of seven patients (3 females, 4 males; mean age 53.1±12.9 years) with an eosinophil count greater than 500/mm³ and group II of seven patients (1 female, 6 males; mean age 53.8±8.8 years) with a normal peripheral eosinophil count.
The time on dialysis was 77.7±46 months in group I and 72±21 in group II (p=NS).

The study was carried out during a regular dialysis session. Cuprophan plate dialysers were used and except for heparin no drugs were administered during the study. Blood samples were drawn immediately prior to initiation of dialysis to determine haematocrit, albumin, globulin fractions, differential leucocytes count, platelet count, fibrinogen, activated partial thromboplastin time (APTT) and antithrombin III (AT-III). A single loading dose (70U/kg) of aqueous sodium heparin (Liquemin, Roche) was injected into the arterial tubing before connecting the patient to the dialyser. Blood samples were then drawn from the dialyser arterial line at 5, 10, 15, 30, 60 and 120 minutes after the start of dialysis for APTT, heparin concentration and differential leucocytes count assay. In five patients in group I the same protocol study was repeated using a polycrylonitrile flat plate dialyser. Heparin and antithrombin III were assayed by COA-TEST (Ortho Diagnostics), white cell counts by Coulter Counter (H 6000, Technicon). The heparin half-life was determined by conventional methods of interpolation on curve, expressing the relationship between the logarithm of heparin concentration and the time after drug injection. The relationship between the heparin concentration and the corresponding APTT was calculated by subtracting the pre-treatment APTT from the APTT obtained on samples drawn after heparin injection and dividing the result (ΔAPTT) by the heparin concentration in the samples. This was called heparin-effect index (HEI).

Results

The main clinical and biochemical pre-dialysis parameters in the two groups of patients are summarised in Table I. The mean leucocyte and eosinophil count of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I mean ± SD</th>
<th>Group II mean ± SD</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Haematocrit (%)</td>
<td>29.01±5.7</td>
<td>31.12±8.2</td>
<td>0.58</td>
</tr>
<tr>
<td>Albumin (gm/dl)</td>
<td>3.8±0.33</td>
<td>4.18±0.32</td>
<td>0.113</td>
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<tr>
<td>a1-globulin (gm/dl)</td>
<td>0.18±0.06</td>
<td>0.17±0.01</td>
<td>0.72</td>
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<tr>
<td>a2-globulin (gm/dl)</td>
<td>0.63±0.13</td>
<td>0.54±0.08</td>
<td>0.16</td>
</tr>
<tr>
<td>b-globulin (gm/dl)</td>
<td>0.66±0.05</td>
<td>0.66±0.07</td>
<td>0.96</td>
</tr>
<tr>
<td>r-globulin (gm/dl)</td>
<td>1.50±0.56</td>
<td>1.20±0.21</td>
<td>0.08</td>
</tr>
<tr>
<td>Leucocytes (cell/mm³)</td>
<td>9214±2764</td>
<td>6028±1372</td>
<td>0.017</td>
</tr>
<tr>
<td>Eosinophils (cell/mm³)</td>
<td>2205±1441</td>
<td>100±55</td>
<td>0.0021</td>
</tr>
<tr>
<td>Platelets (cell/mm³)</td>
<td>185±54×10³</td>
<td>176±32×10³</td>
<td>0.705</td>
</tr>
<tr>
<td>Baseline APTT (sec)</td>
<td>31.7±3.8</td>
<td>28.5±3.9</td>
<td>0.252</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>236±55</td>
<td>267±50</td>
<td>0.293</td>
</tr>
<tr>
<td>Antithrombin III (%)</td>
<td>87±23</td>
<td>88±18</td>
<td>0.90</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>58.7±10.5</td>
<td>64.6±14.4</td>
<td>0.10</td>
</tr>
</tbody>
</table>
patients in group I was significantly greater (p<0.05) than the corresponding value of group II; no other statistically significant differences were found in the other parameters.

The APTT response to the intravenous bolus dose of heparin, during haemodialysis, is shown in Figure 1. The pre-dialysis APTT was 31.7±3.8 seconds in group I and 28.5±3.9 seconds in group II (p=NS). During haemodialysis the APTT values of group I were significantly lower than the corresponding value of group II. The differences were statistically significant (p<0.05) at 5, 15, 30 and 60 minutes from the start of dialysis. The heparin half-life was 83.8±46.3 minutes in group I and 70.5±35.6 minutes in group II (p=NS).

![APTT changes after heparin injection during haemodialysis](image)

The relationship between APTT changes and plasma heparin concentration are shown in Figure 2. A linear dose response curve, within a heparin concentration range of 0.1 to 1.25U/ml, was found in the two groups of patients, but the slope of regression line was significantly lower in patients in group I than those in group II. The mean HEI, during the two hours of the study, was significantly lower in group I (206±117sec/U/ml) compared to group II (523±244 sec/U/ml, p<0.001). In the patients in group I, during cuprophan dialysis but not during polyacrylonitrile dialysis, a significant reduction of circulating neutrophils and eosinophils occurred during the first 15 minutes of haemodialysis. Then, leucocytes and neutrophils returned to pre-dialysis values. Circulating eosinophils also increased but remained at a lower level compared to pre-dialysis values. During cuprophan dialysis, the anticoagulant heparin activity was lesser than
during polyacrylonitrile dialysis and the mean APTT values were significantly lower (p<0.05) than the corresponding values during polyacrylonitrile dialysis (Figure 3).

**Discussion**

During cuprophan dialysis, dialysis patients with eosinophilia showed a lower anticoagulant response to a standard dose of heparin compared to dialysis patients without eosinophilia.

Prior to dialysis, haematocrit, platelets and AT-III levels were similar in patients with and without eosinophilia. This result indicates that eosinophilia per se, rather than other biochemical factors, is responsible for the different anticoagulant action of heparin. The hyposensitivity to heparin appears to be a membrane-related phenomenon: a normal anticoagulant response to heparin was obtained after changing the cuprophan-containing dialysers to polyacrylonitrile dialysers. Both neutrophils and eosinophils were also unaffected by polyacrylonitrile membrane. Hallgren et al [6] reported that dialysers containing cuprophan may induce both aggregation and degranulation of neutrophils and eosinophils. The result of eosinophil degranulation is the release of specific granule constituents such as eosinophil cationic protein (ECP) and major basic protein (MBP) which possesses heparin neutralising activity [5,7].

Therefore it seems likely that haemodialysis using cuprophan membrane results in eosinophil degranulation with a release of MBP neutralising heparin in patients with eosinophilia.
Figure 3. Changes in leucocytes, neutrophils, eosinophils count and activated thromboplastin time (APTT) during cuprophan and polyacrylonitrile dialysis in patients in group I.
The peripheral eosinophil count in patients on maintenance haemodialysis must therefore be taken into consideration in pharmacokinetic models for precise heparin administration. In addition, local degranulation of peripheral eosinophils could be responsible for some ‘start-up’ dialysis-related symptoms.

References

1. Godal HC. *Thromb Diath Haemorrh* 1975; 33: 77
7. Winqvist I, Olofsson T, Olsson I. *Immunology* 1984; 51: 1

Open Discussion

LINDSAY (London, Ontario) I am very interested in your observations. You may recall that some time ago we actually described the presence of a heparin neutralising factor in plasma of dialysis patients which we correlated almost directly with release of platelet factor 4*. The question I have to ask you is whether in some way your association with eosinophils is an epiphenomenon related to a platelet release response, which could easily be with different membranes which have different platelet release capability, or have you indeed demonstrated that if you take eosinophils they release a truly heparin neutralising factor?

ZUCCHELLI We have no data on platelets to answer your question.

RITZ (Heidelberg) Dr Zucchelli could you tell me whether your dialyser was sterilised with ethylene oxide (ETO), if so one might discuss an alternate mechanism for eosinophil degranulation? In epidemiological studies eosinophilia has been shown to be related to ETO hypersensitivity. Consequently reaginic interaction with ETO or rather its haptenic products might trigger eosinophil degrada-

ZUCCHELLI Our dialysers are sterilised with ethylene dioxide.

DE GIULIO (Rome) Did you compare the effect of bolus injections and continuous injections of heparin?

ZUCCHELLI No, we only gave bolus heparin because our study was to compare two different populations with the same loading dose of heparin per kg of body weight.

GRINFELD (Zenica, Yugoslavia) Heparin can induce circulating platelet aggregates and it can affect other coagulation factors. Measuring the Xa inhibitory

factor gives information on plasma free heparin and we can conclude that eosinophilia diminishes plasma heparin.

ZUCCHELLI I agree with you, the aim of our study was not to study the effect of heparin on platelets but to compare two different groups of patients, one with eosinophilia and one without.