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Clopidogrel Resistance After Renal Transplantation

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Abstract. *Background: Leading causes of mortality and morbidity after kidney transplantation are cardiovascular diseases. One of the fundamentals of their prevention is the inhibition of platelet aggregation. Resistance to anti-platelet agents is a well-established phenomenon; however, its causes are yet to be clarified. Patients and Methods: Forty post-transplant patients, who received 75 mg clopidogrel q.d. as a prophylactic measure, were examined using optical aggregometry. Subsequently, logistic regression analysis was performed with 24 variables in order to expose possible causes of resistance. Results: Sixty percent of patients (24) were resistant to clopidogrel therapy; effective thrombocyte inhibition could only be shown in 40% of them (16). Significant correspondence between resistance and variables could not be found. Conclusion: Clopidogrel resistance is expected to occur on a large scale in patients who underwent kidney transplant surgery. Thus, a key component of preventive therapy, which stresses the importance of discovering the cause of resistance so as to decrease mortality rates, is missing.*

Recently it has become apparent that the survival rate of patients suffering from end-stage kidney failure is improved more by transplantation, as opposed to dialysis (1-3). Post-kidney transplant survival is greatly influenced by complications developing due to kidney failure. It is, thus, no surprise that the leading causes of post-transplant mortality are cardiovascular diseases.

Matas and associates examined 2,202 kidney transplant patients and followed them up for a period of 10 years. The main causes of death were as follows: cardiovascular diseases (35 with cadaveric and 38% with living donors), tumors (22 and 18%) and miscellaneous infections (13 and 9%) (4). There are many cardiovascular risk factors during the pre-transplant period: pronounced cardiovascular calcification, increased oxidative stress, micro-inflammatory factors, endothelial dysfunction, hyperhomocysteinaemia, elevated sympathetic activity, an abnormal lipid profile, *etc*. (5). In order to further improve the long-term results of kidney transplantation, it is imperative that pots-transplant cardiovascular preventive measures are taken. Current preventive therapy encompasses three main areas: having an appropriate lifestyle (healthy eating, regular exercise), maintaining blood pressure, sugar and lipid values in target range and following anti-platelet therapy (6). Aggregation inhibition is achieved using cyclooxigenase inhibitor acetylsalicylic acid (ASA) and P2Y12 agonist clopidogrel or prasugrel either alone or in combination according to level IA evidence (6). Inhibition of platelet aggregation decreases the risk of having fatal stroke, myocardial infarctions and other vascular conditions by 22% and the risk of a non-fatal stroke by 25% according to a study that comprised 135,000 patients (7). Resistance to anti-platelet therapy is a well-known phenomenon. Mani and associates summarized their data pertaining to acetylsalicylic acid and clopidogrel resistance. Ineffective anti-platelet therapy was observed in 5-55% of patients receiving ASA and in 4.8-42% of patients undergoing clopidogrel treatment. One of the drawbacks of the analysis was the use of different methods for monitoring resistance and also the inclusion of differently medicated groups in the study, thus making it difficult to draw comparisons between them (8). The most widely accepted method for monitoring platelet functions are the methods of optical aggregometry and platelet function assay (9-10).

The focus of our research was the assessment of clopidogrel resistance in patients that previously underwent renal transplantation. Subsequently, the causes of resistance were investigated relying upon studies available from current scientific literature suggesting the presence of several factors resulting in resistance.

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Table I. *The investigated parameters, which may explain resistance.*

Age	Cellcept	Angiotensin-converting enzyme (ACE) inhibitors
Serum creatinine	Medrol	Antilipid therapy
Serum glucose	Tacrolimus	Steroid resistant acute rejection
Haematocrit	Sandimmun	Chronic allograft nephropathy
Haemoglobin	Myfortic	Type of transplantation
Triglycerides	Certican	Diabetes
Low-density lipoprotein (LDL) cholesterol	Rapamune	Body mass index (BMI)
Platelet count	Calcium channel blockers	Smoking

Patients and Methods

This investigation was approved by the Regional Ethics Committee of the University of Pecs (approval number: 4816) and a written informed consent was signed by all participants

Forty patients, who previously underwent kidney transplantation at the University of Pecs, Surgical Department, were selected to participate in the study between March 2009 and December 2013. Eleven were females and 29 males with an average age of 58.11 years. Due to their cardiovascular history, they were given 75 mg clopidogrel *q.d.* before their operation instead of a daily dose of 100 mg ASA, which is commonly used.

Blood for the aggregometric analysis was drawn from the cubital vein and collected in 3.8% sodium-citrate Vacutainer tubes (Becton, Dickinson and Company, New Jersey USA). Measurements were performed at the University of Pecs, First Department of Internal Medicine. Following multiple stages of centrifugation, first platelet rich plasma (PRP) and, then, platelet poor plasma (PPP) was obtained. Platelet aggregation was induced with adenosine diphosphate (ADP; concentration: 5 mM and 10 mM) and epinephrine (10 mM). Measurements were made using a turbidimetric Carat TX-4 (Carat Diagnosztika Ltd., Budapest, Hungary) platelet aggregometer that calculates the degree of aggregation based on the difference between PPP and PRP optical density.

ADP-induced platelet response was used to examine the effect of clopidogrel. A decrease in maximum aggregation values, *i.e.* if it has fallen outside of the reference range (mean±2SD) typical for the normal (untreated) population, is considered a result of antiplatelet therapy; otherwise therapy is deemed ineffective.

Therapy is considered effective as long as the degree of induced aggregation remains below 50%. Further laboratory tests were performed at the University of Pecs, Department of Laboratory Medicine, to determine serum creatinine, glucose, cholesterol and triglyceride of research subjects. Haemoglobin, haematocrit and platelet count were measured using an automated analyser (Advia 2120i Hematology System, Siemens AG, Germany).

Patients' weight, height and body-mass index (BMI), which derived from the previously mentioned two values, were measured in an outpatient setting. Tobacco use and the number of therapeutic drugs (antilipid drugs, immunosupressants, calcium-channel blockers, angiotensin-converting enzyme (ACE) inhibitors) were assessed based on information obtained from patient interviews, which, according to scientific literature, may influence cardiovascular complications. The incidence of chronic allograft nephropathy and steroid resistant acute rejection, as influencing factors, were investigated as well.

Statistical analysis (logistic regression) of the available data was performed using the 21.0 version of IBM SPSS Statistics software (IBM Corporation, Armonk, NY, USA).

Results

Aggregometric measurements showed clopidogrel resistance in 60% (24 patients) out of the 40 patients examined. Effective treatment was achieved in 40% (16) of them. Subsequently, logistic regression was performed between various factors and resistance in order to uncover possible causes of resistance. Twenty-four variables were monitored as possible causes of resistance, which were selected relying on data from scientific literature (Table I). A significant correlation between resistance and variables could not be found.

Discussion

Multiple factors have been linked to resistance to aggregation inhibitors. Metabolization of clopidogrel into active thiol products is performed *via* the CYP3A4 isoenzyme, the same enzyme that metabolizes certain 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA)-reductase inhibitors (atorvastatin, lovastatin, simvastatin, *etc.*) and, therefore, the concomitant use of both drugs can result in decreased effectiveness (11). Increased rates of resistance have been seen with the over-expression of P2Y1–receptors, high BMI, elevated C-peptide, haemoglobin A1C and von Willebrand factor levels. Greater rates of resistance are present in regular smokers as well because the metabolization of polycyclic aromatic hydrocarbons found in tobacco smoke is carried-out *via* the CYP3A4 and CYP1A2 isoenzymes (12). Experiments on rats have also shown that increased plasma levels of nicotine acts as an inductor of the CYP1A2 isoenzyme (13). One study came to the conclusion that there is a greater rate of clopidogrel resistance amongst people resistant to ASA. Therefore, dual resistance is especially common in women with high BMIs (14). A relationship has been found between plasma levels of

calcium channel blockers, ACE inhibitors and resistance. Higher plasma levels were accompanied by greater rates of resistance (15). The following substances have a blocking effect on the CYP3A4 enzyme: azole-type antifungal drugs, protease inhibitors, macrolide antibiotics (*i.e.* erythromycin) and dihidropyridine-type calcium channel blockers. These drugs also affect anti-platelet therapy (16). Genetic predisposition was proven by another study: the heterogeneous expression of the cytochrome P450 enzyme family (including CYP3A4) influences the effects of clopidogrel (17). Apart from the aforementioned mechanism, the following findings were obtained *via* an extended study: increased platelet activity and production, diabetes or insulin resistance and poor patient compliance (18). The results of optical aggregometry are also affected by the time of the examination and patients' cholesterol levels (19). Other influencing factors can be patients' compliance and heart rate. Educating patients about anti-platelet drug therapy was especially important in this study. Many patients, due to not seeing or feeling any tangible results of the therapy, think compliance with the treatment is not important. The tendency to aggregate is also intensified by elevated heart rates due to the increased sympathetic tone (20).

No significant relationships between examined factors and resistance were found by this study; therefore, no clear explanation can be given concerning resistance. A positive aspect of our measurements is that they were all performed according to the same protocol using the same method. Each of our patients received oral and written information during ambulatory visits as to the importance of taking their medications regularly in order to decrease the possibility of non-compliant behaviour.

Discovering the causes of resistance is crucial, since a significant number of our patients is resistant to the applied antiplatelet therapy and, thus, an important component of cardiovascular prevention is missing. As a result, a significant number of patients with a functioning kidney graft die due to cardiovascular complications. The number of implantable kidneys is well below the number of waiting list candidates; thus, the improvement of long-term results by decreasing post-transplant mortality and morbidity is an important task.

References

1 Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, Held PJ and Port FK: Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. N Engl J Med *341*: 1725-1730, 1999.

2 Meier-Kriesche HU, Schold JD, Srinivas TR, Reed A and Kaplan B: Kidney transplantation halts cardiovascular disease progression in patients with end-stage renal disease. Am J Transplant *4*: 1662-1668, 2004.

3 Nolan CR: Strategies for improving long-term survival in patients with ESRD. J Am Soc Nephrol *16(Suppl 2)*: S120-127, 2005.

4 Matas AJ, Gillingham KJ, Humar A, Kandaswamy R, Sutherland DE, Payne WD, Dunn TB and Najarian JS: 2202 Kidney transplant recipients with 10 years of graft function: What happens next? Am J Transplant *8(11)*: 2410-2419, 2008.

5 Fort J: Chronic renal failure: a cardiovascular risk factor. Kidney Int Suppl *99*: S25-29, 2005.

6 Joep P: European guidelines on cardiovascular disease prevention in clinical practice (version 2012) European Heart Journal *33*: 1635-1701, 2012.

7 Easton JD: Evidence with antiplatelet therapy and ADP-receptor antagonists. Cerebrovasc Dis *16(Suppl 1)*: 20-26, 2003.

8 Mani H and Lindhoff-Last E: Resistenz gegen Azetylsalizylsäure und Clopidogrel. Hämostaseologie *26*: 229-238, 2006.

9 Hayward CP, Pai M, Liu Y, Moffat KA, Seecharan J, Webert KE, Cook RJ and Heddle NM: Diagnostic utility of light transmission platelet aggregometry: results from a prospective study of individuals referred for bleeding disorder assessments. Journal of Thrombosis and Haemostasis *7*: 676-684, 2009.

10 Shah U and Alice D: Tests of platelet function. Curr Opin Hematol *14*: 432-437, 2007.

11 Schroeder WS, Ghobrial L and Gandhi PJ: Possible mechanisms of drug-induced aspirin and clopidogrel resistance. Thrombolysis *22(2)*: 139-150, 2006.

12 Lepäntalo A, Virtanen KS, Heikkilä J, Wartiovaara U and Lassila R: Limited early antiplatelet effect of 300 mg clopidogrel in patients with aspirin therapy undergoing percutaneous coronary interventions. European Heart Journal *25*: 476-483, 2004.

13 Matetzky S, Shenkman B, Guetta V, Shechter M, Beinart R, Goldenberg I, Novikov I, Pres H, Savion N, Varon D and Hod H: Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation *109(25)*: 3171-3175, 2004.

14 Lev EI: Aspirin and clopidogrel drug response in patients undergoing percutaneous coronary intervention: the role of dual drug resistance. J Am Coll Cardiol *47(1)*: 27-33, 2006.

15 Gurbel PA, Bliden KP, Hiatt BL and O'Connor CM: Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation *107(23)*: 2908-2913, 2003.

16 Lau WC, Waskell LA, Watkins PB, Neer CJ, Horowitz K, Hopp AS, Tait AR, Carville DG, Guyer KE and Bates ER: Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction. Circulation *107(1)*: 32-37, 2003.

17 Fitzgerald DJ and Maree A: Aspirin and clopidogrel resistance. Hematology Am Soc Hematol Educ Program 114-120, 2007.

18 Michos ED, Ardehali R, Blumenthal RS, Lange RA and Ardehali H: Aspirin and clopidogrel resistance. Mayo Clin Proc *81(4)*: 518-526, 2006.

19 Hennekens CH, Schror K, Weisman S and FitzGerald GA: Terms and conditions: semantic complexity and aspirin resistance. Circulation *110(12)*: 1706-1708, 2004.

20 Postuła M, Tarchalska-Kryńska B, Filipiak KJ, Kosior D, Serafin A, Huczek Z and Opolski G: Factors responsible for "aspirin resistance" - can we identify them? Kardiol Pol *68(4)*: 403-411, 2010.

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