Anti-heparin–platelet factor 4 antibodies after cardiopulmonary bypass: role of HLA expression

Heparin administration may trigger the formation of antibodies against the heparin-platelet factor 4 complex. This study was conducted to evaluate whether there is a relationship between HLA antigens and antibodies causing heparin-induced thrombocytopenia (HIT) in patients undergoing cardiac surgery. A significant expression of the HLA-DR3 antigen was found among patients who developed HIT antibodies.

Heparin is the most common anticoagulant used in patients undergoing cardiopulmonary bypass (CPB) for cardiac surgery. Heparin administration may cause the formation of antibodies (usuallly IgG) targeted against an antigen complex made up of platelet factor 4 and heparin.[1] Heparin-platelet factor 4 antibodies (anti H-PF4) have been found in 50% of patients after CPB and cardiac surgery.[2] Most commonly no clinical or hematologic alterations are correlated with the presence of anti H-PF4 in the plasma. However anti H-PF4 has been associated with thrombocytopenia in about 3% of the cases and with life-threatening arterial or venous thrombosis in about 1% of the cases.[3] Although many aspects of heparin-induced thrombocytopenia (HIT) have been elucidated,[4] no information is available on the mechanisms regulating such a wide patient population-dependent difference in frequency and clinical significance of anti H-PF4 formation.

Immune response is modulated by the human leukocyte antigen (HLA) system. The amino acid sequence of individual HLA molecules determines whether or not specific peptides will bind and will be presented at the surface of antigen-presenting cells to T-lymphocytes.[5] Class I molecules play a role in the antigen recognition of T-cytotoxic lymphocytes; class II HLA molecules have a role in the antigen recognition of the T-helper lymphocytes. Several studies found evidence of an HLA antigen-associated genetic predisposition to several diseases and autoimmune conditions.[5]

This study wanted to establish whether a genetically-induced mechanism might be involved in the presence of H-PF4 in patients receiving heparin for CPB and cardiac surgery. We tested the hypothesis that the presence of H-PF4 is associated with HLA class II antigens.

All patients undergoing CPB during a 12-month period were invited to enter this study. Of the 86 patients entered, both preoperative and postoperative blood samples were available in only 69 patients. There were 51 males and 18 females; their age ranged between 33 and 79 years. The study was approved by the Ethics Committee and all patients signed informed consent prior to entering the study. All patients received heparin preoperatively, within 45 days from the operation. During the operation porcine heparin [Vister, Parke-Davis, Linate (MI), Italy] was administered at a dose of 300 U/kg to maintain an ACT > 400 seconds. Blood samples were taken 2 hours before surgery (T1), 6 days after surgery (T2) and 3-4 weeks after surgery (T3). Thrombocytopenia was defined as a platelet count decrease of at least 50% compared to the postoperative peak values 5 days after the operation. Anti H-PF4 was measured using an enzyme-linked immunosorbent assay (ELISA) (GTI, Brookfield, WI, USA). This ELISA-assay was shown to be less sensitive but more specific than another commercially available ELISA kit.[6] Class I and class II HLA tissue typing was done using a conventional technique on blood samples taken at T1. A population of 860 healthy local people was used as a control. The association between HLA antigens in patients with and without anti H-PF4 was evaluated by a 2×2 contingency table.

Ten patients had anti-H-PF4 antibodies in at least one of the collected samples. Expression of class II HLA-DR3 antigen was higher in patients with anti-H-PF4 than in patients without (RR=7.4; χ^2=6.1; p=0.01) and in normal controls (RR=5.3; χ^2 Yates =6.1; p=0.01) (Table 1). The distribution of HLA-DR3 did not differ between patients without anti H-PF4 and normal controls. No other significant differences in HLA class I and HLA class II distribution were found (Table 1). None of the anti-H-PF4 positive patients developed thrombosis or thrombocytopenia. The DR3 allele has been shown to be associated with several immune-mediated diseases such as rheumatoid arthritis, diabetes, systemic lupus erythematosus and other conditions.[7] In our study we found that the expression of HLA-DR3 antigens was higher than in the normal population only in those patients in whom administration of heparin caused the formation of antibodies against the heparin-PF4 complex. The presence of an HLA molecule that recognizes the anti-anti-H-PF4 as a non-self epitope would explain the variability in the manifestations of the immune reaction to heparin administration. The establishment of a genetically determined immunologic risk category among patients receiving heparin may lead to new options for the treatment of HIT. Nevertheless, in view of the small number of patients in our series, this finding needs to be confirmed by studies on larger series.

Table 1. Distribution of HLA class II antigens.

DR antigens	Negative 59 (%)	RR	χ^2	p	Positive 10 (%)	RR	χ^2	p	Control 860 (%)
DR 1	8 (13.5)	0.5	0.0	ns	1 (10)	0.6	0.0	ns	134 (15.6)
DR 2 (15)	13 (22)	0.7	0.0	ns	0	0.0	0.9	ns	143 (16.6)
DR 2 (16)	3 (5)	0.0	0.0	ns	0	0.0	0.2	ns	81 (9.4)
DR 3	7 (11.8)	7.4	6.2	0.01	5 (50)	5.3	6.1	0.01	137 (15.9)
DR 4	9 (15.2)	2.3	0.5	ns	3 (30)	2.4	0.7	ns	129 (15.0)
DR 5 (11)	28 (47.4)	0.7	0.0	ns	4 (40)	0.6	0.1	ns	433 (50.3)
DR 5 (12)	3 (5)	2.0	0.0	ns	1 (10)	1.8	0.0	ns	49 (5.7)
DR 6 (13)	15 (25.4)	1.9	0.3	ns	4 (40)	3.3	2.3	ns	145 (16.9)
DR 6 (14)	8 (13.5)	0.0	0.5	ns	0	0.0	0.4	ns	97 (11.3)
DR 7	15 (25.4)	0.3	0.4	ns	1 (10)	0.3	0.4	ns	206 (23)
DR 8	0	0.0	0.0	ns	0	0.0	0.1	ns	60 (4.9)
DR 9	0	0.0	0.0	ns	0	0.0	0.0	ns	13 (1.5)
DR 10	2 (3.4)	0.0	0.0	ns	0	0.0	0.0	ns	34 (3.9)

Frequency and percentage distribution of HLA-DR class II antigens (DR subtypes in brackets) in the antibody negative patients, in the antibody positive ones and in the control population. Relative risk (RR), χ^2 test values using Yates' correction and p values of the comparisons between the groups.

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