HEPARIN IS KNOWN to cause immune-mediated thrombocytopenia.1,2 This condition may be complicated by arterial and, less frequently, venous thrombosis resulting in severe disability or death.2,3 A heparin-dependent antibody is usually present in patients with this disorder2 and platelet activation by this antibody is believed to contribute to the development of the thrombotic complications.2

Management of these patients has proved difficult.2 In most cases, cessation of heparin is required. A common approach is to introduce oral anticoagulant therapy after heparin withdrawal, but this leaves patients with high thrombotic risk without effective anticoagulation cover for two or more days. Several investigators have recently used low molecular weight (LMW) heparin to treat such patients with variable success. They suggested that cross-reaction of the heparin-dependent antibody with the LMW heparin may adversely affect the outcome of treatment,4,5 but confirmation of this is required by formal clinical trials. The reported rate of cross reaction for the heparin-dependent antibody with several LMW heparin preparations has been rather high.4,5 Org 10172 (Organon, Oss, Holland) is a new heparinoid with potent antithrombotic activity,6,7 which may prove to be a safe alternative anticoagulant in this setting; but its cross reaction rate with the heparin-dependent antibody is not known. We describe here studies in which we investigated the cross-reaction rate of the antibody with the heparinoid, the effect of Org 10172 on platelet activation induced by the antibody, and the clinical response of patients with heparin-induced thrombocytopenia and thrombosis treated with this new drug.

MATERIALS AND METHODS

Patients. Seventeen patients were selected for this study from the patients referred to the consultant hematologists/thrombosis and hemostasis specialists at the participating hospitals with suspected heparin-induced thrombocytopenia. These 17 patients were selected because they were considered to have heparin-induced thrombocytopenia, and they fulfilled all the following diagnostic criteria: (1) a normal platelet count or a blood smear showing an "adequate" number of platelets before treatment with standard heparin; (2) thrombocytopenia (platelet count <140 x 10^9/L) occurring during heparin therapy; (3) exclusion of other causes of thrombocytopenia; and (4) a heparin-dependent antiplatelet antibody was detected in the patients' sera/plasma by platelet aggregometry as described in the Methods.

There were ten males and seven females ranging in age from 23 to 88 years (mean 64.9 years). In four patients, the thrombocytopenia occurred seven to 21 days (mean 10.4 days) after commencement of heparin, but in three patients who had intravenous heparin in the preceding fortnight the thrombocytopenia occurred two to four days after recommencing heparin. The thrombocytopenia was often severe with a mean nadir of the platelet counts of 46 x 10^9/L. After withdrawal of heparin, the platelet count returned to normal in 13 patients, but four patients died soon after cessation of heparin. In two of these four patients, the platelet count had risen to near normal levels just before death. Since they were referred patients, the majority of these 17 patients probably represented the more severe cases of heparin-induced thrombocytopenia, as 11 patients (65%) had thrombotic complications, including peripheral arterial thrombosis, venous gangrene, recurrent pulmonary emboli, and cerebrovascular accidents. After obtaining approval from the Australian Department of Health, three of the 17 patients received (with their informed consent) Org 10172 intravenously for three to five days.

Methods. Blood was collected from patients and healthy volunteers, and platelet-rich plasma (PRP) and platelet-poor plasma were prepared as described previously.11 To test for the heparin-dependent antibody in the patient plasma, 340 µL of normal PRP were incubated with 150 µL of patient plasma and 10 µL of standard heparin (Weddell, London) at final concentrations of 0.2 to 1.0 IU/mL in the cuvette of a Paton dual channel aggregometer at 37°C with continuous stirring for at least 15 minutes or until platelet aggregation occurred. The platelet aggregation rate was measured as described in the Methods.

For personal use only.on October 23, 2017. By guest.
HEPARIN-INDUCED THROMBOCYTOPENIA

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 10172 (Anti-Xa U/mL)</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2</td>
<td>0.5</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<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>+</td>
<td>ND</td>
</tr>
<tr>
<td>Patient 14</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patient 15</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Patient 16</td>
<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 &gt;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.

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 heparin-like 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/mL), LMW heparin (0.2 to 10.0 anti-Xa/mL), or saline for two minutes before adding 50 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

---

Fig 1. Platelet aggregation and thromboxane B2 production induced by the heparin-dependent platelet antibody in the presence of standard heparin, LMW heparin, or heparinoid. The results of studies with a representative patient plasma that reacted with standard heparin, LMW heparin, and heparinoid are shown in (A). The results of studies with a representative plasma that reacted with standard heparin and LMW heparin but not with the heparinoid are shown in (B). Platelet aggregation is depicted by the open bars (●), and thromboxane B2 production by closed bars (■).
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.

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-

![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.](image-url)
HEPARIN-INDUCED THROMBOCYTOPENIA

Fig 3. The serial platelet counts of three patients with heparin-induced thrombocytopenia and thrombosis treated with Org 10172. Panels a, b, and c show the serial platelet counts of patient 3, patient 4, and patient 7, respectively. Patients 3 and 4 were given Org 10172 intravenously, 2,400 anti-Xa U as a bolus, followed by 200 anti-Xa U hourly for five days. Patient 7 received Org 10172 1,500 anti-Xa U every 12 hours for three days. (□) HEP, standard heparin; (□) SK, streptokinase; (□) ORG, Org 10172; (□) WAR, warfarin, (□) 0, others (aspirin and dextran 40).

Discussion

Thrombocytopenia and thrombosis are serious complications of heparin therapy.3,23 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.3 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.44 The cross-reaction rate of the heparin-dependent antibody with LMW heparin has been reported to be rather high.45 It has been suggested that cross-reaction of the antibody with LMW heparin may adversely affect the outcome of treatment,46 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.47 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,12 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-
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 microthrombi 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.

ACKNOWLEDGMENT

The authors wish to thank Professor P.A. Castaldi and Dr H.N. Magnani for their generous gift of the LMW heparin (CY216) and the heparinoid (Org 10172), respectively, Phillip Gray for performing the statistical analysis, and D. Hayes for typing the manuscript.

REFERENCES

Heparin-induced thrombocytopenia: studies with a new low molecular weight heparinoid, Org 10172

BH Chong, F Ismail, J Cade, AS Gallus, S Gordon and CN Chesterman

Updated information and services can be found at:
http://www.bloodjournal.org/content/73/6/1592.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml