Heparin-Induced Thrombocytopenia: Studies With a New Low Molecular Weight Heparinoid, Org 10172

By Beng H. Chong, Fawaz Ismail, John Cade, Alex S. Gallus, Susan Gordon, and Colin N. Chesterman

Studies were performed to determine the cross-reaction rate of the heparin-dependent antibody with Org 10172, a new low molecular weight heparinoid, and to investigate the effect of Org 10172 on platelet activation induced by the antibody. The plasmas of 17 patients with thrombocytopenia induced by standard heparin were shown, by platelet aggregation studies, to contain the heparin-dependent antibody. Of these 17 patient plasmas, only three cross-reacted with the heparinoid, producing a cross-reaction rate of 18%. When Org 10172 was added to a reaction mixture containing normal platelet-rich plasma, patient plasma, and standard heparin with non-cross-reacting plasmas, it inhibited platelet aggregation and thromboxane \(B_2\) production induced by the antibody, provided that the ratio of Org 10172 concentration (anti-Xa U/mL) to standard heparin concentration (IU/mL) exceeded 2.5 to 5.0. This inhibitory effect was observed only with platelet activation mediated by the antibody, but not by collagen (2 \(\mu\)g/mL) or ADP (5.0 \(\mu\)mol/L). Additionally, three of 17 patients with serious thrombosis, whose plasma showed no cross-reaction with the heparinoid, received Org 10172 treatment with a good response in each case. These findings suggest that Org 10172 may be a useful drug for the treatment of heparin-induced thrombocytopenia.

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aggregation occurred. Two control experiments were routinely used: in one control normal plasma was substituted for patient plasma, and in the other control saline was substituted for heparin. To test for cross-reaction of the heparin-dependent antibody with LMW heparinoid, Org 10172, LMW heparin, CY 216 (Choay, France), the study was repeated using the heparinoid at concentrations of 0.2 to 4.0 anti-Xa U/mL or the LMW heparin at 0.2 to 1.0 anti-Xa U/mL, instead of standard heparin. Org 10172 is a mixture of heparan sulfate (about 80%), dermatan sulfate (about 10%), chondroitin sulfate (about 5%), and a heparinlike compound (about 4%), with very little variation in its composition from batch to batch (personal communication, Dr J Stiekema Organon, Holland). To avoid the variability of antibody reaction that may be encountered with platelets from different donors, PRP from the same donor was used throughout the testing of a given patient plasma for reactivity with standard heparin, LMW heparin, and heparinoid.

The effect of the LMW heparinoid and LMW heparin on antibody-mediated platelet activation was investigated by incubating normal PRP (330 μL) and patient plasma (150 μL) with 10 μL of the heparinoid (0.2 to 10.0 anti-Xa U/mL), LMW heparin (0.2 to 10.0 anti-Xa U/mL), or saline for two minutes before adding 10 μL of standard heparin (0.05, 0.10, 0.20, or 0.40 IU/mL). To study the effect of Org 10172 on platelet aggregation induced by ADP (Sigma, St Louis) (5 μM) or collagen (Horn, Munchen, FRG) (2 μg/mL), 440 μL of normal PRP were incubated with 10 μL of Org 10172 (0.2 to 10.0 anti-Xa U/mL) or saline before the addition of 50 μL of collagen or ADP.

After completion of the aggregation studies, an equal volume of

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Table 1. Reaction of the Heparin-Dependent Platelet Antibodies With Standard Heparin, LMW Heparin, and Org 10172

<table>
<thead>
<tr>
<th>Patients</th>
<th>Std. Heparin (USP U/mL)</th>
<th>LMW Heparin (Anti-Xa U/mL)</th>
<th>Org 107/2 (Anti-Xa U/mL)</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients 1-10</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>- - -</td>
</tr>
<tr>
<td>Patient 11</td>
<td>- + +</td>
<td>+ + +</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>Patient 12</td>
<td>+ + +</td>
<td>+ + +</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>Patient 13</td>
<td>- + +</td>
<td>+ + +</td>
<td>ND</td>
<td>- - ND ND</td>
</tr>
<tr>
<td>Patient 14</td>
<td>+ + +</td>
<td>- - -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>Patient 15</td>
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<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
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<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>- - -</td>
</tr>
<tr>
<td>Patient 17</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
<td>- - -</td>
</tr>
</tbody>
</table>

Each patient plasma was incubated with normal PRP and standard (Std) heparin/LMW heparin/Org 10172 as described in Methods. A positive reaction (+) is one in which the platelet aggregation tracing showed a definite deviation from the baseline, giving a maximum platelet aggregation of >25%. A negative reaction (−) is one in which the aggregation tracing stayed at the baseline or showed a gentle drift giving a maximum decrease in optical density of 15% or less. No values fell between 15% to 25% maximum platelet aggregation. ND, not done.
ice-cold ethanol was added to the reaction mixture, the precipitate was removed by centrifugation at 1,800 g for 15 minutes, and the supernatant was kept for measurement of thromboxane B₂ by radioimmunoassay as previously described.¹

RESULTS

In vitro studies. When normal PRP was incubated with patient plasma, platelet aggregation was observed with all 17 patient plasmas in the presence of standard heparin (0.2 to 1.0 IU/mL), but in the presence of the LMW heparin (0.2 to 1.0 anti-Xa U/mL) and the LMW heparinoid (Org 10172) (0.2 to 4.0 anti-Xa U/mL), positive reactions occurred with 16 and three patient plasmas, respectively (Table 1). Whenever there was significant platelet aggregation, substantial production of thromboxane B₂ was also observed (Fig 1); however, when the platelet aggregation response to the heparin-dependent antibody was negative, only a negligible amount of thromboxane B₂ was detected.

Further studies were performed to investigate the effect of adding increasing concentrations of Org 10172 or the LMW heparin to the reaction mixture of normal PRP, patient plasma, and standard heparin (0.2 IU/mL). With seven patient plasmas that did not cross-react with Org 10172, the heparin-dependent antibody-mediated platelet aggregation was inhibited when the concentration of Org 10172 reached 0.5 to 1.0 anti-Xa U/mL (Fig 2). When the study was repeated with other concentrations of standard heparin (0.05, 0.10, and 0.40 IU/mL), this inhibitory effect was again present provided the ratio of Org 10172 concentration (anti-Xa U/mL) to standard heparin concentration (IU/mL) exceeded 2.5 to 5.0. However, this inhibitory effect of Org 10172 was not observed with the three patient plasmas that showed cross reactivity with Org 10172. When the heparin-dependent antibody-mediated platelet aggregation was blocked by Org 10172, thromboxane B₂ production was similarly inhibited. In contrast, addition of the LMW heparin to the reaction mixture did not inhibit the antibody-mediated platelet aggregation and thromboxane B₂ produc-

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*Fig 2. Effect of LMW heparinoid (Org 10172) and LMW heparin on platelet aggregation and thromboxane B₂ production induced by the heparin-dependent antibody. Increasing concentrations of LMW heparinoid (Org 10172) or LMW heparin were incubated with normal PRP, patient plasma, and standard heparin (0.2 μ/mL), platelet aggregation and thromboxane B₂ (TxB₂) were then measured as described in Methods. Each point represents the mean ± SEM. The results of seven patient plasmas that did not cross-react with Org 10172, and those of three patient plasmas that did, are represented by closed circles (§) and squares (■), respectively. Statistical analysis by two sample Student's t-test: (1) suppression of platelet aggregation by Org 10172 (0.2 to 1.0 anti-Xa U/mL), P < .0001 for non-cross-reacting plasmas (§), and P > .1 for cross-reacting plasmas (■); and by LMW heparin (0.2 to 1.0 anti-Xa U/mL), P > .2 for both non-cross-reacting and cross-reacting plasmas. (2) Suppression of TxB₂ by Org 10172 (0.2 to 0.8 anti-Xa U/mL), P < .0001 for non-cross-reacting plasma and P > .1 for cross-reacting plasmas, and by LMW heparin (0.2 to 0.8 anti-Xa U/mL) P > .2 for both cross-reacting and non-cross-reacting plasmas.*
HEPARIN-INDUCED THROMBOCYTOPENIA

Thrombocytopenia and thrombosis are serious complications of heparin therapy. The present management with a vitamin K antagonist, plus low molecular weight dextran infusion with or without a platelet suppressive drug, can result in a mortality rate of up to 30%, and a 20% chance of limb amputation. In view of such a disappointing outcome, a number of new drugs have been tried in the treatment of this life-threatening condition. Recently, LMW heparin has been used with variable results. The cross-reaction rate of the heparin-dependent antibody with LMW heparin has been reported to be rather high. It has been suggested that cross-reaction of the antibody with LMW heparin may adversely affect the outcome of treatment, but this has yet to be confirmed by formal clinical studies.

This study shows a low cross-reaction rate of the heparin-dependent antibodies with a new LMW heparinoid (Org 10172), a potentially effective antithrombotic drug with high benefit/risk ratio. Only three of the 17 patient plasmas studied showed positive reaction with Org 10172, giving a cross-reaction rate of 18%, in contrast to a cross-reaction rate of 94% with the LMW heparin. The difference in cross-reaction rates of the antibody with the LMW heparin and the heparinoid may be attributed to the fact that the LMW heparin is derived from standard heparin, whereas Org 10172 is a mixture of heparan sulfate, dermatan sulphate, chondroitin sulfate, and a heparinlike compound. In addition, the two drugs have other differences. Org 10172 has a greater capacity to inactivate thrombin through heparin cofactor II than the LMW heparin, but weight-for-weight the LMW heparinoid has less anti-Xa activity.

Our study also shows that the LMW heparinoid can inhibit platelet activation induced by the heparin-dependent antibody. Org 10172, when added to a reaction mixture of PRP, patient plasma, and heparin, suppressed antibody-induced platelet aggregation and thromboxane production, provided the ratio of the concentration of Org 10172 (anti-

**DISCUSSION**

... (Fig 2). This inhibitory effect of Org 10172 is specific to platelet aggregation and thromboxane production induced by the heparin-dependent antibody, as Org 10172 (0.2 to 10.0 anti-Xa U/mL) did not inhibit platelet aggregation and thromboxane B2 production induced by 5 μM of ADP and 2 μg/mL of collagen (data not shown).

Clinical studies. Three critically ill patients who had heparin-induced thrombocytopenia were treated with Org 10172. These patients' plasmas were tested and found to have no cross reaction with LMW heparinoid. Patient 4, a 23-year-old female, developed multiple and massive pulmonary emboli during heparin therapy, including a large thrombus almost completely occluding the right main pulmonary artery. After heparin was stopped the patient was given systemic streptokinase infusion for 48 hours, followed by Org 10172 intravenously for five days. Patient 5, a 58-year-old man, who had recurrent pulmonary emboli and extensive venous thrombosis of the right leg with incipient venous gangrene, received intravenous Org 10172 for five days after withdrawal of heparin. Patient 7, a 70-year-old female, had a massive right iliofemoral thrombosis with imminent gangrene of the right leg. After cessation of heparin, she received aspirin and dextran 40 infusion without clinical improvement.

In each patient, the platelet count increased steadily during Org 10172 therapy and reached normal levels in five to seven days (Fig 3), confirming in vivo the absence of cross-reactivity of the antibody with the drug demonstrated in vitro. After commencement of LMW heparinoid therapy, the patients' general condition improved with no clinical evidence of further extensions or recurrences of the thromboses. All three patients survived and were clinically well at the time of discharge from hospital. However, patient 7, in whom treatment with Org 10172 was delayed, developed venous gangrene soon after commencement of Org 10172 therapy, and her affected leg was amputated.
Xa U/mL) to the concentration of standard heparin (IU/mL) exceeds 2.5 to 5.0. The inhibitory effect of Org 10172 seems to be specific to platelet activation induced by the heparin-dependent antibody, since the LMW heparinoid at concentrations as high as 10 anti-Xa U/mL did not suppress platelet aggregation and thromboxane B2 production induced by collagen and ADP. This inhibitory effect of Org 10172 may be important for the treatment of heparin-induced thrombocytopenia, and thrombosis as the associated thrombotic complication is believed to be the result of the heparin-dependent antibody-mediated platelet activation and microthrombus formation.

The successful treatment of our three patients with Org 10172 is further evidence that the LMW heparinoid is a potentially useful drug for the management of heparin-induced thrombocytopenia and thrombosis. After commencement with Org 10172, there were no clinically obvious extensions or recurrences of the thrombosis in these three patients. The platelet count in each patient rose steadily during treatment with Org 10172, confirming the lack of cross-reactivity in vivo as well as in vitro of the antibody in these patients with the LMW heparinoid. In view of the small number of patients treated with Org 10172 in this study, the true efficacy of the drug in the treatment of heparin-induced thrombocytopenia and thrombosis will await confirmation by a large prospective controlled study that is currently proceeding.

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