Current Developments in Antithrombotic Therapy: The Role of Antithrombin Agents

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Abstract
Hirudin, the specific thrombin inhibitor from medicinal leeches, is now produced by recombinant technology. r-Hirudin and to a lesser extent polyethyleneglycol-coupled hirudin (PEG-hirudin) have been used in many clinical trials. Hirudin has been shown to be more effective than low molecular weight heparin in the prevention of deep venous thrombosis after total hip replacement. In (acute) coronary syndromes hirudin as well as a synthetic hirudin analogue bivalirudin have been studied in large clinical trials. Higher doses of hirudin were associated with an increased risk of bleeding. A large-scale study with bivalirudin in patients with acute MI (the HERO-2 trial) has not shown a reduction in mortality but a 30% reduction of reinfarction within 96 h. Hirudin and argatroban have been successfully used in patients with heparin-induced thrombocytopenia type II. Several orally active thrombin inhibitors are being developed. The combined use of subcutaneous and oral administration of melagatran in patients with hip or knee replacement has led to promising results. It is likely that in the future thrombin inhibitors will replace other forms of anticoagulation in a number of indications.

Key Words
Thrombin inhibitors · Hirudin · Hirulog · PEG-hirudin · Argatroban · Inogatran · Melagatran · Heparin-induced thrombocytopenia

Introduction
During the last 20 years, low molecular weight heparins have replaced unfractionated heparin (UFH) in many indications. UFH has a similar effect on thrombin and the coagulation factor Xa, but heparins are not direct inhibitors of thrombin and factor Xa. They activate the plasma proteins antithrombin and heparin co-factor II, and this mechanism leads to their anticoagulant effects. Heparins have been used for 40 years in the prophylaxis and treatment of venous or arterial thromboses; improvements in therapy may be anticipated from direct thrombin or factor Xa inhibitors. Specific thrombin inhibitors are independent of the plasma level of antithrombin and of the inactivation of thrombin by heparin cofactor II.

Thrombin plays a central role in regulating thrombotic processes. It is a glycosylated, trypsin-like proteinase, which is formed out of prothrombin by the prothrombinase complex (factors Xa, Va, calcium and phospholipids).

Thrombin influences the permeability of platelet membranes, it has direct effects on the vascular endothelium and many other actions on different cell systems, including the stimulation of synthesis and release of mediators from vascular endothelium. After the characterization of the first specific thrombin inhibitor hirudin by Fritz Markwardt, many other thrombin inhibitors have been detected in different haematophagus animals [1]. Today hirudin is produced by recombinant technology.
and new inhibitors are synthetized. Low molecular weight thrombin inhibitors have been developed later on.

Hirudin and other thrombin inhibitors have been studied in a number of clinical trials and at present several oral thrombin inhibitors are being developed by different pharmaceutical companies.

**Parenteral Thrombin Inhibitors**

**Hirudin**

Hirudin is a specific thrombin inhibitor which was originally obtained from the salivary glands of the medicinal leech (*Hirudo medicinalis*). It was first isolated and characterized by Markwardt [2] in 1957. For approximately 20 years, hirudin has been produced by recombinant technology (r-hirudin). r-Hirudins are as active and specific as natural hirudin. r-Hirudin and a polyethylene-glycol-coupled hirudin (PEG-hirudin) have been investigated in clinical trials in different indications. Hirudin and r-hirudin have a molecular weight of 7,000 D. The molecular weight of PEG-hirudin is 17,000 D. Hirudin-derived molecules, such as hirulog, have been used in different clinical trials [3, 4].

Methods for the Monitoring of Hirudin and Other Thrombin Inhibitors

The measurement of the thrombin-inhibiting effect (anti-F-IIa-activity) in plasma using a chromogenic substrate is probably the most precise method to monitor prophylaxis or treatment with hirudin. However, the ecarin time is simpler, more practical and especially suited for bedside monitoring [5, 6]. The ecarin clotting time (ECT) is well correlated with the inhibition of thrombin as it is measured using chromogenic substrates. Other methods including the activated partial thromboplastin time (aPTT) and the activated clotting time (ACT) can also be used to monitor the effect of thrombin inhibitors. These methods, however, are not specific and are modified by acquired and inborn coagulation defects and in addition they are not well standardized. The thrombin time is too sensitive and therefore less suited for the control of thrombin inhibitors [7].

Hirudin in Coronary Heart Disease

Large clinical trials with r-hirudin in patients with unstable angina [8], patients with acute myocardial infarctions or acute coronary syndromes [9–13] and after coronary angioplasty [14] have been published.

In the two largest studies (GUSTO 2a and TIMI 9a), a relatively high initial intravenous bolus of 0.6 mg/kg hirudin was followed by a continuous infusion of 0.2 mg/kg/h [9, 12]. All patients received aspirin and many were also treated with thrombolytic agents. Both studies were stopped because of increased intracranial bleedings, and in the GUSTO 2a study increased mortality had been observed in the hirudin arms. In the TIMI 9a study, there were 14% major bleedings in the hirudin group compared to 10% in the heparin group.

Both studies were restarted with a much lower initial intravenous bolus of 0.1 mg/kg followed by an intravenous infusion 0.1 mg/kg/h. Following this change, bleeding complications under hirudin were not more frequent than in the heparin group. The clinical efficacy was equivalent to or minimally better than that of heparin [15, 16].

In further studies [17–19], another recombinant hirudin (HBW 023) was used in patients with acute myocardial infarcts together with streptokinase. The patients received either hirudin (i.v. bolus of 0.2 mg/kg, followed by 0.5 mg/kg/h as continuous infusion) or UFH. There were no significant differences in either efficacy or bleeding complications between the two treatment groups. It is possible that the observed hemorrhagic complications in the studies with higher hirudin doses were partially caused by the concomitant use of aspirin.

Interactions of Hirudin and Aspirin

We compared the effect of PEG-hirudin and aspirin alone with a combination of PEG-hirudin and aspirin in healthy volunteers [20]. A mean hirudin level of 1.8 µg/ml was achieved. The bleeding time was minimally prolonged under PEG-hirudin, slightly prolonged under aspirin but very much prolonged in volunteers who received 300 mg aspirin + PEG-hirudin. The effect of the combination was more than additive. We cannot yet explain what causes this synergistic effect of a combination of hirudin and aspirin.

Similar interaction studies with heparin and aspirin have shown that the combined use leads to prolongation of the bleeding time but only to a slight additive effect [21].

Hirudin in the Prophylaxis and Treatment of Deep Venous Thrombosis

Hirudin has been more effective than UFH in the prophylaxis of thrombosis after total hip replacement [22]. In another large study [23], recombinant hirudin (2 × 15 mg/day s.c.) was compared with the low molecular weight heparin enoxaparin (once daily 40 mg s.c.). Enoxa-
parin was administered to 1,023 patients and hirudin to 1,028 patients. The thrombosis rate in the enoxaprin group was 28.5% and 18.4% in the hirudin group; hirudin reduced the incidence of proximal thrombosis (7.5% in the enoxaparin group, 4.5% in the hirudin group). The difference was statistically significant. r-Hirudin was more effective than low molecular weight heparin in this high-risk population. Hirudin has been approved for this indication.

There are few data on the treatment of acute deep venous thrombosis with hirudin [24, 25]. In this indication, low molecular weight heparins probably are more suitable, and their effect has been established in many clinical trials. Moreover, at higher doses hirudin probably needs laboratory monitoring.

**Possible Further Development of Hirudin and PEG-Hirudin**

r-Hirudin and PEG-hirudin are being investigated in new trials in patients with unstable angina. It is expected that PEG-hirudin, because of its longer half-life, will reduce the amount of hirudin needed and will also lead to more stable plasma levels.

In patients with renal insufficiency there is a risk of overdosing because the elimination of hirudin is considerably impaired.

Hirudin can be used instead of UFH in cardiopulmonary bypass. Many cardiac surgeons are concerned about heparin-induced thrombocytopenia (HIT) in patients who have had prior heparin exposure. This risk does not exist with hirudin and in some centers hirudin is successfully used instead of UFH for the anticoagulation in combination with extracorporeal circulation.

**Bivalirudin**

Bivalirudin (hirulog) was compared with UFH in several studies in patients undergoing PTCA in acute coronary syndromes and has shown increased efficacy and reduced bleeding risk in both indications [3, 26–31]. Bivalirudin at a dose of 1 mg/kg every 8 h was effective in a dose-finding study on the prevention of thromboses after major hip or knee surgery [32].

In a recent study (HERO acute MI study) bivalirudin, given as an intravenous bolus of 0.2 mg/kg, followed by 0.5 mg/kg/h for 12 h and 0.2 mg/kg/h up to 60 h, led to an increased TIMI grade 3 coronary flow in a higher percentage of patients and to a reduced incidence of death, MI and cardiogenic shock compared to UFH [33].

In the large HERO 2 trial in 17,000 patients with acute MI bivalirudin has been compared with UFH [33]. Bivalirudin did not reduce mortality compared with UFH but reduced reinfarction by 30% within 96 h. There was a slight increase in mild and moderate bleeding in patients treated with bivalirudin.

**Argatroban**

Argatroban is a synthetic direct thrombin inhibitor derived from L-arginine and was initially developed by Mitsubishi Chemical Industries. In Japan, the drug is approved for Buerger’s disease, arteriosclerosis obliterans, acute cerebral thrombosis and anticoagulation of antithrombin-III-deficient patients undergoing hemodialysis. The use of argatroban in Japan will be discussed later in this issue.

Argatroban is excreted through the liver and therefore does not accumulate in renally impaired patients. Argatroban can be monitored using aPTT. Argatroban is a less potent inhibitor of thrombin than lepirudin, but this fact is associated with a (probably) larger therapeutic window. Argatroban will not produce antibodies and therefore long-term and repeat treatments are possible. Argatroban can easily be monitored [34].

Preliminary studies have shown successful use of argatroban for anticoagulation during percutaneous coronary intervention in HIT II-patients without excessive bleeding [35–40].

**Inogatran**

Inogatran is a low molecular weight specific thrombin inhibitor that has been investigated in patients with myocardial ischemia (TRIM study). In this study, inogatran was administered intravenously; no advantage in comparison to UFH could be demonstrated [41]. After stopping the inogatran infusion, a reactivation of thrombin and increased ischemic reactions have been described [42]. The concomitant use of inogatran and aspirin did not lead to potentiation of anticoagulant effects [43].

**Orally Active Thrombin Inhibitors**

**Melagatran**

H376/95, the prodrug of melagatran, is a small molecule (molecular weight 474 D) with a bioavailability of about 20%. The molecule is absorbed within 15–30 min, the highest melagatran plasma levels are reached within 1–2 h.

In the METHRO II-study melagatran (24 mg) was compared with dalteparin in 1,876 patients with total knee or hip replacement. In both patient groups, melaga-
tran was significantly more effective than dalteparin. The thrombosis incidence was almost halved in patients with total hip replacement or total knee replacement [44, 45]. Melagatran is presently being investigated in several further large clinical trials. One of the most interesting questions is whether thrombin inhibitors will be more effective or as effective as vitamin K antagonists with less bleeding complications.

**Thrombin Inhibitors in the Treatment of Heparin-Induced Thrombocytopenia**

Heparin-induced thrombocytopenia is observed in some patients during the first days of heparin treatment. Their platelet count normalizes after a few days under continued heparin administration. This phenomenon is not antibody related. It also has been named HIT type I (HIT I) or heparin-associated thrombocytopenia type I (HAT I).

Heparin-induced thrombocytopenia type II (HIT II) is triggered by an immune response to heparin. HIT II with thrombotic complications is frequently named HITT or HITTTS. However, many authors use the expression ‘HIT’ for all types of heparin-induced thrombocytopenia with and without thrombotic complications.

For the diagnosis of HIT II, a reduction of platelet count, especially during days 3–21 after onset of heparin treatment, and clinical symptoms are more important than laboratory tests. The frequency of HIT II in different patient groups is not well established. Frequencies of 2–5% of all heparin-treated patients have been described [46–48], but in prospective trials on the treatment of deep venous thrombosis and also in patients undergoing vascular surgery lower percentages have been reported [49–52].

HIT II has been observed in medically and surgically treated adults, during pregnancy and in newborns [53, 54]. The most dramatic and dangerous clinical complications of HIT II are venous and/or arterial thromboses. Patients with HIT-II-associated thrombosis have been reported to have a mortality of 25–30% and an amputation rate of up to 25% [54].

The pathophysiological process of HIT II is associated with the generation of antibodies which bind to a complex of heparin coupled with peptides and proteins on platelet and endothelial cell surfaces [55–62]. The currently accepted hypothesis of the pathophysiology of HIT II is based on the development of an IgG antibody to the heparin-PF4 complex that also recognizes the FcγRIIA receptor on platelets. Binding of this complex causes platelet aggregation and thrombin generation. This concept is probably incomplete because the interactions between endothelium and antibodies are still not fully understood. The antibodies are not heparin specific and have been shown to react with low molecular weight heparins and dermatans [63, 64]. Endothelial cells are probably involved in the mechanism via binding of the antibody to glycosaminoglycans on the cell surface [57, 61, 62] but also to other endothelial epitopes. Damaged endothelial cells probably are the starting point for thrombotic complications.

**Heterogeneity of HIT II Patients**

Patients with HIT II show a large variation of clinical and laboratory symptoms. They may have a fall in platelet count with a positive antibody test with a very good prognosis if heparin is discontinued.

Patients with thrombotic complications may have been treated with heparin for several reasons. They may have already existing thrombosis, they may have a fresh thrombotic complication and the diagnosis of HIT II may have been made after several thrombotic complications in a late stage with a very poor prognosis. In addition, prognosis also depends on underlying diseases. Because of this wide variation, general statements on mortality are misleading.

**‘Prophylaxis’**

If heparin-induced thrombocytopenia type II is suspected based on a fall in platelet count which cannot be otherwise explained and before clinical symptoms appear, heparin should immediately be discontinued and a prophylactic treatment against high-risk thrombosis should be started. There is no consensus as to the optimum prophylactic regimen to prevent imminent thromboses. Early discontinuation of heparin alone does not affect the thrombotic event rate, although early recognition may improve mortality [54]. But since early discontinuation of heparin treatment and an early switch to alternative anticoagulation are likely to prevent thrombotic complications and to reduce mortality, this kind of prophylaxis is clearly recommended.

**General Management**

Patients with HIT II and documented clinical thrombosis have a clear indication for anticoagulation and should be treated with danaparoid (Orgaran®) or a thrombin inhibitor monitored to obtain therapeutic aPTT levels. A coumarin-derivative may be initiated if
the thrombin inhibitor has been given for 4–7 days. Small
doses of the vitamin K antagonist should be used initially
and the thrombin inhibition should be continued until
therapeutic levels of the vitamin K antagonist are docu-
mented [38, 64, 65]. Among other treatment modalities,
two anticoagulants are used in Europe: Orgaran and the
thrombin inhibitor hirudin (lepirudin). In addition, arga-
troban has been released in the US for this indication.

For prophylactic treatment of HIT II patients, a
thrombin inhibitor can be initiated with low level of anti-
coagulation until the thrombocytopenia resolves [34].

Hirudin

Hirudin (lepirudin, Refludan®) has been approved for
the treatment of thrombosis in patients with HIT II in the
USA and Germany. Hirudin does not cross-react with
heparin antibodies as shown by in vitro data and success-
ful treatment of HIT II patients [66–68]. Two multi-cen-
ter trials on lepirudin have been published and have dem-
onstrated a statistically significant reduction in the prima-
ry efficacy endpoint assessed by an overall composite
index (new thrombosis, amputation and death) [69, 70] in
comparison with historic controls. Results on lepirudin
treatment in HIT II patients have also been published
recently [71, 72].

There are problems with hirudin treatment. Because
the drug is renally excreted, hirudin accumulates in pa-
tients with renal insufficiency, a dose reduction is often
necessary and monitoring with dose modifications be-
comes difficult [73, 74].

Another issue is the production of lepirudin-specific
antibodies. Around 40% of patients develop antibodies
against the drug. Furthermore, an investigation of data
from two prospective multicenter studies of lepirudin in
HIT type II patients revealed that the anticoagulant effect
of lepirudin was enhanced in about 45% of the patients in
whom such antibodies were detected [75]. In an investiga-
tion of anti-hirudin antibody isotypes in 23 patients with
HIT type II treated with lepirudin, 13 patients (56%) developed anti-hirudin antibodies of at least one isotype
[75].

In conclusion, the potential for an antibody response
complicates the management of the anticoagulant activity
of lepirudin.

Argatroban

Argatroban was approved in June 2000 in the US for
the prophylaxis and treatment of thromboses in patients
with HIT type II. Efficacy and safety in this indication
were demonstrated, in one historically controlled pivotal
study and one new follow-on supportive study [39].

Orgaran

The low molecular weight heparinoid (danaparoid) Or-
garan is a mixture of nonheparin polysulfated glycosami-
ognoglycans (heparan sulfate, dermatan sulfate, chondroitin
sulfate, low molecular weight heparin). Similar to heparin
in structure, danaparoid differs from heparin in its degree
of sulfation and molecular weight.

In vitro studies using HIT-II-positive sera showed a
cross-reaction in 18 versus 100% for heparin [34]. Danap-
paroid has been approved for the prophylaxis of thrombo-
sis after hip replacement surgery. It was used for many
years off-label for anticoagulation in HIT II-patients [76–
79] but is now approved for the treatment of HIT II-
patients in Europe. Disadvantages of danaparoid are dif-
ficulties in monitoring, lack of well-established dosing
guidelines [80], possibly increased postoperative bleeding
[81] and the possibility of cross-reactions leading to a
symptomatology similar to HIT II [78].

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