J Pharmacol Sci **107**, 451 – 455 (2008)

Journal of Pharmacological Sciences

Short Communication

Thienopyridine Resistance Among Patients Undergoing Intracoronary Stent Implantation and Treated With Dual Antiplatelet Therapy: Assessment of Some Modifying Factors

Nina Djukanovic[1], Zoran Todorovic[2], Aleksandra Grdinic[3], Danilo Vojvodic[4], Milica Prostran[2,*], and Miodrag Ostojic[1]

[1]*Department of Cardiology, Institute for Cardiovascular Diseases, Clinical Centre of Serbia, Belgrade, Serbia*
[2]*Department of Pharmacology, Clinical Pharmacology and Toxicology, School of Medicine, University of Belgrade, Belgrade, Serbia*
[3]*Department of Cardiology, Clinical Center Dr. Dragisa Misovic, Belgrade, Serbia*
[4]*Institute for Medical Research, Military Medical Academy, Belgrade, Serbia*

Received March 19, 2008; Accepted June 12, 2008

Abstract. In this open, prospective study we assessed the prevalence of antiplatelet resistance among patients subjected to intracoronary stent implantation. In patients treated with aspirin + thienopyridine (N = 32), platelet reactivity index (PRI) significantly decreased after 2 and 7 days of dual antiplatelet treatment in comparison with the same patients on aspirin monotherapy ($P<0.001$, both). After 7 days of aspirin + thienopyridine treatment, insufficient antiplatelet response was observed in 28% (9/32) of the patients. High interindividual variability in response to aspirin + thienopyridine treatment emphasizes the significance of thienopyridine resistance, while the influence of statins on such a treatment should be reassessed.

Keywords: antiplatelet agent, stent, vasodilator-stimulated phosphoprotein (VASP) assay

Thienopyridine resistance is still a matter of debate (1). There is no widely accepted definition, but it could be described as a failure of antiplatelet drugs to produce an expected biological response (platelet inhibition) or their failure to inhibit the target ($P2Y_{12}$ receptor-related response in platelets) (2). Thienopyridine resistance may be explained by extrinsic and/or intrinsic mechanisms. The former mechanisms may involve patient non-adherence, underdosing or inappropriate dosing of clopidogrel, and drug-drug interactions involving CYP3A4 isoforms, while the latter may be related to genetic polymorphisms (e.g., $P2Y_{12}$ receptors or CYP3As), increased release of ADP, or alternate pathways of platelet activation (3).

Since thienopyridines irreversibly inhibit ADP binding to the platelet $P2Y_{12}$ receptor and prevent subsequent phosphorylation of vasodilator-stimulated phosphoprotein (VASP), the increase in VASP phosphorylation could be a useful marker of thienopyridine resistance (4).

The aim of our study was to assess the prevalence of thienopyridine resistance in patients subjected to intracoronary stent implantation in Serbia, since there is almost no data on this subject. Also, the influence of selected modifying factors to such a prevalence would be analyzed. Thienopyridine resistance was tested with the VASP phosphorylation assay and assessed by platelet reactivity index (PRI). In this open, prospective study, 20 healthy volunteers (14 males, 6 females), aged 49.4 ± 11.4 years, without any medication, were compared to 32 patients (27 males, 5 females), aged 57.1 ± 8.5 years, with ischemic heart disease, undergoing elective percutaneous coronary intervention (PCI) at the Clinical Centre of Serbia (Belgrade, Serbia). In the latter group, all the patients had one or more cardiovascular risk factors, 26 had a previous history of vascular event, and 22 had previous myocardial infarction.

The study was conducted according to the Declaration of Helsinki.

All the patients were treated with aspirin (100 mg

*Corresponding author. mprostran@doctor.com
Published online in J-STAGE
doi: 10.1254/jphs.08077SC

/day) for three consecutive days. Subsequently, they were randomized to aspirin (100 mg/day) + ticlopidine (2 × 250 mg/day) or aspirin (100 mg/day) + clopidogrel (75 mg/day) (N = 15 and 17, respectively). The dual antiplatelet treatment was administered continuously, from 7 days before stent implantation up to 12 months after the intervention (one month in patients after bare-metal stent implantation and 9 – 12 months in patients after drug-eluting stent implantation).

All the patients received unfractioned heparin (5000 – 6000 IU or 700 IU/kg, i.v.) and antibiotic (1 g ceftazidim or 1.5 g cefuroxim, i.v.) immediately before the PCI intervention (direct stenting was routinely used through *a. femoralis*). The use of other cardiovascular drugs (beta-blockers, ACE inhibitors, Ca^{2+}-channel blockers, diuretics) was allowed.

We used a standardized flow cytometric assay [Platelet VASP®; Diagnostica Stago (Biocytex), Asnières, France] to determine the VASP phosphorylation state of the whole blood (5). Blood samples were collected in 0.129 M sodium citrate vacutainer tubes and incubated with PGE1 alone or PGE1 and ADP, before fixation with paraformaldehyde. Platelets were subsequently permeabilized with non-ionic detergent and labeled with a monoclonal antibody 16C2, specifically directed against serine 239–phosphorylated VASP, followed by a staining reagent, polyclonal anti-mouse antibody IgG-FITC (fluorescein isothiocyanate). The samples were analyzed on a Coulter Epics XL flow cytometer, at a medium rate. The platelet population was identified for its forward and side scatter distribution and 10,000 platelets were gated.

PRI was calculated using mean fluorescence intensities (MFIs) in the presence of either PGE1 or PGE1 + ADP according to the following formula (4):

$$PRI = ((MFI_{(PGE1)} - MFI_{(PGE1+ADP)}) / MFI_{(PGE1)}) \times 100$$

There is an inverse correlation between thienopyridine treatment efficacy and PRI.

Results are expressed as the mean ± S.D. (standard deviation). Comparisons between groups were analyzed using the chi-square test, *t*-test, Mann-Whitney test, and one-way analysis of variance (ANOVA), followed by Bonferonni's *post-hoc* test, when appropriate. Standard regression analysis was used to investigate relationships between the response to thienopyridine treatment and other modifyng factors (e.g., age, gender, type of angina, etc.). A *P* value of less than 0.05 was considered significant.

There was no significant difference in PRI between healthy donors and patients who were treated with aspirin only (79.55 ± 9.99% *vs* 85.08 ± 9.20%, $P>0.05$). However, in patients treated with dual antiplatelet therapy (aspirin + thienopyridine), PRI decreased in a time-related manner. In other words, PRI was significantly altered by the addition of thienopyridine to aspirin treatment (55.89 ± 19.29% or 37.60 ± 25.28% *vs* 85.08 ± 9.20%, both at $P<0.001$) (Fig. 1).

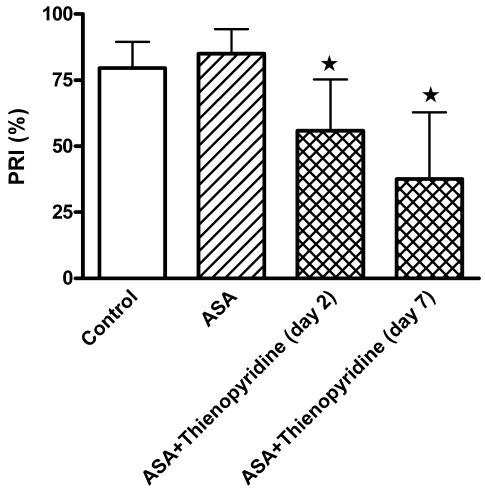

Fig. 1. Analysis of platelet VASP phosphorylation by flow cytometric assay in healthy volunteers and patients on single or dual antiplatelet therapy. Control: healthy volunteers (N = 20). ASA: patients subjected to PCI treated with aspirin in monotherapy (N = 32). ASA + Thenopyridine: patients subjected to PCI treated with aspirin + thienopyridine (test was performed on days 2 and 7). Vertical bars represent the mean ± S.D. of 20 – 32 observations. **P*<0.001, in comparison with the ASA group.

PRI has not similarly changed in all of the patients.

First, both good and bad responders were identified according to their remaining PRI after 7 days of dual antiplatelet treatment (cut-off value of 50%) (6). Bad response (PRI ≤50%) was observed in 4/15 (27%) patients treated with aspirin + ticlopidine and in 5/17 (30%) patients on aspirin + clopidogrel combination (Fig. 2).

Second, rapid response (PRI ≤50% after ≤2 days) was achieved in 4/15 patients in the aspirin + ticlopidine group and in 7/17 patients in the aspirin + clopidogrel group (27% and 41%, respectively). However, in the former group, 1 of 4 patients with rapid response became a bad responder on day 7. On the other hand, in the latter group, all the patients with rapid response still had PRI ≤50% (good responders) on day 7.

In three patients (20%) treated with aspirin + ticlopidine, PRI increased between days 2 and 7. A similar pattern was observed in one patient (6%) on clopidogrel + aspirin treatment, but without significant increase between the 2nd and 3rd measurement (days 2 and 7, respectively) (Fig. 2).

The outcome of the dual antiplatelet treatment showed no correlation with the choice of thienopyridine (ticlopidine or clopidogrel), age, gender, type of angina, and

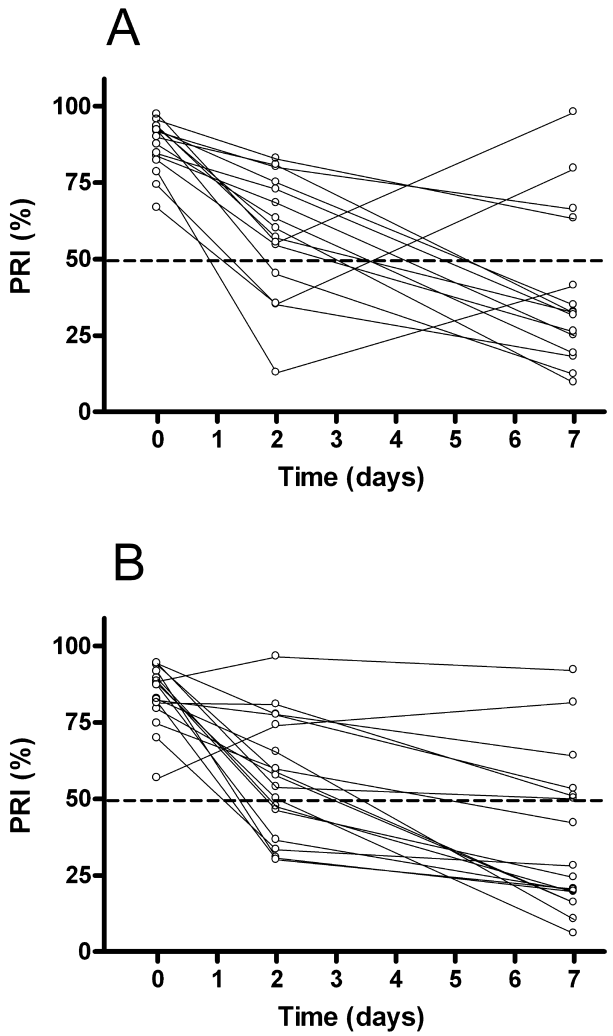

Fig. 2. Distribution of platelet reactivity in patients treated with aspirin + ticlopidine (N = 15) or aspirin + clopidogrel (N = 17) (panels A and B, respectively). Good responders: ADP receptor reactivity ≤50% after 7 days of aspirin + thienopyridine treatment; bad responders: ADP receptor reactivity >50% after 7 days of aspirin + thienopyridine treatment. Points represent the baseline values of ADP-receptor reactivity (day 0), as well as values of ADP-receptor reactivity after 2 and 7 days of dual antiplatelet therapy.

risk factors for cardiovascular diseases (hypertension, hyperlipoproteinemia, diabetes mellitus, and smoking) (Table 1).

Response to thienopyridine treatment was significantly better in patients who received statins in comparison with patients without statins ($P<0.05$)

Periprocedural myocardial infarction during PCI, myocardial infarction and/or revascularisation did not occur in good or bad responders during the follow-up period of one year. However, one patient with bad response to thienopyridines died during the follow-up period, but the cause of death was congestive heart failure, that is, it was not related to the stent thrombosis (Table 1).

Currently, there is no "gold standard" among the tests for detection of thienopyridine resistance (7). For a long time, platelet aggregometry used to be the first line option for measuring platelet inhibition, despite its disadvantages (e.g., complex methodology and high variability of results).

The main results of our study confirm that the flow cytometric assay is a sensitive method for detection of patients with diminished response to thienopyridine treatment. Also, such an analysis of ADP-receptor reactivity reveals large interindividual variability in response to thienopyridines. Therefore, flow cytometric measurements are more specific and reliable for evaluation of the efficacy of thienopyridine treatment.

Clinical studies have demonstrated high antiplatelet resistance variability, which occurred in 5% – 30% of patients 24 h after clopidogrel administration (2, 7). In addition, poor responsiveness to thienopyridines seems to be a drug-, but not class-, specific phenomenon because nonresponders to both ticlopidine and clopidogrel were rarely observed (8). On the other hand, aspirin-resistant patients, as a group, have reduced response to clopidogrel (9).

The present results fit into the range mentioned above, with 27% – 30% bad responders after 7 days of dual antiplatelet treatment. However, different patterns of ADP-receptor reactivity among bad responders may indicate different mechanisms and/or causes of resistance to thienopyridines. In other words, distributions of ADP-receptor reactivity after two and seven days of dual antiplatelet treatment were different in several ways. First, a significant number of rapid responders was observed in both PCI groups, and second, the rapid response after two days did not always indicate the good response after seven days. In addition, our results confirm both intra- and interindividual variability of the response to dual antiplatelet treatment over time; the ADP receptor reactivity even increased between days 2 and 7 in some patients, despite the treatment administered.

The results of our study only partially agree with those reported by Barragan et al. (10), despite the concordance in the test used and sample involved. The pattern of decrease in ADP-receptor reactivity is similar, but the values obtained after 2 and 7 days of dual antiplatelet treatment are different in the former and latter case (60.14% and 48.37% *vs* 55.89% and 37.60%, respectively). Further investigation is needed to explain this discrepancy.

CYP3A4 metabolized statins were supposed to diminish the response to clopidogrel, but clinical trials did not confirm such a hypothesis (11, 12). In our study,

Table 1. Demographic and procedural characteristics of good or bad responders treated with dual antiplatelet therapy (aspirin + ticlopidine or aspirin + clopidogrel)

Parameter		Good response	Bad response	*P*
		n = 23	n = 9	
Gender	Male	20	7	NS
	Female	3	2	
Age (years)		56.95 ± 8.72	57.44 ± 8.54	NS
Unstable angina	yes	8	6	NS
	no	15	3	
Previous MI	yes	16	6	NS
	no	7	3	
Statins	yes	14	1	<0.05
	no	9	8	
ASA		100 %	100 %	NS
Predilatation	yes	14	5	NS
	no	9	4	
Stent length (mm)		18.96 ± 5.83	19.77 ± 5.04	NS
Stent diameter (mm)		3.20 ± 0.47	3.0 ± 0.5	NS
Maximum balloon pressure (bars)		16.82 ± 2.19	16.44 ± 0.88	NS
Number of stents per patient		1.39 ± 0.94	1.11 ± 0.33	NS
Stents	drug-eluted	4	1	NS
	bare metal	19	8	
Myocardial infarction <1 years		0	0	0
Death <1 years		0	1	NS

statins even seemed to potentiate the response to thienopyridines: only one of seven patients treated with both clopidogrel and statins were bad responders compared to 4/8 patients from the clopidogrel group without statins. Pleiotropic (lipid-lowering–independent) effects of statins might contribute to such a response (13, 14).

In conclusion, this study suggests a high prevalence of resistance to thienopyridines among the patients subjected to intracoronary stent implantation, even in those with a significant rapid response to antiplatelet therapy. Statins do not seem to contribute to such a resistance. This study also shows that the flow cytometric assay is a very reliable and sensitive method for detection of thienopyridine resistance, while clopidogrel monitoring would be justified only if it was demonstrated that poor responders benefit from dose increases (15). However, insufficient number of participants and open design preclude firm conclusion from this data.

Acknowledgment

This work was supported by the Ministry of Science, Republic of Serbia.

References

1 Patrono C, Bachmann F, Baigent C, Bode C, De Catherina R, Charbonnier B, et al. Expert consensus document on the use of antiplatelet agents. The Task Force on the Use of Antiplatelet Agents in Patients with Atherosclerotic Cardiovascular Disease of the European Society for Cardiology. Eur Heart J. 2004;25: 166–181.

2 Gurbel PA, Tantry US. Clopidogrel resistance? Thromb Res. 2007;120:311–321.

3 Nguyen TA, Diodati JG, Pharand C. Resistance to clopidogrel: a review of the evidence. J Am Coll Cardiol. 2005;45:1157–1164.

4 Aleil B, Ravanat C, Cazenave JP, Rochoux G, Heitz A, Gachet C. Flow cytometric analysis of intraplatelet VASP phosphorylation for the detection of clopidogrel resistance in patients with ischemic cardiovascular diseases. J Thromb Haemost. 2005;3: 85–92.

5 Schwarz UR, Geiger J, Walter U, Eigenthaler M. Flow cyto-

metry analysis of intracellular VASP phosphorylation for the assessment of activating and inhibitory signal transduction pathways in human platelets – definition and detection of ticlopidine/clopidogrel effects. Thromb Haemost. 1999;82: 1145–1152.

6 Bonello L, Paganelli F, Arpin-Bornet M, Auquier P, Sampol J, Dignat-George F, et al. VASP phosphorylation analysis prior to percutaneous coronary intervention for exclusion of post-procedural major adverse cardiovascular events. J Thromb Haemost. 2007;5:1630–1636.

7 Siller-Matula J, Schrör K, Wojta J, Huber K. Thienopyridines in cardiovascular disease: focus on clopidogrel resistance. Thromb Haemost. 2007;97:385–393.

8 Campo G, Valgimigli M, Gemmati D, Percoco G, Catozzi L, Frangione A, et al. Poor responsiveness to clopidogrel: drug-specific or class-effect mechanism? Evidence from a clopidogrel-to-ticlopidine crossover study. J Am Coll Cardiol. 2007;50: 1132–1137.

9 Lev EI, Patel RT, Maresh KJ, Guthikonda S, Granada J, DeLao T, et al. Aspirin and clopidogrel drug response in patients undergoing percutaneous coronary intervention: the role of dual drug resistance. J Am Coll Cardiol. 2006;47:27–33.

10 Barragan P, Bouvier JL, Roquebert PO, Macaluso G, Commeau P, Comet B, et al. Resistance to thienopyridines: clinical detection of coronary stent thrombosis by monitoring of vasodilator-stimulated phosphoprotein phosphorylation. Catheter Cardiovasc Interv. 2003;59:295–302.

11 Lau WC, Waskell LA, Watkins PB, Neer CJ, Horowitz K, Hopp AS, et al. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction. Circulation. 2003;107:32–37.

12 Steinhubl SR, Akers WS. Clopidogrel-statin interaction: a mountain or a mole hill? Am Heart J. 2006;152:200–203.

13 Schulz R. Pleiotropic effects of statins: acutely good, but chronically bad? J Am Coll Cardiol. 2005;45:1292–1294.

14 Nesic Z, Todorovic Z, Stojanovic R, Basta-Jovanovic G, Radojevic-Skodric S, Velickovic R, et al. Single-dose intravenous simvastatin treatment attenuates renal injury in an experimental model of ischemia-reperfusion in the rat. J Pharmacol Sci. 2006;102:413–417.

15 Gachet C, Aleil B. Testing antiplatelet therapy. Eur Heart J. 2008;10 Suppl A:A28–A34.