

DRUG-INDUCED PLATELET ANTIBODIES

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Introduction

Hypersensitivity to drugs may lead to the development of drug-induced platelet antibodies and consequently thrombocytopenia and bleeding, usually in the form of purpura. The association of drug and purpura was reported by Vipari⁽¹⁾ even before the role of platelets in haemostasis was recognised. The relationship between drug ingestion and thrombocytopenia was demonstrated by Rosenthal in 1928 when he rechallenged a patient who had just recovered from quinine-induced thrombocytopenia

of drug-induced thrombocytopenia was confirmed by Ackroyd in 1951.⁽³⁾ In his pioneering experiments, he demonstrated the presence of drug-induced antibodies in the plasma of patients with serdomid-induced thrombocytopenia. He showed that incubation

with complement and platelet agglutination in its absence. Since then a large number of drugs (> 50) have been reported to cause immune thrombocytopenia. Some of the commonly implicated drugs include quinine, quinidine, heparin, co-trimoxazole,

cimetidine. Of these drugs, only antibodies induced by quinine/quinidine and heparin have been extensively studied.

Drug-induced platelet antibodies usually belong to the IgG immunoglobulin class, but occasionally IgM and/or IgA antibodies may be present. Most IgG antibodies belong to IgG1 subclass, but a few patients may also have IgG3 antibodies. Arepally et al⁽⁶⁾ reported that the heparin-induced antibody is almost exclusively of the IgG2

subclass, usually fix complement and, in fact, this characteristic was the basis of some of the earlier in vitro tests for the detection of the antibodies. Following complement fixation, the

⁽⁷⁾ whereas the heparin-
⁽⁸⁾ instead. The majority of drug-

induced antibodies agglutinate platelets, but a small proportion (0-25%) of quinine/quinidine-induced antibodies do cross-react with the stereoisomer of the drug that originally provokes the immune

⁽⁹⁾ In contrast, heparin-induced antibodies are less specific. They will react with platelets in the presence of a range of highly sulphated polymeric saccharides of

pentosan polysulphate and dextran sulphate, in addition to unfractionated heparin.

Antibody-Drug-Platelet Interaction

In the past, two mechanisms have been postulated for the interaction of drug-induced antibodies with platelets and the drug. Ackroyd (1951)⁽³⁾ postulated that the drug acting as a hapten reacted with platelets to form a drug-platelet antigenic complex. The antibody then bound to the antigenic complex and caused platelet lysis or agglutination. Later, Shulman (1958)⁽⁷⁾ proposed an alternative mechanism based on his studies on quinidine-induced antibodies. He suggested that the antibodies reacted first with the drug to form drug-antibody complexes which then became nonspecifically adsorbed to platelets. The platelets, in this hypothesis, are 'innocent bystanders' as the antibodies have no specificity for platelets. Shulman's hypothesis, widely known as the 'innocent bystanders' mechanism, became widely accepted for the next 20 years. However, the validity of Shulman's hypothesis has recently been challenged in the light of new experimental data.^(4,9,15) It is now clear that, in general, the drug first binds to a platelet membrane protein and induces a conformational change so that a cryptic epitope becomes accessible or a neo-epitope (new epitope) which is previously absent, is formed. The drug-induced antibody then reacts with and binds to the neo-epitope. However, the 'innocent bystanders' hypothesis still holds true with the heparin-induced antibody,^(11,14) which appears to be different from other drug-induced antibodies.

Quinine/Quinidine-Induced Antibodies

There is now little doubt that the quinine/quinidine-induced antibodies recognise platelet membrane glycoprotein (GP) Ib-IX^(9,12) and GP IIb-IIIa⁽¹⁵⁾ complexes as target antigens. These are major platelet membrane glycoproteins. GP Ib-IX is the platelet receptor for the von Willebrand factor and GP IIb-IIIa is the platelet fibrinogen receptor. In our hands, the antibody in almost every patient with quinine/quinidine-induced thrombocytopenia has specificity for GP Ib-IX complex and in about a third of the patients there is another antibody that reacts with GP IIb-IIIa complex.⁽¹²⁾ However, other investigators have found that the antibody with specificity for GP IIb-IIIa is much more widely distributed.⁽¹⁵⁾ We have recently attempted to map the epitope for the quinine/quinidine-induced antibodies on GP Ib-IX complex.⁽⁹⁾ In the majority of patients with quinine/quinidine-induced thrombocytopenia, the antibodies recognise the remnant of GP Ib-IX complex after a major portion of the a-chain of GPIb (termed glycolalicin) has been removed by protease. This suggests that the epitope is situated on GP IX, the b-chain of GP Ib or the stump of GP Iba (GPIba fragment which remains associated with the platelet membrane after proteolytic cleavage). We now have several lines of experimental evidence which indicate that the epitope is probably on GP IX.⁽⁹⁾ First, the binding of the antibody to platelets is completely blocked by the anti-GP IX monoclonal antibody, SZ 1. Second, the antibody binding is partially inhibited by another anti-GP IX monoclonal antibody, FMC 25. Third, we found that all quinine/quinidine-induced antibodies bound strongly and drug-dependently to intact purified GP Ib-IX complex, but when we dissociated the purified glycoprotein complex into GP Ib and GP IX, only one antibody reacted weakly with GP IX and no antibodies reacted with GP Ib. These data suggest that the antibodies recognise a neo-epitope on GP IX which becomes available only after the binding of quinine /quinidine to GP Ib-IX complex and the neo-epitope is disrupted or concealed when GP IX is dissociated from GP Ib.

recognises a quinine/quinidine-dependent epitope in the amino-terminal portion of the a-chain of GP Ib.

Visentin and co-workers have characterised the binding domains of the
(15) They have identified

a GP IIb-IIIa complex-specific epitope while others recognised an epitope on GP IIb or an epitope on GP IIIa.

for GP V have also been detected in the sera of patients with quinidine-induced thrombocytopenia. The clinical significance of these antibodies, however, is unclear as they bind to GP V independently of the drug.

to premature clearance of platelets by macrophages in the reticulo-endothelial system and will result in thrombocytopenia. Although complement fixation and platelet lysis

uncertain whether platelet lysis occurs in vivo and contributes to the thrombocytopenia.

Heparin-Induced Antibody

thrombocytopenia recognises an epitope on platelet factor 4 (PF4),(11,14) a protein in the a-granules of platelets. The antibody reacts with PF4 and heparin to form a trimolecular
(17,18) It

activation, release of granular constituents, thromboxane biosynthesis and platelet aggregation. The potent platelet activating property of this antibody is the basis of the laboratory tests used to detect the antibody. The antibody also induces the formation of platelet microparticles. Platelet activation induced by the antibody probably also occurs in vivo as we have shown elevated plasma levels of b-thromboglobulin and P-
(21) In vivo platelet

in patients with HIT.⁽¹⁹⁾

induced antibody in vitro and the reasons for this are still uncertain.⁽²²⁾
polymorphic forms of FcγRII A, namely one with histidine and another with arginine at position 131 of the protein. There is conflicting evidence as to whether platelets carrying
(23,24)

However, three groups of investigators have reported an increased proportion of individuals with FcγRIIAHis131 genotype compared with individuals with

former genotype to the development of HIT. Besides FcγRIIA polymorphism, increase in platelet Fc receptor numbers (which occurs for example in acute inflammatory state) may

expressed on each platelet is increased up to 3- to 4-fold above normal levels during the

acute phase of HIT.⁽²⁶⁾ Platelets with such high Fc receptor density have been shown to be more reactive to the heparin-induced antibody, although the slight variation in platelet Fc receptor numbers among healthy individuals does not significantly influence antibody-platelet interaction.

The antibody also binds to PF4 that is attached to heparan sulphate of the surface of endothelial cells.⁽¹⁴⁾ This occurs in the absence of exogenous heparin. The binding of the antibody to endothelial cells may cause immunoinjury to the cells and it has been suggested that this may contribute to thrombosis in patients with HIT, even though the thrombotic complications in these patients usually resolve or cease to progress after heparin withdrawal.

Other Drug-Induced Antibodies

There have been only a few studies on other drug-induced antibodies besides those induced by quinine/quinidine and heparin. These antibodies, including those induced by cephalosporins, bind to platelets probably by a mechanism similar to that of quinine/quinidine-induced antibodies. Like quinine/quinidine-induced antibodies, they may also have specificity for the major glycoproteins on platelets such as GP IIb-IIIa and GP Ib-IX. For instance, Curtis et al⁽²⁷⁾ reported antibodies induced by sulfamethoxazole and sulfisoxazole which recognise calcium-dependent epitopes on GP IIb-IIIa complex.

Clinical Aspects

Clinical Features

Patients with drug-induced thrombocytopenia, except those with HIT, usually present with purpura and mucosal bleeding which may take the form of epistaxis, blood blisters in the mouth and occasionally haematemesis and melaena. Fortunately, serious bleeding such as intracranial and retroperitoneal haemorrhage are uncommon.⁽⁴⁾ In patients with quinine/quinidine-induced thrombocytopenia, the onset of symptoms is usually abrupt and the thrombocytopenia severe ($< 10 \times 10^9/L$). The onset of thrombocytopenia induced by other drugs is often less abrupt and thrombocytopenia less severe. The patients may be asymptomatic and the thrombocytopenia may be detected on routine blood counts. The symptoms, if present, often occur after the patients have been taking the drugs for several days or weeks, continuously or intermittently. In HIT, the onset usually occurs 4-14 days after institution of heparin.⁽¹⁴⁾ If there is a previous exposure to the drug, the thrombocytopenia may develop within hours or 1-2 days of drug administration. After drug withdrawal, the platelet count rises to normal levels in 5-7 days but occasionally recovery may take longer, sometimes up to a month. It has been suggested that in these cases the antibody may recognise a drug-independent epitope or the drug, such as gold, is sequestered in the tissues and takes a long time to be eliminated from the body.

Unlike patients with other drug-induced thrombocytopenias, patients with HIT have a high risk of developing venous and less frequently arterial thrombo-embolic complications.⁽¹³⁾ These may include deep venous thrombosis (DVT), pulmonary embolism, arterial occlusion of the leg causing limb ischaemia, acute myocardial

infarction and stroke. The thrombosis tends to occur at sites of pre-existing pathology, for example post-operative patients are more likely to develop DVT. The thrombosis may be extensive and may occur at multiple sites. In fact, very unusual forms of thrombosis have been described in association with HIT, including multiple hepatic infarcts, haemorrhagic necrosis of the adrenals, biventricular thrombi, transient global amnesia and skin necrosis. In a small proportion of patients, disseminated intravascular coagulation may occur. Patients with thrombotic complications associated with HIT have an approximately 30% mortality rate and 20% risk of leg amputation.

Another serious complication associated with drug-induced thrombocytopenia is haemolytic uraemic syndrome (HUS), which has recently been described in patients with quinine-induced thrombocytopenia.⁽²⁸⁾ The patients presented with chills, diaphoresis, nausea and vomiting, abdominal pain and petechiae following quinine ingestion. All patients experienced anaemia, severe thrombocytopenia, increased lactate dehydrogenase, elevated serum creatinine and oliguria. Quinine-dependent platelet antibodies and, in some patients, red cell and granulocyte antibodies were detected.

Diagnosis

Diagnosis of drug-induced thrombocytopenia is usually made clinically based on the following criteria^(4,13): (1) Development of thrombocytopenia during drug therapy; (2) Resolution of thrombocytopenia after drug withdrawal; (3) Exclusion of other causes of thrombocytopenia; and (4) Recurrence of thrombocytopenia on rechallenge with the offending drug. It is now not ethical to rechallenge the patient with the drug because of the risk of inflicting harm upon the patient. Furthermore, it may not be possible in some patients to exclude other causes as the patients may have co-morbid conditions, such as infection, which are also known to cause thrombocytopenia. It is important, particularly in these patients, to demonstrate the presence of a drug-dependent antibody in vitro using a specific and sensitive laboratory test. If the patient is taking a number of drugs that may potentially cause thrombocytopenia, the test may help to identify the offending drug.

There are two types of tests for detection of drug-dependent antibodies. The first type is a functional assay^(4,13,29) and the second is an immunochemical assay.^(9,14,30) The former is based on the fact that binding of the antibody to platelets in the presence of the drug will induce an effect such as ¹⁴C-serotonin release, platelet aggregation or platelet lysis⁽³⁰⁾ with release of ⁵¹Cr. These types of tests, particularly ¹⁴C-serotonin release and platelet aggregation, are very useful for the detection of the heparin-induced antibody^(22,29) but not other drug-induced antibodies. For maximum sensitivity and specificity, the tests should be performed with washed platelets from a donor whose platelets are known to react well to the heparin-induced antibody⁽²²⁾ in a two-point format described by Sheridan et al.⁽²⁹⁾ An enzyme-linked immunosorbent assay (ELISA), which measures antibody against PF4-heparin complex, is also highly sensitive and specific for the detection of the heparin-induced antibody.⁽¹⁴⁾ Since neither test will detect the antibodies in all patients with HIT, some investigators have recommended that the two tests be used together to obtain the best results.

For the detection of other drug-induced antibodies such as the quinine/quinidine-induced antibodies, the best methods are immunochemical assays such as flow cytometry

and antigen-capture ELISA, e.g., the monoclonal antibody-specific immobilisation of platelet antigen assay, popularly known as MAIPA assay.^(9,30)

Management

The most important step in the management of patients with drug-induced thrombocytopenia is cessation of the offending drug. In patients with severe thrombocytopenia and bleeding, glucocorticosteroid and platelet transfusion are frequently given but their clinical usefulness is unclear. In patients with HIT, platelet transfusion is contra-indicated as it may precipitate a thromboembolic event. After cessation of heparin in patients with HIT, particularly in those with an acute thrombosis, treatment with an alternative anticoagulant is required.⁽¹³⁾ Orgaran (a LMW heparinoid), ankyrod, hirudin or its analog may be used and should be administered until the acute thrombosis is under control. Thereafter, a vitamin K antagonist such as warfarin may be added. A LMW heparin is not recommended because of its high cross-reactivity rate with the heparin-induced antibody.⁽¹³⁾

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