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

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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 B₂ 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 μg/mL) or ADP (5.0 μmol/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.

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 of 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 10⁹/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 14 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 10⁹/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. 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 (Weddel, 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. 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, Munich, 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 (■).](image-url)
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-

![Graph showing results](image-url)
tion (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 intravenous Org 10172 for five days. Patient 7 received 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 of the thrombosis. These two drugs were ceased and the patient was then treated with Org 10172.

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.

**DISCUSSION**

Thrombocytopenia and thrombosis are serious complications of heparin therapy.2,3 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.4 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.4,5 The cross-reaction rate of the heparin-dependent antibody with LMW heparin has been reported to be rather high.4,5 It has been suggested that cross-reaction of the antibody with LMW heparin may adversely affect the outcome of treatment,4,5 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.5-8 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 heparin-like 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.

REFERENCES


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.
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