REDUCTION OF UNFAVOURABLE EFFECTS OF HEPARIN
WITH USE OF GABEXATE MESILATE IN DIALYSIS

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Summary

Gabexate mesilate (GM), a potent synthetic proteinase inhibitor has been evaluated as an alternative anticoagulant to heparin for haemodialysis. GM haemodialysis was achieved at a dose of 1,600mg/hr. GM was found to be dialysable and so rapidly degraded that dialysis with GM anticoagulation was analogous to regional heparinisation.

Although heparin induced considerable lipolytic activity, GM showed no particular effect on lipids. Some patients reported mild nausea and there was evidence of slight clot formation around the top of the venous drip chamber.

GM anticoagulation is easier than the conventional unreliable techniques of regional heparinisation.

Introduction

Although heparin is widely used as an anticoagulant in haemodialysis, it has some limitations. In order to be effective as an anticoatulant, heparin needs antithrombin III and it does not effectively inhibit the plasma kallikrein/kinin system, which is activated by dialysis [1]. In addition, heparin induces lipolytic activity [2], which might play a role in abnormal lipid metabolism, and it possesses antigenicity [3]. It is not easy to perform effective regional anticoagulation with heparin due to potential imbalance between heparin and protamine sulphate [4]. To overcome these problems, we have investigated the possible use of gabexate mesilate (GM), a synthetic proteinase inhibitor, as both a substitute and an anticoagulant to be used in combination with heparin.

Materials and Methods

Whole blood clotting time was measured by the kaolin activated clotting time (KCT), because GM is rapidly degraded and the anticoagulant activity of GM
is apt to be underestimated by the less accurate and more cumbersome Lee-White method.

The dialysability of GM was evaluated at a GM infusion rate of 1,000mg/hr and at various blood flow rates. GM was analysed using photometric procedures. Inactivation of GM in normal plasma was observed by serial determination of thrombin time after the addition of GM at a concentration of 250 μg/ml.

The effect of GM on the KCT system was evaluated at various doses, ranging from 30 to 1,000 μg/ml.

The clinical studies were done on uraemic patients stabilised on maintenance haemodialysis. Dialysis was thrice weekly, with a treatment duration of five hours. Conventional dialysate electrolyte levels were used with acetate added as buffer base. Glucose was also present in the dialysate at a concentration of 11mmol/L. Dialysers of various brands were used, but the membranes were always regenerated cellulose. Initial clinical trials were done in a group of 22 patients with GM (600mg/hr) in combination with heparin infusion (600 IU/hr). In the subsequent clinical experiments, heparin was gradually substituted by GM, using patients as their own control. In the final studies heparin was completely withdrawn and only GM was used.

To evaluate effects on the coagulation system, KCTs were determined before and during dialysis. Blood samples were collected, both upstream and downstream of the GM infusion line. Samples in the venous return line were also collected.

Platelet counts and fibrinogen levels were determined before and after dialysis, and as a control, dialysis solely with heparin (heparin study) was also evaluated. When GM was used without heparin the procoagulant activities of factors II, V, VII, VIII, IX, X, XI and XII were also analysed before and after dialysis.

To compare and contrast changes in lipid metabolism with and without heparin, free fatty acid and triglyceride levels were observed. Post-dialysis samples were frozen within ten minutes of collection, since heparin samples have considerable lipolytic activity.

**Results**

With a single passage through the dialyser, nearly half the GM disappeared. This would be due to both its dialysability and rapid degradation. When added to normal human plasma at a concentration of 250 μg/ml, only 40% of GM remained intact after 2 minutes. After 6 minutes, GM was not detectable. KCT was moderately prolonged at a GM concentration of 30 μg/ml, the lowest level tested. KCT reached a level of more than double the baseline value at a dose of GM 125 μg/ml.

Haemodialysis with GM only was achieved at a dose of 1,600mg/hr. As illustrated in Figure 1, KCT was sufficiently prolonged at the inlet to the dialyser but was almost at normal values at the outlet. The KCT of systemic blood was also normal. Thus, the use of GM as anticoagulant during dialysis is almost analogous to regional heparinisation.

Some clot formation was observed even at the highest dose of GM (1600mg/hr), reflecting rapid degradation of the agent. However, clot formation was only
Figure 1. KCT profile during haemodialysis with Gabexate Mesilate

observed in the venous drip chambers. About half the patients also complained of nausea during anticoagulation with GM.

Post-dialysis platelet counts were significantly decreased (10%) in both the heparin and GM (no heparin) studies, but there was no significant difference in the degree of reduction between the two studies. Fibrinogen levels were not significantly different between the studies and the values before and after dialysis were the same.

Furthermore the procoagulant activities of factors II, V, VII, VIII, IX, X, XI and XII did not change significantly before and after dialysis with GM (Table I).

TABLE I. Changes of Procoagulant Activities (%) of Coagulation Factors II, V, VII, VIII, IX, X, XI and XII during Haemodialysis with Gabexate Mesilate

<table>
<thead>
<tr>
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<th>Predialysis (m. ± SD)</th>
<th>Postdialysis</th>
<th>P</th>
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<tbody>
<tr>
<td>II</td>
<td>80 ± 10</td>
<td>81 ± 10</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>V</td>
<td>107 ± 12</td>
<td>110 ± 22</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>VII</td>
<td>121 ± 41</td>
<td>135 ± 27</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>VIII</td>
<td>179 ± 75</td>
<td>164 ± 54</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>IX</td>
<td>111 ± 15</td>
<td>110 ± 26</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>X</td>
<td>95 ± 11</td>
<td>96 ± 13</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>XI</td>
<td>94 ± 29</td>
<td>115 ± 32</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>XII</td>
<td>115 ± 32</td>
<td>112 ± 33</td>
<td>&gt;0.05</td>
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The changes in lipid studies revealed that heparin has a remarkable effect. Free fatty acid (FFA) levels were increased from 260±160 μEq/L (mean ± S.D.) to 620±200μEq/L when patients were on heparin and triglycerides were decreased from 133±58mg/100ml to 83±43mg/100ml. In contrast, no changes were observed in either FFA or triglyceride levels when the patients were anticoagulated with GM (Figure 2).

![Graphs showing FFA and TG levels before and after heparin and GM](image)

**Figure 2. Effects on free fatty acid (FFA) and triglyceride (TG) in haemodialysis: heparin vs Gabexate Mesilate (GM)**

**Discussion**

GM is a synthetic proteinase inhibitor with a molecular weight of 417 daltons and is rapidly metabolised in the blood. Ki (inhibitory coefficient) values were 0.2 μM for human plasma kallikrein, 0.87 μM for human thrombin and 8.5 μM for human activated factor X.

At concentrations used clinically, heparin showed inhibition of plasma kallikrein in the contact phase of the intrinsic coagulation system. In fact, kallikrein was activated by dialysis resulting in bradykinin release, one of the potent vaso- dilators derived from high molecular weight kininogen. The use of GM was expected to have some inhibitory effect on the plasma kallikrein/kinin system.

KCT profiles revealed that haemodialysis with GM alone produced almost the
same effect as regional heparinisation. The normalisation of KCTs after the
dialyser was due to a combination of dialysability, degradation and consumption
of the drug.

Although we frequently observed slight clot formation around the top of the
venous drip chamber, coagulation factor activities were not significantly changed
in systemic blood. This suggests that the clot formation was a reflection of rapid
degradation of GM.

Regional heparinisation cannot be performed with a great deal of confidence
using heparin and protamine sulphate and a potentially dangerous post-dialysis
rebound in anticoagulation activity is possible. Anticoagulation with GM might
therefore be preferable to attempts at regional heparinisation in such situations
as dialysis of patients with a severe bleeding tendency.

The effect of dialysis on lipid metabolism is still a controversial topic. Never-
theless, the role of heparin on lipid metabolism must be taken into consideration.
In our study with GM, we observed no significant change in free fatty acid or tri-
glyceride levels.

Glucose and acetate in the dialysate are also suspected to have a potentially
detrimental role in the abnormal lipid metabolism of maintenance dialysis patients
[5,6]. In our study, however, there were no significant changes in lipid levels
although both glucose and acetate were present in the dialysate. In fact, it could
be said that heparin contributed, to some extent, to abnormal lipid metabolism.

Adequate anticoagulation during dialysis was achieved with GM without the
use of heparin.

Although heparin-free anticoagulation was fairly successful, the observation
of slight clot formation in the venous drip chamber as well as some patients’ com-
plaint of mild nausea were negative aspects of GM anticoagulation.

Nevertheless heparin also has considerable limitations and in the light of these
studies, its use as the only effective anticoagulant in chronic haemodialysis should
be reassessed.

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Open Discussion

KRAMER (Göttingen) Gabexate mesilate has a very short half life of only one minute. For the clinical situation this may be too short if one considers that something might go wrong with the GM infusion resulting in clotting of the dialyser within 2-3 minutes. What is the upper therapeutic tolerance limit of GM administration? Would it be possible to double the infusion rate in order to increase the safety period?

NAKAGAWA So far as degradation is concerned you are correct, but so far clinically we have no such clotting problems with the dialyser. The effect on thrombin time was always kept to normal on GM, so I do not have any anxiety for the future. And for the second part of the question we think we cannot double the dosage because the patient shows nausea.

KRAMER I would conclude that if anything goes wrong with the infusion of this GM you would have clotting of the dialyser within 5 minutes.

NAKAGAWA So far the clotting is on the venous side, it is very slight, so we do not see any problem.

RUMPF (Göttingen) I wonder whether you actually measured plasma kallikrein and not plasma prekallikrein in your patients, since, as far as I know, plasma kallikrein is not detectable under normal conditions in human plasma. Could you please comment on this?

NAKAGAWA We have not studied the prekallikrein in plasma.

RUMPF But normally plasma kallikrein is not active in plasma, so my question is, how could you determine plasma kallikrein under these conditions?

NAKAGAWA We studied kallikrein activity in vitro, we did not measure pre-kallikrein levels in plasma.

RUMPF So you activated prekallikrein in vitro and then measured kallikrein activity, is that correct?

NAKAGAWA Yes.

WILL (Nottingham) Could you indicate what studies have been done with this material relating to bone mineralisation?

NAKAGAWA We don’t have any data on bone mineralisation.

WILL Do you know if there are any animal studies?

NAKAGAWA No.