Recombinant hirudin (HBW 023) produces stable anticoagulation unaffected by circadian variation in patients with thrombolysis for acute myocardial infarction

U. Zeymer, M. Mateblowski and K.-L. Neuhaus
Medizinische Klinik II, Städtische Kliniken Kassel, Germany

Background Circadian variations have been described for a number of haemostatic and physiological factors, all of which might predispose towards clotting in the late morning. The anticoagulation effect of heparin has been shown to respond in a circadian manner, resulting in minimal prolongation of the activated partial thromboplastin time (aPTT) in the morning.

Methods Recombinant hirudin (HBW 023) given as a bolus of 0.07, 0.1, 0.2 or 0.4 mg . kg⁻¹ followed by an infusion of 0.05, 0.06, 0.1 or 0.15 mg . kg⁻¹ over 48 h was used as conjunctive therapy to thrombolysis with front-loaded recombinant tissue-type plasminogen activator (100 mg . 90 min⁻¹) in 40 patients with acute myocardial infarction. aPTT, activated clotting time and free hirudin plasma levels were determined at baseline and at 8, 12, 16, 20, 24, 32, 40 and 48 h.

Results The prolongation of aPTT and activated clotting time was dose-dependent and stable. In 82.5% of the patients, aPTT values were ranged between the highest and the lowest aPTT of <30 s. When the results were divided into four time intervals (0000-0600, 0600-1200, 1200-1800, 1800-2400) neither in the individual patients nor in the mean values of the four different dose groups was any significant circadian variation in aPTT or activated clotting time prolongation observed. The pharmacokinetic studies of free hirudin plasma levels revealed no circadian rhythm either. All but one patient (97.5%) had a patent vessel (TIMI grade 2/3) at the end of the hirudin infusion.

Conclusions Recombinant hirudin, in contrast to heparin, does not show any circadian variation in its anticoagulation effect. This might, in part, explain the more stable and predictable anticoagulation achieved by hirudin, which is associated with a reduced rate of reocclusions after thrombolysis.

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Key Words: Thrombolysis, acute myocardial infarction, anticoagulation, recombinant hirudin.

Introduction

Several studies have shown a peaking in the onset of acute myocardial infarction, myocardial ischaemia and sudden death in the morning between 0600 and noon[11-13]. Circadian variations that may predispose to thrombotic occlusion of coronary arteries in the morning hours have been described for platelet aggregation, coronary flow, viscosity, cortisol and epinephrine levels[4-7]. Furthermore, there are reports of circadian variations in the level of endogenous tissue-type

plasminogen activator, plasminogen activator inhibitor-1 (PAI-1) and the efficacy of recombinant tissue-type plasminogen activator (rt-PA)[8-10]. Thrombin is thought to play a major role in the development of acute coronary syndromes[11,12]. Therefore, thrombin inhibition is one of the widely used therapies in unstable angina and acute myocardial infarction[13]. Heparin, the only thrombin inhibitor approved to date, has several drawbacks, including large inter-individual and intra-individual variations in its anticoagulation effect[14,15]. A circadian response to fixed-dose heparin regimens has been reported, with the minimal effect of activated partial thromboplastin time (aPTT) prolongation in the late morning[16,17], when the incidence of ischaemic events is peaking.

Hirudin, a specific direct thrombin inhibitor is able to inhibit clot-bound thrombin as well as free
were obtained after 30, 60 and 90 min and 36-48 h. APTT and activated clotting time were measured at baseline and at 8, 12, 16, 20, 24, 32, 40 and 48 h. APTT was measured with an automated centrifugal coagulometer (MLA Elektra 1000C, Medical Laboratory Automation Inc., Pleasantville, New York, U.S.A.). The upper normal aPTT with this method is 35 s.

The activated clotting time, a bedside test of global coagulation, was determined with the Hemochrome 801 clot-detecting system (International Technidyne Corporation, Edison, NJ, U.S.A.) using FTCA 510 test tubes, while following the manufacturer’s instructions closely. Free r-hirudin plasma levels were determined at baseline and at 8, 12, 24 and 48 h with a double monoclonal antibody validated for free hirudin, using an enzyme-linked immunosorbent assay (Enzygnost TMB, Behringwerke, Marburg, Germany).

Statistics

Results are expressed as mean ± standard deviation. Friedman two-way analysis of variance (ANOVA) was used to test differences between more than two groups. For all the analyses, a two-sided probability value of 0.05 or less was considered as statistically significant.

Results

Laboratory findings

The mean aPTT levels at baseline were 23 s. During r-hirudin infusion, the mean aPTT was prolonged to 96-6 s, 100-69 s, 120-0 s and 128-4 s in dose groups 1, 2, 3 and 4, respectively. There was stable prolongation of aPTT throughout the 48 h of hirudin treatment in the four dose groups (Fig. 1(a)). There was no significant difference between highest and lowest mean aPTT in dose groups 1, 2 and 3. In dose group 4 there was a consistent, statistically significant decline in aPTT with time, possibly due to the high initial bolus of 0.4 mg . kg$^{-1}$.

Variations of less than 30 s between the lowest and highest aPTT were seen in 82.5% (33/40) of the patients and in more than half of the patients (23/40 = 57.5%) this range was less than 20 s. The prolongation of aPTT was dose-dependent and correlated with the free hirudin plasma levels (Fig. 2).

The activated clotting time was determined in the HIT II study (dose 2–4) only. The mean activated clotting time level at baseline was 102 s and increased during the r-hirudin infusion to 166-5 s, 204-25 s and 225-2 s in dose groups 2, 3 and 4, respectively. As shown in Fig. 1(b) the activated clotting time was constantly prolonged throughout the hirudin treatment.

When the aPTT and activated clotting time levels were divided into four pre-defined periods of the day, no significant differences between mean values in the four different periods (00:00-06:00, 06:00-12:00, 12:00-18:00, 18:00-24:00) were observed (Tables 2 and 3).

Although there were some minor fluctuations in aPTT prolongation in the individual patients, no circadian rhythm or variation was seen in any patient.

### Methods

#### Patients

Forty patients enrolled at one centre as a subcohort of the multicentre ‘Hirudin for the Improvement of Thrombolysis’ (HIT I and II) studies, form the basis for this analysis. Both studies were approved by the ethics committee of the University of Göttingen, Germany. HIT I was an uncontrolled, open multicentre pilot trial and HIT II was conducted as a multicentre dose-ranging study with a sequential design. Patients aged between 25 and 75 years, with onset of symptoms suggestive of acute myocardial infarction of less than 6 h, and ST-segment elevations ≥ 0.2 mV in at least two precordial leads (V$1$–V$6$) and/or ST-segment elevations ≥ 0.1 mV in at least two limb leads of the ECG, were eligible for inclusion into the studies. Main exclusion criteria were any of the recognized contraindications to thrombolytic therapy and renal insufficiency.

#### Treatment protocol

After inclusion in the study, patients received a bolus of hirudin (Table 1). Subsequently, thrombolytic therapy with the front-loaded regime of rt-PA (actilyse, 100 mg . 90 min$^{-1}$) was administered. Recombinant (r-)hirudin (HBW 023) was given intravenously over 48 h (Table 1). The target range for aPTT prolongation was 1-5 to 2-5-fold control in the HIT I and 1-5 to 4-0-fold control in the HIT II study. In cases where aPTT fell below the target range, an additional bolus of 0-1 mg . kg$^{-1}$ was given, while in cases where aPTT was above the target range the r-hirudin infusion was stopped for 2 h. Angiograms of the infarct-related artery were obtained after 30, 60 and 90 min and 36–48 h.

### Laboratory investigations

APTT and activated clotting time were measured at baseline and at 8, 12, 16, 20, 24, 32, 40 and 48 h. APTT was obtained after 30, 60 and 90 min and 36-48 h.
Figure 1  Time course of mean activated partial thromboplastin time (aPTT) ± SD and mean activated clotting time (ACT) ± SD levels at baseline and during hirudin treatment in the different dose groups. • = dose group 1 (0-05 mg kg⁻¹ h⁻¹); ⋆ = dose group 2 (0-06 mg kg⁻¹ h⁻¹); ⋄ = dose group 3 (0-1 mg kg⁻¹ h⁻¹); ▲ = dose group 4 (0-15 mg kg⁻¹ h⁻¹).

There was no significant difference in the time course of aPTT regardless of the time of initiation of therapy.

The free hirudin plasma levels were determined in the patients of groups 2–4, increased with the dose (Fig. 2) and showed no significant circadian variability (Table 4).

Additional bolus injections and interruptions of therapy

Additional bolus injections to maintain the constant level of anticoagulation were necessary in only 20% of the patients. More than one additional bolus injection for aPTTs <1.5-fold control were given in 2, 4, 2 and no patient in dose groups 1, 2, 3 and 4, respectively. Interruptions of the infusion for aPTTs >4-fold control were necessary in one patient of dose group 2 and in three patients of dose group 4, all within the first 12 h after thrombolytic therapy.

Figure 2 Correlation of mean aPTT and mean free r-hirudin plasma levels (Hir) in dose groups 2 and 4 during the 48-h hirudin infusion.

- ▲ = hirudin dose group 4 (0-15 mg kg⁻¹ h⁻¹);
- ■ = aPTT dose group 4 (0-15 mg kg⁻¹ h⁻¹);
- ⋄ = hirudin dose group 2 (0-06 mg kg⁻¹ h⁻¹);
- ⋆ = aPTT dose group 2 (0-06 mg kg⁻¹ h⁻¹).

Table 2 Mean aPTT levels in sec during hirudin infusion at the four pre-defined periods of the day

<table>
<thead>
<tr>
<th>Time of day</th>
<th>0000–0600</th>
<th>0600–1200</th>
<th>1200–1800</th>
<th>1800–2400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose group 1</td>
<td>53±6 ± 12±6</td>
<td>52±1 ± 10±5</td>
<td>51±7 ± 7±8</td>
<td>53±4 ± 11±3</td>
</tr>
<tr>
<td>Dose group 2</td>
<td>57±4 ± 8±1</td>
<td>56±4 ± 8±4</td>
<td>57±1 ± 8±4</td>
<td>55±2 ± 4±3</td>
</tr>
<tr>
<td>Dose group 3</td>
<td>62±3 ± 11±9</td>
<td>61±0 ± 14±0</td>
<td>60±2 ± 6±3</td>
<td>62±5 ± 14±4</td>
</tr>
<tr>
<td>Dose group 4</td>
<td>73±9 ± 14±3</td>
<td>74±5 ± 9±5</td>
<td>70±8 ± 10±5</td>
<td>74±1 ± 12±0</td>
</tr>
</tbody>
</table>

Table 3 Mean activated clotting time levels in sec during hirudin infusion at the four pre-defined periods of the day

<table>
<thead>
<tr>
<th>Time of day</th>
<th>0000–0600</th>
<th>0600–1200</th>
<th>1200–1800</th>
<th>1800–2400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose group 2</td>
<td>176±20</td>
<td>169±19</td>
<td>173±17</td>
<td>165±7</td>
</tr>
<tr>
<td>Dose group 3</td>
<td>187±24</td>
<td>207±39</td>
<td>201±40</td>
<td>202±31</td>
</tr>
<tr>
<td>Dose group 4</td>
<td>232±27</td>
<td>237±38</td>
<td>226±46</td>
<td>222±37</td>
</tr>
</tbody>
</table>
Table 4  Free hirudin plasma levels (mean ± standard deviation) in ng . ml⁻¹ during the four pre-defined periods of the day

<table>
<thead>
<tr>
<th>Time of day</th>
<th>0000-0600</th>
<th>0600-1200</th>
<th>1200-1800</th>
<th>1800-2400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose group 2</td>
<td>524 ± 117</td>
<td>527 ± 117</td>
<td>606 ± 157</td>
<td>506 ± 142</td>
</tr>
<tr>
<td>Dose group 3</td>
<td>971 ± 259</td>
<td>886 ± 83</td>
<td>897 ± 256</td>
<td>984 ± 262</td>
</tr>
<tr>
<td>Dose group 4</td>
<td>1359 ± 613</td>
<td>1256 ± 347</td>
<td>1363 ± 432</td>
<td>1287 ± 523</td>
</tr>
</tbody>
</table>

**Angiographic findings and reocclusions**

Angiograms of the infarct-related artery after 90 min and 36-48 h (mean 44.2 h) were available in all 40 patients. An open infarct vessel (TIMI grade 2 or 3 flow) after 90 min was seen in 36 (90%) of the 40 patients. Percutaneous transluminal coronary angiography (PTCA) after the 90 min angiography was carried out in four patients. Reocclusion during the hirudin treatment occurred in one patient only in dose group 1, who had had a PTCA after the 90 min angiogram. After 36-48 h 39 (97.5%) of the patients had TIMI grade 2 (17.5%) or 3 (80%) flow in the infarct-related artery.

**Discussion**

The main finding of this study is that hirudin provided stable prolongation of aPTT and activated clotting time without a circadian variation, in patients treated with thrombolysis for acute myocardial infarction. Hirudin plays a central role in the pathogenesis of coronary artery thrombosis and rethrombosis[11,13]. Thrombolytic therapy can induce thrombin activation, resulting in failure of lytic therapy or recurrent thrombosis[29]. Most of the reocclusions after successful thrombolysis occur within the first 24 h after initiation of the therapy. Therefore effective anticoagulation and thrombin inhibition should be achieved throughout the first 24 to 48 h after thrombolysis to diminish the rate of reocclusion.

Thrombin inhibition by heparin is highly variable, regardless of the mode of administration[30,31]. This might, in part, be due to the circadian pattern of response to heparin, with a minimal effect on aPTT prolongation in the morning when the risk for ischaemic events is greatest and the need for effective thrombin inhibition is highest. In a study of Violaris et al., a fixed high dose (12 500 IU twice daily) of subcutaneous heparin regimen after intravenous streptokinase therapy in patients with acute myocardial infarction showed a marked individual variation in the response. Over 70% of the patients were inadequately anticoagulated and there was an obvious circadian pattern of response[15]. A similar circadian variability in aPTT was seen in patients on intravenous heparin therapy for venous thrombosis[16]. In both studies the lowest aPTTs values were observed in the morning between 0600 and noon. The mechanism for this circadian rhythm is unclear. There may be a circadian rhythm in the pharmacokinetics of this agent, as has been shown for many drugs[23]. Furthermore the factors which are able to neutralize heparin, such as platelet factor 4 and other plasma proteins, might have their peak in the morning leading to a reduced heparin response. Consistent with this are findings on peak platelet aggregability in the morning[44]. Additionally, there is a relative increase in precoagulant activity in the morning, with a reduction of fibrinolytic activity and an increase in blood viscosity[15,30]. Because of the increased incidence of acute coronary syndromes in the morning, a thrombin inhibitor without any circadian variation in its anticoagulation effect would be preferable to heparin.

Hirudin binds directly, in contrast to heparin, independent of anti-thrombin III to thrombin, has no natural inhibitor and is not inactivated by platelet factor 4 or other plasma proteins[17,18]. In our study, no circadian variation in the plasma levels of free r-hirudin could be observed. These features might partly explain the absence of circadian variation in the response to four different doses of intravenous hirudin. R-hirudin provided stable and effective anticoagulation even in the time between 0600 and noon. The stable response to r-hirudin was demonstrated both with aPTT and activated clotting time; the latter may be more appropriate than the aPTT for monitoring the hirudin therapy[29]. Variations of more than 30 s between the lowest and highest aPTT values were observed in only 17.5% of the patients. In the TIMI 5 and 6 trials, which compared hirudin and heparin after thrombolysis with alteplase or streptokinase, 87.5% and 75% of the heparin-treated and 63.8% and less than 30% of then hirudin-treated patients had aPTTs with variations of more than 30 s, respectively[20,21]. Furthermore, it was only necessary in our study to give additional bolus injections, to maintain the level of anticoagulation, to 20% of the patients. This indicates that a constant infusion of r-hirudin provides a stable level of anticoagulation, which might reduce the need for numerous controls of coagulation parameters.

The number of patients investigated in this trial was too small to draw relevant conclusions with regard to angiographic patency. Nevertheless, the high late (36-48 h) TIMI 2/3 patency rate of 97.5% and the low reocclusion rate of 2.5% underscores the effective and stable anticoagulation and thrombin-inhibition achieved by r-hirudin.

The results of this study are confirmed by the preliminary findings of the TIMI 5 study, in which CPG 39393, a similar recombinant hirudin, was used, and no circadian variation in the anticoagulation response to CPG 39393 was observed[28].

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This stable and effective anticoagulation by hirudin throughout the whole treatment period was associated with a remarkably low rate of reocclusion after rt-PA thrombolysis in the TIMI 5 and HIT II studies\textsuperscript{[20,21]}. The absence of circadian variability might partly explain this stable and effective anticoagulation.

**Conclusion**

R-hirudin provides stable anticoagulation after thrombolysis in patients with acute myocardial infarction. In contrast to heparin, r-hirudin does not show any circadian variability in its anticoagulation effect. Even in the late morning, when the incidence of ischaemic events is peaking, r-hirudin provides stable anticoagulation and might therefore be preferable to heparin in the treatment of acute coronary syndromes.

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**References**


