Prospective Randomised Open-label Comparison of Danaparoid with Dextran 70 in the Treatment of Heparin-induced Thrombocytopenia with Thrombosis

A Clinical Outcome Study


The Australian HIT Study Group

Keywords

Heparin-induced thrombocytopenia, thrombosis, danaparoid, heparinoid, dextran

Summary

Aim: To compare clinical outcomes in a randomised comparison of treatment with danaparoid sodium (a heparinoid), or dextran 70, for heparin-induced thrombocytopenia (HIT) plus thrombosis. Methods: Forty-two patients with recent thrombosis and a clinical diagnosis of probable HIT who presented at ten Australian hospitals during a study period of six and one half years were randomly assigned to open-label treatment with intravenous danaparoid or dextran 70, each combined with oral warfarin. Thirty-four patients (83%) had a positive platelet aggregation or 14C-serotonin release test for HIT antibody. Twenty-five received danaparoid as a bolus injection of 2400 anti-Xa units followed by 400 units per hour for 2 h, 300 units per hour for 2 h, and then 200 units per hour for five days. Seventeen received 1000 mL dextran 70 on day one and then 500 mL on days 2-5. Patients were reviewed daily for clinical evidence of thrombus progression or resolution, fresh thrombosis or embolism, bleeding or other complications. The primary trial endpoint was the proportion of thromboembolic events with complete clinical resolution by the time of discharge from hospital. Results: With danaparoid, there was complete clinical recovery from 56% of thromboembolic events compared with 14% after dextran 70 (Odds Ratio 10.53, 95% Confidence Interval 1.6–71.4; p = 0.02). Clinical recovery with danaparoid was complete or partial in 86% of thromboembolic events compared with 53% after dextran 70 (Odds Ratio 4.55, 95% Confidence Interval 1.6–71.4; p = 0.02). Overall clinical effectiveness of danaparoid was rated as high or moderate in 88% of patients compared with 47% for dextran 70 (p = 0.01). One patient given danaparoid died of thrombosis compared with three patients given dextran 70. The platelet count returned to normal after a mean of 6.7 days with danaparoid and 7.3 days with dextran 70. There was no major bleeding with either treatment. Conclusion: danaparoid plus warfarin treatment for HIT with thrombosis is effective, safe, and superior to dextran 70 plus warfarin.

Introduction

Heparin-induced thrombocytopenia (HIT) is an immune-mediated syndrome that develops in as many as 1-3% of patients receiving heparin (1). The syndrome is characterized by a moderate decrease in platelet count beginning four or more days after the start of a first exposure to heparin treatment and by the presence of heparin-associated antibodies (2). 30-75% of patients with HIT develop thrombosis, with venous events about four times as common as arterial events (1, 3, 4), and approximately 20-30% of HIT patients with thrombosis die or suffer serious morbidity (e.g., the loss of a limb) (5, 6). HIT with thrombosis is managed by immediately stopping heparin and giving another antithrombotic drug.

Danaparoid is a glycosaminoglycan antithrombotic agent isolated from porcine intestinal mucosa. It is registered in many countries for preventing post-operative deep vein thrombosis (DVT) (7). Danaparoid contains no heparin fragments and its main active ingredients are heparan sulphate (84%) and dermatan sulphate (12%). The predominant anticoagulant effect is anti-factor Xa activity generated by heparan sulphate through its interaction with antithrombin. Anti-Xa activity has a half-life of 24.5 h, with 40-50% explained by renal clearance (8). Dermatan sulphate contributes some antithrombin activity mediated through heparin cofactor II (9). The ratio of anti-Xa to anti-IIa activity is about 20:1 (10). Danaparoid has little or no effect on INR and therefore does not interfere with warfarin dosing. The drug has minimal or no effects on platelet function in vitro or in vivo but in vitro studies show that it suppresses platelet aggregation induced by heparin-associated antibodies (9, 11). These properties make danaparoid a good candidate for the treatment of patients with HIT plus thrombosis.

Danaparoid has been used for a number of years to treat HIT (11-16). When HIT is accompanied by venous or arterial thrombosis, danaparoid is usually given with an oral anticoagulant like warfarin. By far the largest experience reported with danaparoid is of 811 patients treated in a compassionate use program supported by the drug’s manufacturer (NV Organon, Oss, The Netherlands), where treatment appeared to be successful in 94% of the evaluable patient (16, 17).
Other drugs used to treat HIT plus thrombosis with apparent success include hirudin, a directly acting antithrombin (18-20) and argatroban, a synthetic thrombin inhibitor (21). Both of these drugs have recently been evaluated in large cohort studies of HIT patients with or without thrombosis where clinical outcomes, judged by a composite endpoint of death, amputation or new thrombosis, were compared with those in historical controls. To date, therefore, the information about treatment for HIT with thrombosis has come from cohort studies that were uncontrolled (danaparoid) (12-16) or had historical controls (hirudin and argatroban) (18-21). We now report on the first randomised prospective comparative trial of antithrombotic therapy for patients with HIT and thrombosis. The trial compared the clinical efficacy and safety of danaparoid plus warfarin with that of dextran 70 and warfarin. Dextran 70 can prevent venous thrombosis and pulmonary embolism after surgery (22) and was considered to be the standard treatment for HIT in Australia at the time this study was designed (hirudin and argatroban were not then available for clinical use).

**Methods**

**Study Population.** Patients were enrolled if they had a clinical diagnosis of highly probable HIT, based on the onset of thrombocytopenia during heparin treatment (platelet count less than 100 × 10⁹/L) and exclusion of other clinically probable causes of thrombocytopenia (23). To be eligible, the patients were also required to have a thrombotic condition requiring continued anticoagulant therapy after stopping heparin. A serum sample obtained at the time of presentation was assayed for the presence of heparin-dependent platelet antibody using platelet aggregometry or the ¹⁴C-serotonin release test. A positive HIT antibody test result was not needed before study entry and a negative test did not exclude patients from the study if the clinical features strongly suggested HIT. Patients were excluded if they had started warfarin and the INR had reached its target threshold of 2.0 at the time when heparin-induced thrombocytopenia was recognised. Other reasons for exclusion were renal or hepatic insufficiency, recent surgery or trauma, a need for thrombolytic therapy or inferior vena cava interruption, pregnancy, refusal or inability to give informed consent, and a presentation at times when randomisation was not feasible.

The study was conducted from January 1988 to June 1994 at 10 Australian teaching hospitals where it received approval by each hospital’s Ethics Committee. Procedures were in accord with ethical standards espoused in the Statement on Human Experimentation by the National Health and Medical Research Council of Australia and with the Helsinki Declaration of 1975, as revised in 1983.

**Randomisation.** Consecutive eligible patients at each hospital who gave informed consent were randomly assigned to treatment with danaparoid plus warfarin or dextran 70 plus warfarin. The 1:1 randomisation was stratified for city (Sydney, Melbourne or Adelaide) and for the presence of mild or serious thrombosis according to predefined criteria. *Mild thrombosis* included (i) stable calf vein thrombosis, (ii) stable proximal leg vein thrombosis, (iii) stable or minor pulmonary embolism and (iv) other stable or minor thrombosis e.g. skin necrosis or upper limb vein thrombosis. *Serious thrombosis* included (i) progressing or extending deep vein thrombosis, frequently with evidence of limb ischaemia or impending gangrene, (ii) major or progressing/recurrent pulmonary embolism, (iii) arterial occlusion causing limb ischaemia or impending gangrene, and (iv) vascular occlusion causing vital organ infarction or insufficiency e.g. myocardial infarction. After receiving a patient’s consent, investigators telephoned their city’s coordinating centre for treatment allocation. Treatment was not blinded because of major differences between administration of the two agents.

**Study medications.** Treatment with danaparoid (Orgaran™; Organon, Oss, The Netherlands) consisted of a bolus intravenous (i.v.) injection of 2400 anti-Xa units, followed by continuous i.v. infusion of 400 units/hour for 2 h, 300 units/hour for the next 2 h, and then 200 units/hour for 5 days. This dose regimen was that recommended by the manufacturer for treatment of established thrombosis in HIT (17). The dose was not adjusted for anti-factor Xa activity. Dextran 70 (Baxter Healthcare, Australia) was given as an i.v. infusion of 1000 mL on day 1, followed by infusions of 500 mL daily for the next four days. Patients also received 10 mg of warfarin on the first two days and 5 mg on the third day, with subsequent doses adjusted to achieve an International Normalized Ratio (INR) of 2-4. Study drugs were to be started within 12 h after stopping heparin and were to be continued for at least 72 h.

**Clinical and laboratory assessments.** Each patient had their medical history and physical examination recorded before the start of study treatment. Clinically suspected venous thrombosis, pulmonary embolism and artery occlusion was confirmed whenever possible by objective testing with Doppler ultrasound examination, venography, ventilation/perfusion lung scanning, pulmonary angiography or other site-specific angiography.

The presence of HIT antibody in a baseline sample was sought by platelet aggregometry or ¹⁴C-serotonin release assay, with testing done before or after randomisation as time permitted. Cross-reactivity of HIT antibody with danaparoid was not tested before randomisation.

The platelet count was repeated on alternate days until it returned to normal or the patient died. Blood cell counts, coagulation profiles, blood urea, creatinine, electrolytes and liver function tests were measured at baseline and then daily during trial therapy. Other procedures to assess the progress of affected organs were performed at the discretion of the investigator. These included cardiac enzyme levels when clinically indicated. The INR was used to monitor warfarin therapy.

Patients were reviewed daily until their death or discharge from hospital, to assess the clinical outcomes of initial thrombotic events and seek clinical evidence of new thromboembolic events, bleeding or other complications. At the end of clinical followup investigators recorded the treatment response of each thromboembolic event as complete recovery, partial recovery, no change or deterioration/progression. Investigators also made a subjective overall clinical assessment of treatment effectiveness for each patient. Treatment was rated as ineffective, slightly effective, moderately effective, or highly effective (response grades of 0 to 3). No guidelines or criteria were set by the study for this assessment.

**Clinical trial endpoints and statistical analyses.** Analyses were done on an “intention to treat” basis. Three outcomes were measured: 1) clinical response of initial thromboembolic events to treatment, 2) the number of days taken for the platelet counts to return to normal levels, and 3) overall clinical response of the patients to treatment. The primary clinical trial endpoint was the proportion of initial thromboembolic events with complete clinical resolution by the time of discharge from hospital. Other analyses were secondary.

1) **Clinical response of thromboembolic events to treatment.** Clinical outcomes were coded as 1 = complete recovery or 0 = partial recovery, no change or deterioration, and as 1 = complete or partial recovery or 0 = no change or deterioration. Data were analysed using a form of ordinal regression that allows for repeated measures. Analyses were adjusted for age, gender, severity of disease (mild or serious thrombosis) and type of thrombotic events (e.g. arterial or venous thrombosis). The statistics packages were ACCord (Analysis of Censored and Correlated Data) and SPIDA (Statistical Package for Interactive Data), both from the Statistical Computing Laboratory, Eastwood, NSW, Australia.

2) **Number of days taken for the platelet count to return to normal levels.** Cox regression analysis was used to compare the number of days taken to return to a normal platelet count when patients were treated with danaparoid or dextran 70.

3) **Overall clinical response to treatment.** The chi-square test was used to compare the proportion of patients with a positive (highly or moderately effective) or negative (slightly or not effective) overall clinical response in the two treatment groups.

**Results**

**Patient characteristics.** Forty-two patients entered the study and were included in the “intent to treat” analyses. Thrombocytopenia was detected an average of 11 days (range 1-23 days) after the start of heparin treatment. The mean of the minimum platelet counts before...
randomisation was $43 \times 10^9/L$ (range 1-108 $\times 10^9/L$), and the average percentage decrease of the platelet count during heparin treatment was 81% (range 44-99%).

Twenty-five patients were randomised to be given danaparoid (12 with mild and 13 with serious thromboembolism) and 17 to receive dextran 70 (seven mild and ten serious). This difference between the number of patients allocated to the two treatment groups arose by chance. Baseline demographic characteristics are comparable (Table 1) and all patients were followed and are accounted for. All patients had recent or new thromboembolic events that required urgent therapy. There were 43 new or recent thromboembolic events in the 25 danaparoid treated patients, compared with 36 events in the 17 patients given dextran 70. Venous events predominated and the proportion of venous to arterial events was similar in both groups (Table 2). Patients with “serious” thromboembolism were more likely to have multiple thromboembolic events. Three patients randomised to receive danaparoid presented with venous gangrene and none progressed. No new episodes of venous gangrene were reported in the danaparoid group. One dextran-treated patient with extensive venous thrombosis progressed to artery occlusion and limb gangrene in the affected leg.

Retrospective review indicates that one patient (patient 14) from the danaparoid group did not satisfy the inclusion criteria. This 59 year-old man was admitted with dizziness, sweating and hypotension. Three days later he became febrile, blood culture grew E. coli, and gentamicin was commenced. Six days after admission he complained of chest pain and dyspnea, a ventilation/perfusion lung scan suggested pulmonary embolus and he was treated with i.v. unfractionated heparin. His platelet count, which was $241 \times 10^9/L$ at presentation, dropped to $16 \times 10^9/L$ on that day. He was thought to have HIT, entered the study, and was randomized to receive danaparoid and warfarin even though heparin-induced antibody testing by platelet aggregometry was negative. He continued to deteriorate, progressing to respiratory and renal failure, and died after three days. Autopsy showed necrotic and perforated diverticula in the sigmoid colon with a large abscess and

### Table 1 Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>danaparoid + warfarin</th>
<th>dextran 70 + warfarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Males/Females</td>
<td>14/11</td>
<td>7/10</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>67.7 ± 6.5 yr</td>
<td>71.5 ± 7.2 yr</td>
</tr>
<tr>
<td>Minimum platelet count before starting study drugs (mean ± SD)</td>
<td>$44.6 \pm 31.5 \times 10^9/L$</td>
<td>$39.7 \pm 21.8 \times 10^9/L$</td>
</tr>
<tr>
<td>Thrombosis Severity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Serious</td>
<td>13</td>
<td>10</td>
</tr>
</tbody>
</table>

None of the apparent differences between the treatment groups is statistically significant.

### Table 2 Recent or fresh thromboembolic events at presentation. Some patients had more than one event when they entered the trial. The distribution of thrombotic events between the two treatment groups is not statistically different

<table>
<thead>
<tr>
<th>Treatment group and presentation</th>
<th>Pulmonary embolism</th>
<th>DVT</th>
<th>Leg artery occlusion</th>
<th>Ischaemic stroke</th>
<th>Other #</th>
<th>Total Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danaparoid plus warfarin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild Thrombosis</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>Severe Thrombosis</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>All Patients</td>
<td>14</td>
<td>14</td>
<td>7</td>
<td>1</td>
<td>8</td>
<td>44</td>
</tr>
<tr>
<td>Dextran 70 plus warfarin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild Thrombosis</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Severe Thrombosis</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>All Patients</td>
<td>12</td>
<td>12</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>36</td>
</tr>
</tbody>
</table>

# Digital ischaemia/gangrene of hands, subclavian vein thrombosis, acute myocardial infarction, coronary artery occlusion, renal ischaemia/impairment, adrenal insufficiency, ventricular thrombus, and disseminated intravascular coagulation.
peritonitis, and extensive infarction of the left upper lobe of the lung but no evidence of pulmonary emboli. It is most likely the thrombocytopenia was caused by sepsis and not HIT.

**HIT antibody testing.** Thirty-four patients (81%) had positive tests by platelet aggregometry or 14C-serotonin release, seven (17%) had negative tests (including patient 14 who probably did not have HIT), and one patient was not tested. Antibody testing was positive in 19/25 patients given danaparoid (76%) and 15/17 patients given dextran 70 (88%).

**Resolution of thrombocytopenia.** Thrombocytopenia resolved in 23/25 patients given danaparoid (92%) and 15/17 patients given dextran 70 (88%). Four patients (including patient 14) died within a few days after the diagnosis/presumed diagnosis of HIT, while still thrombocytopenic. The mean time to resolution of thrombocytopenia in the remaining 38 patients was slightly sooner with danaparoid than dextran 70 but the trend is not statistically significant (Table 3).

**Clinical response to treatment of thromboembolic events.** The significant treatment effect in favor of danaparoid remains after adjusting for age, gender, and the severity and type of thrombosis. Complete clinical resolution of thromboembolic events was 10 times more likely with danaparoid than dextran 70 (Odds Ratio 10.53, 95% confidence interval...
Venous thrombotic events were almost three times more likely to achieve complete clinical recovery than arterial events (Odds Ratio 2.9, 95% confidence interval 1.05–8.1, p = 0.04). Complete clinical resolution from pulmonary embolism was three and one half times more likely than after other episodes (Odds Ratio 3.5, 95% confidence interval 1.04–11.7, p = 0.04), while complete or partial clinical recovery was about four times more likely (Odds Ratio 4.2, 95% confidence interval 1.2–14.6, p = 0.02).

**Overall clinical assessment.** Investigators considered danaparoid to be moderately or highly effective (a positive response) in 88% of HIT patients. This contrasts with a positive overall clinical outcome in 47% of patients given dextran. The difference is statistically significant and is more marked for patients with “serious” thromboembolism (Table 5b). The subjective estimates of overall treatment effect (Grades 0 to 3) are recorded in Table 5a.

**Deaths.** There were four deaths among patients who received danaparoid plus warfarin. One patient presenting with serious thrombosis (massive pulmonary embolism) died of HIT-associated thrombosis, and three patients (including Patient 14) died of their underlying diseases e.g. sepsis. There were four deaths among patients who received dextran 70 plus warfarin. Three who presented with serious thrombosis died of their HIT-associated thrombosis. The fourth had mild thrombosis and HIT contributed to but was not the only cause of his death.

**Other adverse experiences.** There were no reports of major bleeding attributed to danaparoid, dextran 70 or warfarin. No other adverse events were attributed to danaparoid. One patient developed vomiting and flushing during dextran 70 infusion but completed treatment.

**Excluded patients.** 24 patients with clinically suspected HIT, laboratory confirmation of HIT, and recent thrombosis were excluded during the study period at participating hospitals from the danaparoid/dextran 70 comparison, but then received danaparoid on compassionate grounds. Their clinical outcomes were obtained by Dr. H Mignani from end of treatment case records returned to Organon. Treatment was considered “successful” in 20 patients (83%). 2 patients (8%) had ongoing thrombosis, 1 (4%) had major bleeding and 1 (4%) had minor bleeding. Two of these patients died of causes unrelated to their thrombosis.

**Discussion**

Danaparoid with its demonstrated antithrombotic effect, long half-time, minimal effect on the INR, and relative lack of cross-reactivity with HIT antibody in vitro, is an attractive drug for the treatment of HIT with thrombosis. This randomised prospective clinical endpoint trial of treatment for thrombosis associated with HIT suggests that, when given together with warfarin, danaparoid is superior to dextran 70.

To minimise the chances of an unequal distribution of poorer prognosis patients between treatment groups, the randomisation was prestratified to favor an equal allocation of patients with serious or mild thrombosis. Table 1 confirms this was achieved and the treatment groups were also similar with regards to age, male/female ratio and sites of thromboembolism. Furthermore, the clinical superiority of danaparoid remains after adjusting for age, gender and thrombosis site.

Venous thrombosis appeared to have a better clinical outcome than arterial thrombosis. This is not unexpected, as most arterial thromboses caused lower limb artery occlusions that often resulted in gangrene of the limb. Once gangrene became established, the process was irreversible regardless of the treatment given.

Because there was no large difference in the time taken for thrombocytopenia to resolve in the two treatment groups, it is likely that its resolution was largely determined by stopping heparin. After stopping heparin, HIT antibody ceases to interact with antigen (a complex of platelet factor 4 with heparin) and no further drug-antibody complexes are formed to cause premature platelet removal (7). As a result, platelet counts gradually return to normal levels within a mean time of 6-7 days of stopping heparin, irrespective of subsequent antithrombotic treatment provided this does not cross-react with HIT antibody in vivo and the patient survives long enough.

The observed mortality of 19% is roughly consistent with previously published data (5, 6). However, three patients in the danaparoid arm and one patient given dextran 70 arm died of causes like cardiac failure and infection that are not preventable with antithrombotic therapy. If only deaths attributed to serious HIT-associated thrombosis are considered (deaths attributed to failure of antithrombotic drug treatment), then this occurred in 1 of 25 patients treated with danaparoid (4%) and 3 of 17 given dextran 70 (18%) – a statistically nonsignificant trend. Safety of the two treatments was comparable.

This study has several potential weaknesses. One is the small number of patients who took part and this reflects the rarity of HIT with thrombosis. Nevertheless, despite the small patient numbers, highly statistically significant results were obtained. A second is reliance on subjective clinical endpoints. Clinical endpoints were chosen because the investigators believe they are more relevant to clinical practice than more objective surrogate endpoints, like a change in the thromboembolic burden measured with repeated venography or lung scanning. The third and most important limitation comes from the open study design, which invites bias when investigators report clinical treatment outcomes. The difficulty of double-blindness two very different treatment regimens and the very slow patient accession rate made this unavoidable (the ten centres averaged four patients each during the six and one half years of study recruitment). Sadly, there were insufficient resources for a central and blinded clinical endpoint adjudication. Lastly, the trial had no predetermined sample size but was stopped when a first analysis done after six years of recruitment indicated there were dramatic differences in clinical outcomes between danaparoid and dextran 70 treatment.

Our results are important despite these limitations. They come from the first randomised prospective evaluation of clinical outcomes after antithrombotic treatment for HIT accompanied by recent thromboembolism and demonstrate that danaparoid plus warfarin is effective and safe, and is clearly better than dextran 70 plus warfarin.

**Authors**

All of the manuscript’s authors helped design and execute the Australian HIT Study and all contributed patients. The team leader was Professor BH Chong. Professors BH Chong and AS Gallus prepared the manuscript for publication with special assistance from Dr. H Mignani.

**Acknowledgements**

This work was supported, in part, by an unrestricted grant from Organon, Oss, The Netherlands. The authors wish to thank Dr. Val Gebski and Ms. Jackie Fabri of the National Health & Medical Research Council Clinical Trials Centre, Camperdown, NSW, Australia, for the statistical analyses. We are also indebted to the physicians who allowed their patients to participate in the study and to the other doctors/health care staff who assisted in various ways.
References


Received March 13, 2001  Accepted after revision July 4, 2001