Review Paper

Heparin-induced thrombocytopenia

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Introduction

Heparin is widely used in medicine in the prevention and treatment of thromboembolic disease. While there is no denying its usefulness, one potentially fatal immune complication of heparin that is often not thought of and missed is heparin-induced thrombocytopenia. This condition has been associated with fatal thromboembolism involving both the venous and arterial systems. Recently a new syndrome of warfarin-induced acral tissue necrosis in patients with HIT and deep venous thrombosis was described. This paper will review recent developments in pathogenesis, laboratory testing and management of this disorder.

Definition

There are two clinically distinct forms of heparin-induced thrombocytopenia (HIT): type I HIT and type II HIT (Chong *et al.*, 1982; Chong, 1992; Chong, 1995). In type I there is an early onset (usually within 48 hrs of commencing heparin treatment) and is characterized by mild thrombocytopenia, the platelet count rarely dropping below $100x10^9/l$. The thrombocytopenia may return to normal even with the continuation of heparin treatment. The underlying cause is thought to be non-immune in nature and a possible mechanism is the platelet-proaggregating effects of heparin (Chong & Castaldi, 1986). This form of HIT is not associated with thrombotic complications and is of no known clinical significance. Currently there is no available laboratory test. The tested serum is HIT-Immunoglobulin G (IgG) negative.

HIT type II, on the other hand, is an immune-mediated reaction caused by an immunoglobulin (usually IgG) that occurs 5-14 days after the commencement of heparin. This is the type known to be associated with thrombotic complications.

In a recent paper aiming towards consensus on HIT (Warkentin *et al.*, 1998) the authors felt that HIT type I should be renamed non-immune heparin-associated thrombocytopenia and that the name Heparin-induced thrombocytopenia should be reserved strictly for the type II HIT.

Incidence

A recent review paper quotes the incidence for HIT as ranging from 0-30% (Chong, 1995). A number of factors including differences in the type of heparin used, the definition of thrombocytopenia and the patient populations studied are thought to contribute to this wide variation in incidence. A pool analysis (Hirsh *et al.*, 1995) and a recent analysis paper of 665 patients (Warkentin *et al.*, 1995a) showed the incidence to be closer to 3%. All forms of administration of heparin have been shown to cause HIT. This includes intravenous and subcutaneous heparin, heparin flushes used to maintain patency of indwelling catheters (Doty *et al.*, 1986) and heparin-coated catheters (Laster & Silver., 1988). The incidence is affected by the route and dosage administered. Schmitt & Adelman (1993) showed in their meta-analysis that there were more HIT cases in patients receiving intravenous heparin compared to those receiving subcutaneous heparin. Studies comparing porcine with bovine heparin have reported a higher incidence of HIT in patients receiving the latter (Schmitt & Adelman., 1993; Bell & Royall, 1980). The incidence of HIT with low molecular weight heparin (LMWH) has been shown to be significantly lower than heparin (Warkentin *et al.*, 1995a). However LMWH should not be used to treat HIT as several studies have shown 100% cross reactivity between LMWH and the antibody that causes HIT (Warkentin *et al.*, 1995a; Chong *et al.*, 1989a; Greinacher *et al.*, 1992a). Danaparoid has been shown to have a lower cross reactivity rate (10%) with the heparin antibody (Chong *et al.*, 1989a; Chong & Magnani, 1992).

Pathogenesis

It has been clearly demonstrated that the target antigen recognized by the HIT-IgG is a heparin/platelet factor 4 complex (Amiral *et al.*, 1992; Kelton *et al.*, 1994; Visentin *et al.*, 1994; Greinacher *et al.*, 1994). Platelet factor 4 (PF 4) is a heparin-binding protein found in the alpha granules of platelets. Unlike other IgG-mediated thrombocytopenic disorders in which the Fc 'tail' of the IgG is orientated away from the platelet (leading to platelet clearance by Fc receptor-bearing endothelial cells), in HIT, the Fc tail of the IgG interacts with platelet receptors (Warkentin, 1998) causing platelet activation (Chong *et al.*, 1982). IgG binding to platelets is via the platelet Fc receptors (Kelton *et al.*, 1988; Chong *et al.*, 1989b). This results in the generation of procoagulant platelet-derived microparticles (Warkentin *et al.*, 1994),

which is thought to account for the marked increase in thrombin generation seen in HIT (Warkentin, 1996a). This, in turn, results in the increased venous and arterial thrombotic complications in heparin-induced thrombocytopenia. Platelet microparticles are measured by flow cytometry (Lee *et al.*, 1996) and are not specific to HIT as they have been described in unstable angina (Katopodis *et al.*, 1997). Other drug dependent thrombocytopenias do not bind to Fc receptors (Christie *et al.*, 1985).

There is an increased expression of platelet IgG Fc receptors in HIT and it has been suggested that this may contribute to the pathogenesis of HIT as patients with serious thrombotic complications and those who died shortly after the diagnosis had a greater increase in platelet Fc receptors (FcRs) than those who had mild HIT without any serious thrombotic complications (Chong *et al.*, 1993).Sires of pre-existing pathology are also thought to contribute to thrombosis. It has also been shown that there is a significant association between cardiovascular complications and the occurrence of arterial thrombosis (Boshkov *et al.*, 1993), and a strong association of venous thrombosis with recent surgery (Warkentin *et al.*, 1995a; Boskov *et al.*, 1993; Warkentin & Kelton, 1996).

Activation of endothelial cells via heparan sulphate-PF4 complexes is also believed to contribute to the clinical picture of HIT (Visentin *et al.*, 1994; Greinacher *et al.*, 1994; Cines *et al.*, 1987). The HIT-antibody is not heparin-specific, as heparin can be substituted by a variety of sulphated oligosaccharides dependent on their grade of sulphation. (Greinacher *et al.*, 1992a; 1992b; 1993). Clinically pentosan polysulphate (Tardy-Poncet *et al.*, 1994) and polysulphated chondroitin sulphate (Greinacher *et al.*, 1992b) have been associated with thrombocytopenia and thrombosis.

Type II HIT can also occur only in the presence of IgM and/or IgA anti-PF4/heparin antibodies (Amiral *et al.*, 1996a) or antibodies directed against PF4-related chemokines (Amiral *et al.*, 1996b). It is interesting and intriguing that only a subset of patients on heparin therapy develop HIT. While there is still no clear-cut answer, Burgess *et al.* (1995) suggested that a single amino acid mutation of the Fcg receptor is associated with the development of HIT. In their study they found that all of the HIT patients examined had at least one copy of the $FcgRIIA^{His[31]}$ allele and that none of the HIT patients were found to be $FcgRIIA^{Arg[31]}$ homozygous. However, other studies have failed to show an association between $FcgRIIA_{His[31]}$ and HIT (Arepally *et al.*, 1997; Bachelor-Loza *et al.*, 1998).

Warkentin & Kelton have put forward an 'iceberg model' for HIT (Warkentin, 1996b;Warkentin, 1997). In this model, they have suggested (i) only some anribodies formed with heparin use trigger thrombocytopenia; (ii) thrombotic events take place almost exclusively in patients with HIT antibodies and thrombocytopenia; and (iii) a number of biological and clinical factors interact to bring about the pathogenic potential of HIT antibodies.

Clinical Features of HIT

A plarelet fall beginning after day 5 to 8 of heparin therapy should alert the clinician to the possibility of HIT (Warkentin *et al.*, 1995a). It has been shown that a >50% platelet count fall after >5 days of heparin correlates best with HIT-IgG formation in postoperative patients at risk of HIT (Warkentin *et al.*, 1995b). These patients have increased risk of thrombosis. Unexplained heparin resistance is an unusual presentation. This is most likely due to the release of PF4 which binds tightly to heparin and hinders its anticoagulant action.Heparin has been shown to be strongly associated with thrombotic events (Warkentin *et al.*, 1995a; Boshkov *et al.*, 1993). The thrombosis can occur despite normal platelet counrs (Hach-Wunderle *et al.*, 1994; Klement *et al.*, 1996). Previous studies have emphasized the close association of HIT with arterial thrombosis (Towne *et al.*, 1979; Abu Rahma *et al.*, 1991; Cimo *et al.*, 1979;Laster *et al.*,1987). A 14-year study of HIT (Warkentin & Kelton, 1996), however, found that venous thrombotic events were far more common than arterial thrombotic events (4:1 ratio). Moreover, it was observed that the cohort of patients who were initially recognized with isolated thrombocytopenia were at high risk (approximately 50%) of developing a thrombotic event over the subsequent 30 days following the diagnosis of HIT. Unusual thrombotic complications that have been described include mesenteric artery or vein occlusion, dural sinus thrombosis, adrenal haemorrhage and infarction and venous thrombosis (Kelton & Warkentin, 1998).

Patients on heparin who develop new areas of thrombosis should alert the clinician to the possibility of HIT. Recently a new syndrome of warfarin-induced acral tissue necrosis in patients with HIT and deep vein thrombosis was described (Warkentin *et al.*, 1997). A number of features are characteristic of this syndrome. Firstly, the patients have invariably received warfarin at the onset of their gangrene. Secondly, there is a marked anticoagulant response to warfarin. Thirdly, there is a marked decrease in the protein C levels and marked thrombin-antithrombin complexes during the initiation of warfarin therapy. None of the patients who developed this syndrome had a congenital thrombophilia.

Heparin-induced skin lesions have been described (Platell & Tan, 1986; Ulrick & Manoharan, 1984). The skin lesions may be erythematous plaques or necrosis (Warkentin, 1996c). Formation of heparin-dependent, platelet- activating IgG is a consistent feature of patients who develop heparin-induced skin lesions, even in the absence of thrombocytopenia (Warkentin 1996c; Warkentin *et al.*, 1995b). About 30% of patients wirh skin lesions at heparin injection sites develop thrombo-

cytopenia (Warkentin *et al.*,1996c). This subset of patients is at risk of thrombosis, particularly arterial thrombosis (Warkentin, 1997b). LMWH has also been shown to cause skin nectosis at the site of injection (Real *et al.*, 1995; Ojeda *et al.*, 1992) and also distant from heparin injection sites (Tietge *et al.*, 1998; Balestra *et al.*, 1994).

Acute life-threatening systemic reactions (Warkentin *et al.*, 1992) and transient global amnesia (Warkentin *et al.*, 1994b) have been described following administration of intravenous heparin.

Laboratory tests

Laboratory tests for HIT can be categorized into 3 groups. Tests in group 1 include those measuring platelet aggregation or activation as endpoints. Tests in group 2 include those which measure platelet-associated IgG, or demonstrate immunoglobulin binding to platelet membrane in the presence of heparin. Tests in group 3 measure immunoglobulin binding to isolated PF4-heparin complexes (Griffiths & Dzik, 1997). In practice only tests in group 1 and 3 are useful in the diagnosis of HIT. Group 1 tests are functional (or activation) assays. The 2 most commonly used functional methods are the serotonin release assay (SRA) and the platelet aggregation test (PAT). Both these tests are essentially similar in principle, the main difference being the endpoint measured. The underlying principle is that normal platelets are activated *in vitro* in the presence of therapeutic concentrations of heparin. In the case of PAT, the test is positive when there is greater than 20% increase in light transmission with therapeutic doses of heparin while aggregation is inhibited in the presence of high dose heparin (Kelton *et al.*, 1984; Favaloro *et al.*, 1992). The two-point SRA, first introduced by Sheridan *et al.* (1986) is considered positive only if there is greater than 20% release of ^{14C} serotonin at therapeutic concentration of heparin and the release is inhibited with high dose heparin. Because there is so much variability in donor reactivity to HIT serum (Watkentin *et al.*, 1992b), it is important to select only donor whose platelets are easily activated by the HIT antibody. The use of washed platelets increases the sensitivity in patients with clinical HIT (Sheridan *et al.*, 1986; Greinacher *et al.*, 1994b). Another important quality control measure is to ensure that appropriate controls are used. This should include a 'weak positive' HIT sera to ensure that weak but significant HIT sera are not missed. The assay used should be a two-point assay, that is, a positive result at 0.1 to 0.3 U/ml heparin and a negative result (ie no activation of platelets) at 10 to 100 U/ml heparin. With a two-point assay, the specificity is at least 95% (Warkentin *et al.*, 1995a; Favaloro *et al.*, 1992; Sheridan *et al.*, 1986; Chong *et al.*, 1993b).

The SRA is generally considered the 'gold standard' for functional tests. It's clinical usefulness was shown in a large trial involving 665 patients (Warkentin *et al.*, 1995a). However it involves radioactive material and is a much more tedious procedure. As a result, more laboratories prefer the use of platelet aggregation tests.

Group 3 tests are antigenic tests. These assays use the enzyme-linked immunosorbent assay (ELISA) whereby the patient immunoglobulin (antibody) recognizes the heparin/platelet factor 4 complex (antigen).

Currently the recommendation would be to use both functional and antigenic assays for the diagnosis of HIT. This is because there have been discrepancies in results between the 2 types of assays in up to 20% of patients (Greinacher *et al.*, 1994b; Arepally *et al.*, 1995). This can be partly explained by the fact that functional assays detect antigens other than heparin-PF4 such as interleukin-8 and neutrophil-activating-peptide 2 (Amiral *et al.*, 1996b) which are missed by antigenic assays. On the other hand, antigenic assays tend to pick up IgA or IgM antibodies against heparin-PF4 complex, which are missed by functional assays (Amiral *et al.*, 1996a; Warkentin, 1997).

Treatment

Because there is increased generation of thrombin in HIT, treatment should be aimed either at stopping thrombin generation (such as danaparoid or LMWH) or inhibiting thrombin (such as hirudin or argatroban. Currently the 2 favoured treatment agents are danaparoid and hirudin.

Danaparoid is a heterogenous mixture of glycosaminoglycans extracted from porcine intestinal mucosa (Chong *et al.*, 1989a). It does not contain heparin but consists of a mixture of heparan sulphate, dermatan sulphate and chondroitin sulphate (Chong, 1995). Its mechanism of action is predominantly through anti-III inhibition of activated factor Xa (Meuleman, 1992). In contrast to LMWH, dapanaroid has a low cross-reaction rate (10%) with the heparin-dependent antibody (Chong & Magnani, 1992). Patients who have failed treatment with LMWH have been successfully treated with danaparoid (Greinacher *et al.*, 1992a; Greinacher *et al.*, 1992c). An advantage of danaparoid is that it specifically inhibits platelet aggregation and thromboxane B_2 induced by the HIT antibody (Chong *et al.*, 1989c). However some patients have developed new and fatal episodes of HIT whilst being treated with this drug (Magnani, 1993). For this reason some authors recommend that the patient's serum be tested against danaparoid for cross reactivity prior to its usage. Others like Ortel TL and Chong BH (in press) do not recommend routine cross reactivity testing. Instead they have recommended that the cross reactivity test be performed only for the following indications: if there is failure of the thrombocytopenia to respond or if thrombocyropenia recurs or is aggravated; if there is extension of the thromboembolic event or a new

thromboemboli appears. Routine monitoring of plasma anti-Xa activity is not essential. Ortel and Chong (in press) have, however, recommended that plasma anti-Xa activity be monitored in certain selective groups of patients including underweight (<55 kg) or juvenile patients, patients with severe renal dysfunction, and gross overweight (>100 kg) patients.

Hirudin is another option in the treatment of HIT. It is extracted from the salivary glands of the medicinal leech *Hirudo medicinalis*. It has several advantages. Firstly it is a drug that is structurally different from heparin and hence there is no risk of cross reactivity. Secondly it is a thrombin-specific inhibitor which binds specifically to thrombin forming a noncovalent, irreversible 1:1 stoichiometric complex. When bound by hirudin, thrombin is incapable of either binding to fibrinogen or cleaving substrate (Markwardt, 1991). Hirudin has been shown to be effective in the treatment of HIT (Schiele *et al.*, 1995; Schmidt & Lang, 1997). In a recent study Greinacher *et al.* (1996) treated 82 HIT patients with hirudin. They looked at the incidence of new thromboembolic complications, limb amputations and death as the combined endpoint and compared this with a historical control of 120 patients treated before hirudin was available. At day 7 they found that the endpoint for hirudin was 10% compared to 23% for the historical group. By day 35,the percentage had risen to 35% for hirudin but this was still lower than the 52% reported for the historical group. They concluded that r-hirudin was an effective anticoagulant allowing rapid recovery of platelet counts and that it had a strongly reduced incidence of combined endpoint of mortality, limb amputation and new thromboembolic complicationsas compared to the historical group. However there are 2 disadvantages associated with r-hirudin therapy. Firstly, some patients can develop an IgG antibody resulting in a prolonged APTT (Eichler & Greinacher, 1996). Secondly, it is excreted mainly by the kidneys and is a disadvantage in patients with renal failure.

Argatroban is another thrombin inhibitor that could be potentially used for the treatment of HIT. There have been reports of its successful use in HIT (Lewis *et al.*, 1998; Lewis *et al.*, 1997a; Matsuo *et al.*, 1992; Lewis *et al.*, 1997b). An advantage of argatroban is that it excreted normally even in patients with renal failure (Hursting *et al.*, 1996). Currently argatroban looks promising.

Ancrod, an extract of snake venom that is a potent defibrinogenating agent, has been used in the treatment of HIT (Demers *et al.*, 1991). There are, however, two disadvantages to its use. Firstly it does not block thrombin generation, and secondly it requires monitoring by a clotting (Clauss) technique which may not be available. Warkentin *et al.* (1998) pointed out that warfarin-induced venous limb gangrene has occurred in patients receiving ancrod (Warkentin, 1997) and hence they would not recommend the use of ancrod.

A number of other treatments have been used in the treatment of HIT.These include Intravenous Immunoglobulin G (Frame *et al.*, 1989; Nurden *et al.*, 1991), plasmapheresis (Bouvier *et al.*, 1988; Nand & Robinson, 1988; Brady *et al.*, 1991; Poullin *et al.*, 1998), thrombolytic therapy (Cohen *et al.*, 1985; Fessinger *et al.*, 1984; Mehta *et al.*, 1991), aspirin (Janson *et al.*, 1983), iloprost (Kappa *et al.*, 1990) and surgical procedures such as thrombectomy or embolectomy (Towne *et al.*, 1979; Laster *et al.*, 1987) for limb salvage. Currently these treatments are best used as adjuncts rather than first line treatment for HIT.

Conclusion

In summary HIT is a not uncommon, potentially lethal condition of heparin. It should be suspected in any patient on heparin who develops unexplained thrombocytopenia, heparin resistance and new thrombosis. Heparin should be immediately stopped and replaced with another antithrombotic agent such as danaparoid or hirudin.

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