The incidence of aspirin resistance in heart transplantation recipients

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Abstract

Introduction: Coronary allograft vasculopathy can cause as many deaths as infections or rejection episodes within 3 years following heart transplantation.
Aim: To compare the aspirin resistance rate in an allograft heart transplantation population and in a control group by laboratory tests including the Aspirin-Resistant Patients Identification Test (ASPItest).
Material and methods: A total of 24 heart recipients (20 men and 4 women) at a mean age of 48 ±13 years who underwent routine clinical follow-up were consecutively enrolled in group 1. The control group consisted of 24 patients (19 men and 5 women) at a mean age of 64 ±7 years waiting for coronary artery bypass grafting in our department. All patients were treated with a standard dose of 75 mg aspirin (ASA) daily.
Results: Aspirin resistance was evaluated by the Multiplate platelet function test. The ASPItest revealed a mean value of 27 ±22 U in the transplant group. Results above 30 U were obtained in 8 (34%) patients, with a mean value of 50.3 ±20.6 U, indicating aspirin resistance. In the control group ASPItest results above 30 U were obtained in 5 (20%) patients, with a mean value of 43.3 ±6.4 U.
Conclusions: There is a high incidence (34% vs. 20%, NS) of ASA resistance in heart transplantation recipients and in the general population, respectively.
Key words: heart transplantation, aspirin, ASPItest.

Streszczenie

Wstęp: Choroba naczyniowa graftu niesie ze sobą ryzyko zgonu porównywalne ze śmiertelnością z przyczyn infekcyjnych czy związanych z epizodami odrzucania w pierwszych 3 latach po operacji transplantacji serca.
Cel: Porównanie oporności na kwas acetylosalicylowy (ASA) u osób poddanych przeszczepowi serca i osób poddanych operacji bezpośredniej rewaskularyzacji mięśnia sercowego jako grupy kontrolnej. Do oceny oporności na ASA zastosowano ASPItest.
Materiał i metody: Grupę transplantacyjną stanowiły 24 osoby (20 mężczyzn i 4 kobiety) w wieku średnio 48 ±13 lat poddane procedurze przeszczepienia serca techniką klasyczną Lower-Shumway. Do grupy kontrolnej włączono 24 osoby (19 mężczyzn i 5 kobiet) w wieku 64 ±7 lat poddanych operacji rewaskularyzacji bezpośredniej mięśnia sercowego. Wszyscy pacjenci otrzymywali standardową, dobową dawkę ASA 75 mg.
Wyniki: W badaniu ASPItest w grupie transplantacyjnej uzyskano średni wynik 27 ±22 U. Wyniki o wartości powyżej 30 U, wskazujące na oporność na ASA, otrzymano u 8 (34%) pacjentów z wartością średnią 50,3 ±20,6 U. W grupie kontrolnej oporność na ASA potwierdzono u 5 (20%) pacjentów z wartością średnią 43,3 ±6,4 U.
Wnioski: Badanie potwierdziło wysoką oporność na ASA zarówno u pacjentów poddanych transplantacji serca, jak i poddanych operacji bezpośredniej rewaskularyzacji mięśnia sercowego.
Słowa kluczowe: transplantacja serca, kwas acetylosalicylowy, ASPItest.

Introduction

Coronary allograft vasculopathy can cause as many deaths as infections or rejection episodes within 3 years following heart transplantation. Although changes in the coronary artery wall consist of diffuse artery lumen narrowing, routine use of aspirin is a standard preventive therapy.

Platelets are primary factors in clot formation and consequent acute coronary events. Thrombocytes are activated by several agents including thromboxane A2, collagen, thrombin and adenosine-5-diphosphate (ADP). Aspirin (ASA) acts by irreversibly inactivating cyclooxygenase-1 and thus decreasing the conversion of arachidonic acid to prostaglandin H2 and thereby the formation of platelet-derived thromboxane A2 (TxA2). Uncertainty remains regarding the incidence of the ASA resistance phenomenon among heart transplantation recipients.

Aim

The aim of the study was to compare the aspirin resistance rate in an allograft heart transplantation population and in a control group by laboratory tests including the Aspirin-Resistant Patients Identification Test (ASPItest).

Material and methods

A total of 24 patients (20 men and 4 women) at a mean age of 48 ±13 years who underwent routine clinical follow-up after allograft heart transplantation were consecutively enrolled in the study. All patients were treated with a standard dose of 75 mg ASA daily. Patient compliance regarding aspirin intake was proven by family members' supervision.

The control group consisting of 24 patients (19 men and 5 women) with a mean age of 64 ±7 years waiting for coronary artery bypass grafting in our department was enrolled in the study. All patients in group 2 were treated with a standard dose of 75 mg ASA daily. The diagnosis of dyslipidemia was related to basic results. Detailed demographic and clinical information is presented in Table I.

The aim of the study was to evaluate the aspirin resistance rate in both groups by laboratory tests including the Aspirin-Sensitive Patients Identification Test (ASPItest).

Platelet aggregation tests was performed on Multiplate platelet function analyzer on whole blood samples (Roche Diagnostic, Germany). The method is based on measurements of electric resistance that occur during platelets' ad-

hesion and aggregation to two electrodes, which is recorded for 6 consecutive minutes [1]. The results are presented as area under the curve (AUC). Values of ASPItest below 30 U are related to strong inhibition of cyclooxygenase-1 enzyme (COX-1) on the surface of the platelets, which was an indicator of aspirin sensitivity [2].

The laboratory tests including whole blood count and kidney and liver functions tests were evaluated.

The indication for heart transplantation was end stage heart failure due to ischemic (n = 10), dilated idiopathic (n = 12) or hypertrophic (n = 2) cardiomyopathy. The mean time since transplantation was 48 ±28 months. Patients were treated with two different immunosuppressive regimens including tacrolimus and cyclosporine (both calcineurin inhibitor-based protocols) which are presented in detail in Table I. All patients were clinically stable in NYHA class I (22 patients) and II (2 patients). When the diagnostic measurements were performed none of the patients were suffering either from a rejection episode or infection.

The control group consisted of patients awaiting a coronary artery bypass grafting procedure. The indication for surgical intervention was left main disease in 8 (33%) patients, two-vessel disease including proximal left descending artery disease in 12 (50%) patients and three-vessel disease in 4 (17%) more patients.

Laboratory tests were performed consisting of the standard coagulation test and liver and kidney tests followed by complete blood count. The coagulation test included activated partial prothrombin time (APTT), international ratio (INR) and the ASPItest. The laboratory kidney function test consisted of serum creatinine, and liver tests included serum albumin concentration, overall protein serum concentration, aspartate and alanine transaminases (AST and ALT). Complete blood count included white cell serum concentration, hemoglobin and thrombocyte concentrations.

Statistical analysis

Interval data were presented as means and standard deviations. Nominal data were presented as percentages. To compare clinical parameters between analyzed groups – control vs. heart transplantation recipients – Student's t test (parametric) was used when data followed a normal distribution (Shapiro-Wilk test). If data did not follow a normal distribution the Mann-Whitney rank sum test (nonparametric) was used. The dependence between groups and nominal data was analyzed using Fisher's exact test.

The percentages of sensitivity and resistance among control patients vs. heart transplantation recipients was compared with the test for proportions.

Statistical analyses were performed with Statistica 10 (StatSoft Inc., Poland). All tests were considered significant at a p-value of less than 0.05.

Results

The platelet function test (ASPItest) revealed a mean value of 27 ±22 U in the transplant group. In the heart re-

Tab. I. Clinical and demographic data

Parameter	Transplant group (n = 24)	Control group (n = 24)	P-value
Age [years]	48 ±13	64 ±7	< 0.05
Gender (M/F)	19 (80%) 5 (20%)	19 (80%) 5 (20%)	
Transplantation indications:			
Ischemic cardiomyopathy	10 (42%)		
Dilated cardiomyopathy	12 (50%)		
Hypertrophic cardiomyopathy	2 (8%)		
Coronary bypass grafting indications:			
Left main stenosis		8 (33%)	
2-vessel disease		12 (50%)	
3-vessel disease		4 (17%)	
Concomitant diseases:			
Arterial hypertension	10 (42%)	15 (62%)	NS
Kidney dysfunction	7 (29%)	7 (29%)	NS
Diabetes mellitus	8 (33%)	8 (33%)	NS
Peripheral artery disease	2 (8%)	2 (8%)	NS
Chronic obstructive pulmonary disease	1 (4%)	2 (8%)	NS
Dyslipidemia	3 (12%)	11 (46%)	< 0.05
Stroke in medical history	1 (4%)	2 (8%)	NS

cipient group ASPItest values below 30 U were found in 16 (66%) patients with a mean value of 13.8 ±6.1 U. ASPItest results above 30 U were obtained in 8 (34%) patients, with a mean value of 50.3 ±20.6 U.

There were 5 (50%) of 10 patients diagnosed with ASA resistance in the ischemic cardiomyopathy subgroup who were referred for heart transplantation.

The comparison of ASA resistance and sensitivity in both groups is presented in Figure 1.

The platelet function test (ASPItest) revealed a mean value of 24 ±13 U in the control group. In the control group ASPItest values below 30 U were found in 19 (80%) patients with a mean value of 19.6 ±9.3 U. The results obtained in aspirin-sensitive subgroups were statistically not significant in the transplant versus control group (13.8 ±6.1 U vs. 19.6 ±9.3 U, NS). ASPItest results above 30 U were obtained in 5 (20%) more patients, with a mean value of 43.3 ±6.4 U.

There was no difference in other coagulation parameters, e.g. prothrombin time (97.7 ±19.2 s vs. 101 ±9.6 s in group 1 and 2, respectively (NS)). The mean value of INR ratio was 1.2 ±0.8 vs. 1 ±0.1 in group 1 and 2, respectively (NS). There was no statistically significant difference in liver function test including aminotransferase – ALT (29.1 ±10.9 IU/l vs. 32 ±15 IU/l in group 1 and 2, respectively (NS)) and aminophosphatase – AST (25.5 ±8.7 IU/l vs. 29.2 ±10.1 IU/l in group 1 and 2, respectively (NS)). Serum protein concentration (76.6 ±5.4 g/l vs. 76.2 ±3.3 g/l; NS) and serum albumin (43.5 ±3.1 g/l vs. 45 ±1.7 g/l; NS) were not different between groups. Kidney function estimated by serum creatinine concentration was not statistically significantly different between groups (147 ±54 µmol/l vs. 138.2 ±47 µmol/l in group 1 and 2, respectively). Laboratory results are presented in Table II.

There was no difference in whole blood count between groups; white blood count was 6.4 ±1.3 × 1000/mm^3 vs. 6.5 ±1.4 × 1000/mm^3 (NS) and hemoglobin serum concentration 8.4 ±1.1 mmol/l vs. 8 ±0.7 mmol/l in group 1 and 2, respectively (NS). The serum thrombocyte concentration was 185 ±44 × 1000/mm^3 and 189 ±63 × 1000/mm^3 in group 1 and 2, respectively.

Patients were under two different immunosuppressive protocols but all based on calcineurin inhibitor therapy. There were 20 (83.3%) patients treated with tacrolimus with a measured mean serum concentration of 12.5 ±3 mg/dl and 4 (16.7%) on cyclosporine therapy with a mean serum concentration of 90 ±20.2 mg/dl. Mean serum mycophenolate mofetil concentration was 2.45 ±1.9 µg/ml.

Discussion

Although heart transplantation is the optimal treatment option for patients suffering from heart failure, prevalence is estimated to be as high as 8% in the population, and its long-term complications limit the results [3, 4]. Coronary allograft vasculopathy can cause as many deaths as infections or rejection episodes within 3 years following heart transplantation [5]. Accelerated coronary artery disease and myocardial infarction complicate up to 50% of patients
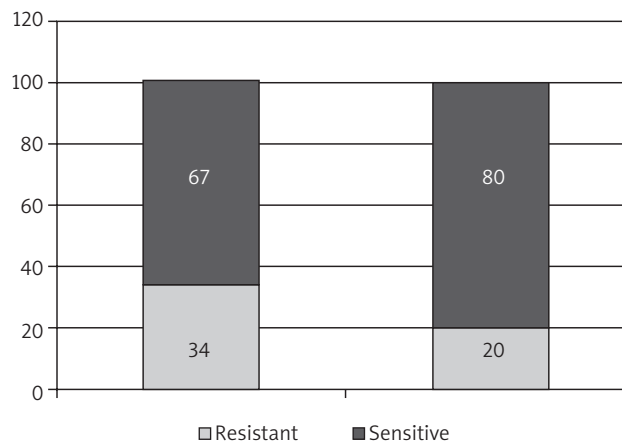

Fig. 1. Aspirin sensitivity and resistance in each group

within 5 years following transplantation [6]. Hyperlipidemia and hypertension are not among main risk factors of ischemic disease in heart transplantation recipients [7]. Although cardiac allograft vasculopathy is one of the major causes of death among heart transplant recipients, the pathogenesis of this cardiovascular complication, involving chronic inflammatory processes, is not fully clarified [8].

Platelets play an important role in thrombus formation but several studies point to their involvement in inflammatory processes [9–13]. ASA is integral to the secondary

Tab. II. ASPItest and coagulation test results in transplant group (group 1), and control (group 2)

Parameter	Group 1 (n = 24)	Group 2 (n = 24)	P-value
Laboratory coagulation tests:			
APTT (s)	97.7 ±19.2	101 ±9.6	NS
INR	1.2 ±0.9	1 ±0.1	NS
Mean ASPI value [U]	27 ±22	24.3 ±13	< 0.05
Aspirin:			
Sensitivity:	16 (67%)	12 (80%)	
Mean ASPI value [U]	13.8 ±6.1	19.6 ±9.3	< 0.05
Resistance:	8 (33%)	3 (20%)	
Mean ASPI value [U]	50.2 ±20.6	43.3 ±6.4	< 0.05
Liver function tests:			
ALT [IU/l]	29.1 ±10.9	32.1 ±15	NS
AST [IU/l]	25.5 ±8.7	29.2 ±10.1	NS
Whole protein [g/dl]	76.6 ±5.4	76.2 ±3.3	NS
Albumin [g/dl]	43.5 ±3.2	45 ±1.7	NS
Kidney function test:			
Creatinine [µmol/l]	147 ±54	138 ±47	NS
Complete blood count:			
White cells [× 10^9/dl]	6.43 ±1.3	6.5 ±1.4	NS
Hemoglobin [mmol/dl]	8.4 ±1.1	8 ±0.7	NS
Thrombocytes [× 10^3/dl]	185 ±63	189 ±63	NS

prevention of cardiovascular disease and acts to impair the development of platelet-mediated atherothromboembolic events by irreversible inhibition of platelet cyclooxygenase-1 (COX-1). Inhibition of this enzyme prevents the synthesis of the potent pro-aggregatory prostanoid thromboxane A$_2$ [14–16]. Thrombi at different stages of organization were also found in histological examinations of transplanted hearts [17–19].

A large number of patients continue to experience atherothromboembolic events despite aspirin therapy, so-called 'aspirin treatment failure', and this is multifactorial in etiology [20].

Three types of ASA resistance are defined. Type I, called pharmacokinetic resistance, occurs when aspirin fails to inhibit COX-1 despite its adequate dosing. It is explained by aspirin malabsorption or genetic polymorphisms in COX-1. In type II, named pharmacodynamic resistance, continued thromboxane production occurs despite adequate COX-1 inhibition. Type III is ASA pseudoresistance that is related to inadequate drug intake mainly due to noncompliance.

There are several laboratory options for measuring platelet function in patients receiving aspirin therapy. The Aspirin-Sensitive Patients Identification Test (ASPI-test) is a Multiplate electrode aggregometry (MEA) test. It is based on a development of Cardinal and Flower's 1979 impedance aggregometry method [21]. Values < 30 U indicate strong COX-1 inhibition by aspirin. This level is considered a cut-off for strong inhibition of COX-1 by aspirin [22]. The ASPItest is confirmed to be a reliable tool in aspirin resistance diagnosis [23–26]. A high frequency (34%) of aspirin resistance in heart transplantation recipients was found in the present study.

The increased platelet aggregation in heart transplantation recipients has already been described in previous human and animal studies [27–29]. The enhanced platelet response to ADP was reported in patients following heart transplantation. It is associated with changes in platelet fatty acids and a high cholesterol-to-phospholipid ratio, which is associated not only with decreased membrane fluidity but also platelet hyperreactivity [30–32]. There was no statistically significant difference in ASA resistance between the transplant and control group. Although there was a significant age difference between groups, we found no correlation between ASA resistance and age in multivariate analysis. The study revealed high occurrence of aspirin resistance in each population. The only difference noted in the present study concerned the results obtained in aspirin sensitive subgroups (13.8 ±6.1 U vs. 19.6 ±9.3 U, $p < 0.05$).

Calcineurin inhibitor based triple-drug immunosuppression therapy, despite reducing the number of acute graft episodes, does not reduce the incidence of post-transplant coronary disease. It is partially explained by the calcineurin inhibitor influence on platelet lipid metabolism and aggregability [33]. High incidence of ASA resistance point to the underdiagnosed problem in the heart transplant population.

We believe that routine ASA resistance evaluation in both heart transplant and coronary artery bypass grafting groups should be routine clinical practice. It may be very helpful in pointing out those who should be treated with other antiplatelets drugs. The heart transplant recipients who are resistant to ASA should have their immunosuppressive regimens modified so as to reduce the risk of graft vasculopathy. They should probably be treated with higher doses of immunosuppressive drugs to slow or prevent chronic humoral rejection in future.

Conclusions

There is a high incidence of ASA resistance in heart transplantation recipients and the general population that indicates the need for routine diagnosis in patients referred for aspirin therapy.

Disclosure

Authors report no conflict of interest.

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