Heme Arginate: Effects on Hemostasis

By Liisa Volin, Vesa Rasi, Elina Vahtera, and Raimo Tenhunen

Hematin, the drug used for acute porphyrnic attacks, has been shown to cause disturbances in hemostasis, mainly because of its degradation products. Lately a new heme compound, heme arginate, has been developed for the treatment of porphyrias. In experimental animal studies as well as in clinical use it has proved to be well tolerated. To find out whether heme arginate has any effects on hemostasis we have studied a number of parameters of coagulation and fibrinolysis after a heme arginate infusion in seven healthy volunteers. All parameters studied remained practically unchanged except the coagulation factor X, which showed a transient, insignificant decrease during the maximal heme concentration. We believe that the lack of side effects is due to a better stability of heme arginate, the degradation rates being 1% for heme arginate and 61% for hematin in four hours. Our data favor the use of heme arginate in acute porphyrias as well as in other deficiency states of heme.

MATERIALS AND METHODS

Seven healthy volunteers were included in the study; there were three females and four males with a mean age of 40.4 ± 7.5 (mean ± SD) years and mean weight of 67.9 ± 7.9 kg. An informed consent was obtained from each subject before the study. The study was approved by the Ethic Committee of the Third Department of Medicine, Helsinki University Central Hospital.

Heme arginate infusion concentrate (Normosang, 25 mg/mL; Leiras-Medica, Huhtamäki Pharmaceuticals, Ltd, Turku, Finland) was diluted in 100 mL physiological saline, and 3 mg heme/kg body weight (the normal therapeutic dose) was infused for 30 minutes into a forearm vein.

Serial venous blood samples were drawn before and 15 minutes, 1, 5, and 24 hours after infusion for coagulation and fibrinolysis tests, the measurement of plasma heme and hemopexin concentrations, and for blood cell and platelet counts. In addition, extra samples were taken for plasma heme and hemopexin concentrations 30 minutes and two hours after infusion, and for blood cell and platelet counts two hours after infusion. In addition, samples were taken for the measurement of plasma heme and hemopexin concentrations 30 minutes and two hours after infusion and samples for complete blood screens and platelets two hours after infusion. Blood samples for the measurement of plasma thromboxane B2 concentrations were taken before and 15 minutes, 1, 2, and 5 hours after infusion.

Heme concentrations were measured spectrophotometrically, hemopexin by immunodiffusion (Nor-Patigene, Behringwerke AG, Marburg, Germany), and thromboxane B2 in plasma by radioimmunoassay. Complete blood screens and platelet counts were done by using the routine methods of the University Hospital. The following tests of coagulation and fibrinolysis were performed at the Laboratory of Hemostasis in the Finnish Red Cross Blood Transfusion Service: activated partial thromboplastin time (APTT); prothrombin-proconvertin (P+P); prothrombin time (Quick); thrombin time:

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Submitted May 11, 1987; accepted October 19, 1987.

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fl-thromboglobulin could antigen; fibrinopeptide A (FPA, radioimmunoassay); and functional test and measurement of prothrombin antigen; activities of fibrinogen; fibrin(ogen) degradation products (FDP); ethanol gelation and measurement of hemopexin values fell in parallel with those of heme (Fig 2).

The plasma heme concentration-time curves after the seven single heme arginate (3 mg heme/kg) infusions are shown in Fig 1. The maximal plasma heme concentrations of 51.5 ± 10.0 μg/mL (mean ± SD) were reached in 30.0 ± 21.2 minutes, and the elimination half-life for heme was 11.0 ± 2.2 hours. The plasma hemopexin values fell in parallel with those of heme (Fig 2).

The effects of the heme arginate infusions on platelets are shown in Table 1. The mean platelet count remained stable throughout the observation period. No activation of platelets could be found on the basis of the measurement of plasma β-thromboglobulin and plasma thromboxane B2 levels.

The overall function of the coagulation mechanism tested by APTT, P+P, prothrombin time, and thrombin time was not influenced by the heme arginate infusions. FPA levels remained normal, and ethanol gelation was negative (Table 2).

The activities of the individual coagulation factors at different times after the heme arginate infusions are shown in Table 3. They remained quite constant, except for factor X, which showed a transient, insignificant decrease in all volunteers during the maximal heme concentrations, from 90% ± 7% before infusion to 76% ± 13% one hour after infusion.

The levels or activities of the inhibitors of coagulation, ie, antithrombin III and protein C, did not change after the heme arginate infusions (Table 2).

The results of the tests of fibrinolysis, eg, fibrinogen, FDP, and tPA, remained unchanged (Table 2). The heme arginate infusions did not cause signs of phlebitis or other clinical side effects.

### RESULTS

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### DISCUSSION

The present study showed that one therapeutic heme arginate infusion had no adverse effects on hemostasis in seven healthy volunteers.

In this respect the present results differ markedly from those of Glueck et al that were obtained with hematin in porphyria patients. They discovered that, after a therapeutic hematin infusion (4 mg heme/kg), the clotting times measured (prothrombin time, partial thromboplastin time, thrombin time, Reptilase time) were prolonged, the platelet count and levels of coagulation factors V and VIII and fibrinogen were decreased, and the titer of FDP was doubled during the maximal plasma hematin concentrations. Platelet aggregation studied with ADP was impaired. Further studies of the same group indicated that hematin expressed the anticoagulant activity by inactivating thrombin and complexing with clotting factors and induced platelet aggregation by release of storage pool ADP. In vitro studies showed...
vascular endothelium when judged by volunteers have to our knowledge been carried out, and thus on the hemostatic effects of hematin infusions in healthy however, not probable. The platelet count or any signs of platelet activation is, our study, Tokola et al found a similar elimination half-life.

Marked in vivo platelet aggregation, which sensitively reflect the activation of platelets, remained stable. Platelet aggregation could not be tested with the routine light transmission technique because of the disturbing effect of the dark color of the plasma heme concentration followed a monoequivalent course. The comparisons in vitro of heme arginate infusions with the routine light transmission could not be tested in the study of Glueck et al were, on an average, higher than those after the hematin (4 mg heme/kg) infusions (50 vs 40 μg/mL). The elimination half-lives of heme arginate and hematin in these studies were about the same. In accord with our study, Tokola et al found a similar elimination half-life for hematin arginate, 10.8 ± 0.6 hours (mean ± SEM), and the decline of heme arginate concentration followed a monoequivalent course.

The heme arginate infusions did not have any thrombocytopenic effect. The plasma β-thromboglobulin and thromboxane B2 concentrations, which sensitively reflect the aggregation of platelets, remained stable. Platelet aggregation could not be tested with the routine light transmission technique because of the disturbing effect of the dark color of heme arginate during the maximal plasma heme concentration. Marked in vivo platelet aggregation without a decrease in the platelet count or any signs of platelet activation is, however, not probable.

In the present study there were no signs of activation of the vascular endothelium when judged by the stability of the different parameters of the factor VIII complex and by the normal tPA levels. In practice, the venous tolerance of heme arginate infusions has proved to be excellent on the basis of the experience of over 500 infusions thus far given to humans by us.

No marked effects of the heme arginate infusions on the overall coagulation or fibrinolysis could be found by the parameters measured as shown in Table 2. The transient slight decrease of factor X levels during the maximal plasma heme concentration has no clinical significance.

Jones and Goetsch and Bissell have shown that the anticoagulant effects of heme are caused by its degradation products. The comparisons in vitro of heme arginate concentrate and lyophilized hematin (Panhematin, Abbot Laboratories, Chicago) diluted to the same concentration (7.3 mg heme/mL) revealed that only 1% of the heme of heme arginate, but 61% of the heme of hematin, had degraded after four hours. The rapid degradation of hematin may explain the adverse effects of even freshly made hematin solutions both in animal studies and in humans. It also explains why a smaller dose of heme arginate causes a larger dose of heme arginate than does a larger dose of hematin. In clinical practice it is also of importance that the maximal degradation rate of heme arginate as a concentrate is less than 4% in 2.5 years, which makes heme arginate readily usable in hospital dispensaries.

Table 2. The Effect of Heme Arginate Infusion (3 mg Heme/kg) on Parameters of Coagulation and Fibrinolysis (Mean ± SD) at Different Time Points After Administration in Seven Healthy Volunteers

<table>
<thead>
<tr>
<th>Study</th>
<th>Reference Range</th>
<th>Before</th>
<th>15 min</th>
<th>1 h</th>
<th>5 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTT (s)</td>
<td>30-42</td>
<td>35 ± 3</td>
<td>36 ± 2</td>
<td>36 ± 3</td>
<td>35 ± 2</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>P + P (%)</td>
<td>77-118</td>
<td>95 ± 9</td>
<td>95 ± 8</td>
<td>95 ± 9</td>
<td>95 ± 7</td>
<td>94 ± 10</td>
</tr>
<tr>
<td>Prothrombin time (Quick)</td>
<td>17-19</td>
<td>18 ± 2</td>
<td>18 ± 2</td>
<td>18 ± 2</td>
<td>19 ± 2</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>1.7-3.6</td>
<td>2.4 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>FDP (μg/mL)</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>tPA (U/mL)</td>
<td>0.01-0.12</td>
<td>0.03 ± 0.03</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
</tbody>
</table>

Table 3. Percent Activities of Coagulation Factors (Mean ± SD) at Different Time Points After Heme Arginate (3 mg Heme/kg) Infusion in Seven Healthy Volunteers

<table>
<thead>
<tr>
<th>Study</th>
<th>Reference Range</th>
<th>Before</th>
<th>15 min</th>
<th>1 h</th>
<th>5 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin activity</td>
<td>92 ± 7</td>
<td>90 ± 7</td>
<td>91 ± 9</td>
<td>93 ± 5</td>
<td>92 ± 5</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>105 ± 16</td>
<td>110 ± 20</td>
<td>103 ± 18</td>
<td>110 ± 16</td>
<td>105 ± 8</td>
<td></td>
</tr>
<tr>
<td>F VII</td>
<td>90 ± 5</td>
<td>90 ± 8</td>
<td>91 ± 10</td>
<td>91 ± 8</td>
<td>90 ± 9</td>
<td></td>
</tr>
<tr>
<td>F VIII:C</td>
<td>78 ± 15</td>
<td>73 ± 15</td>
<td>74 ± 14</td>
<td>74 ± 16</td>
<td>85 ± 18</td>
<td></td>
</tr>
<tr>
<td>vWF:Ag</td>
<td>95 ± 15</td>
<td>93 ± 17</td>
<td>94 ± 18</td>
<td>96 ± 17</td>
<td>105 ± 26</td>
<td></td>
</tr>
<tr>
<td>F VIII:Rc</td>
<td>84 ± 20</td>
<td>81 ± 21</td>
<td>84 ± 21</td>
<td>85 ± 15</td>
<td>91 ± 19</td>
<td></td>
</tr>
<tr>
<td>F X</td>
<td>95 ± 12</td>
<td>88 ± 17</td>
<td>88 ± 13</td>
<td>89 ± 18</td>
<td>90 ± 17</td>
<td></td>
</tr>
<tr>
<td>F X</td>
<td>90 ± 7</td>
<td>81 ± 7</td>
<td>76 ± 15</td>
<td>86 ± 15</td>
<td>87 ± 11</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: F, factor.
In experimental porphyria studies with animals, heme arginate has proved to be at least as antiporphyrinogenic as hematin but without side effects like thrombophlebitis, which is rather often caused by hematin. In clinical use heme arginate has also been effective, safe, and well tolerated without any adverse effects on hemostasis. In our opinion it might be the treatment of choice, not only for acute porphyrias but also for other heme deficiency states. In fact, heme arginate has already proved to be efficient in the treatment of some patients with a myelodysplastic syndrome. Heme arginate has not caused any disturbances in hemostasis, even in these patients with compromised hemostasis.

REFERENCES

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