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Casos clínicos

Prasugrel resistance may be linked to the sticky platelet syndrome. Report of one case

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RESUMEN

Antecedentes: el prasugrel es un antiagregante plaquetario más potente que el clopidogrel y es una alternativa para el tratamiento y prevención de los síndromes coronarios agudos. Pese a la eficacia de estos fármacos, puede sobrevenir una respuesta insuficiente para disminuir la actividad de las plaquetas medida a través de la prueba de agregación plaquetaria.
Pacientes y método*:* se estudiaron seis pacientes consecutivos sometidos a intervención coronaria percutánea que recibieron una dosis de carga de 60 mg de prasugrel. Se les realizó agregación plaquetaria y como agonistas ADP y epinefrina. Se determinaron polimorfismos de los genes ABCB1, CYP2C19, CYP3A5 y P2RY12 y se les midió factor tisular mediante la técnica de ELISA.
Resultados: uno de los seis casos mostró hiperactividad plaquetaria aún después del tratamiento con prasugrel y clopidogrel, así como 3.6 veces más factor tisular en plasma en comparación con los enfermos estudiados sin hiperactividad plaquetaria. Ningún caso presentó los polimorfismos estudiados.
Conclusión: se comunicó el caso clínico de un enfermo que muestra respuesta disminuida al prasugrel y al clopidogrel manifestándose con hiperactividad plaquetaria.
Palabras clave: prasugrel, clopidogrel, síndromes coronarios agudos, prevención, tratamiento.

ABSTRACT

Background: Prasugrel is the most potent antiplatelet agent, even more than clopidogrel and is an alternative for the treatment and prevention of acute coronary syndromes. Despite the effectiveness of these drugs, it could be an inadequate response to reduce the hyperactivity of platelets measured by platelet aggregation test.
Patients and Methods*:* We studied six consecutive patients undergoing percutaneous coronary intervention which received a 60mg loading dose of prasugrel. Platelet aggregation was performed using as agonists ADP and epinephrine, were identified gene polymorphisms ABCB1, CYP2C19, CYP3A5 and P2RY12 and tissue factor was measured by ELISA.
Results: One of the six patients showed platelet hyperactivity even after treatment with prasugrel and clopidogrel, and 3.6 fold tissue factor in plasma compared with patients studied without platelet hyperactivity. No patient had the polymorphisms studied.
Conclusion: We report the case of a patient showing decreased response to prasugrel and clopidogrel manifesting with platelet hyperactivity.
Key words: Sticky platelets, prasugrel resistance, microvesicles, tissue factor and hyperreactive platelets.

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Cardiovascular disease is one of the leading causes of mortality worldwide. It had been classically linked to atherosclerosis in addition to other inflammatory stimuli that play a role in the progression of the plaque destabilization and subsequent rupture, resulting in atherothrombosis. Platelets are intimately linked to this process. That's why the antiplatelet drugs, clopidogrel and aspirin are the mainstream treatment in acute coronary syndromes, especially in patients undergoing percutaneous coronary intervention.

However, clopidogrel has at least three disadvantages: delayed onset of action, irreversibility of its inhibitory effect on platelets and a large interindividual variability in platelet response, shown as a high prevalence of drug-resistance (4- 30%)[1].

This has led to the development of a novel agent, prasugrel[2] that has proven it effectiveness in several studies in which a loading dose of 60 mg of prasugrel resulted in a greater platelet inhibition compared with 600 mg of clopidogrel after cardiac catheterization[3,4,5] and also reducing the risk of myocardial infarction and other vascular events.[6,7] The hepatic cytochrome P450 enzymes CYP2B6, CYP3A4, and unlike clopidogrel CYP2C9 and CYP2C19 to a lesser extent, transform prasugrel into the active metabolite (R-1387279)[8],[9] with a rapid and extensive absorption.[10,11,12] So, prasugrel has emerged as a better alternative to clopidogrel in acute coronary syndromes undergoing percutaneous coronary intervention.[13]

The cytochrome alleles that reduce the effectiveness of clopidogrel could interfere with the activity of prasugrel as well. The insufficient response could also be caused by hyperactive platelets.

Multiple factors may increase platelet activity, the prevailing blood flow conditions, the platelet-platelet interaction and thrombotic prone pathologies.

The sticky platelet syndrome, first described by Mammen et al,[14] is an autosomal dominant platelet disorder, associated with arterial and venous thrombotic event.[15,16] It has been characterized in vitro, by exaggerated platelet aggregation in response to low dose platelet agonists (ADP, epinephrine). For comprehensive review[17] in this number.

Three different types of the syndrome are described, whereas type I show hyperaggregability with ADP and epinephrine, type II only with epinephrine and type III only with ADP.

In Mexico, it has been reported the sticky platelet syndrome as the most common inherited thrombophilia condition. It is present in 57% of people who have a clinical marker of primary thrombophilia.[14,15]

The treatment of this condition might be a challenge, because only anecdotally treatment with aspirin had been reported, in addition that there is not a large reported series of patients with this condition.

In our Institution, the use of prasugrel recently initiated, and we have identified in a patient, a poor response to both drugs prasugrel and clopidogrel, associated with a high level of tissue factor, and the Mammen's criteria fulfilled for the type III sticky platelet syndrome.[16]

MATERIAL AND METHODS

To date, we have studied six consecutive patients with ischemic heart disease, who underwent a coronary angiography at the Hemodynamic Department of the National Institute of Cardiology "Ignacio Chávez". All of them received a loading dose of 60 mg prasugrel before catheterization. We recorded anthropometric measurements, traditional risk factors and clinical variables. Table 1 shows the demographic and clinical characteristics of patients.

For each patient, we obtained a blood sample 24 hour after the administration of 60mg loading dose of prasugrel. Venous blood was extracted by antecubital puncture and placed in a tube with 0.129 mol/L sodium citrate for the platelet aggregation test. EDTA was employed for DNA analysis.

Platelet Aggregation. Samples with sodium citrate were centrifuged at 900 rpm for 1.5 min at room temperature to obtain platelet-rich plasma (PRP). The platelet-poor plasma (PPP) was obtained by centrifuging the sample at

Table 1. Demographic and clinical characteristics of patients.

Variables	*Patients (n = 6)*
Age: (min-max)	57.67±6.8 (45-63)
Gender M/F	6 /0
Smokers (n/%)	1 (16.7)
Diabetes Mellitus type 2 (n/%)	1 (16.7)
Dyslipidemia (n/%)	3 (50)
Hypertension (n/%)	3 (50)
BMI Kg/m^2	25.39±2.93

The variables are expressed as the mean ± standard deviation (SD).
BMI = body mass index, M/F = male/female

3500 rpm for 20 minutes at room temperature. The PRP was adjusted with PPP to obtain a platelet count of 200 000 platelets /mL. The aggregation was performed in a Lumi Aggregometer (Model 560 CA; software Model 810 AGGRO/LINK Chrono-log, Havertown, PA, USA), using 10 mM of ADP as the agonist agent to diagnose prasugrel resistance and then using 0.58 mM, 1.17 µM, 2.34 µM of ADP and 0.55µM, 1.1µM, 11µM of epinephrine in order to diagnose the sticky platelet syndrome as described by Mammen[16]. In this technique, the maximum aggregation is expressed as the percentage of light transmitted through the sample.

Genotyping assay and allelic discrimination. DNA was extracted from peripheral blood leukocytes by a no enzymatic method.[18]

Allelic variants were determined using real-time PCR. ABCB1 linked to drug absorption (rs1045642). CYP2C19*2 (rs4244285), CYP2C19*3 (rs4986893), linked to oxidation reactions, CYP3A5*3 (rs776746) and three polymorphic sites of the P2RY12 gene (rs16846673, rs6809699 and rs6785930) linked to the ADP platelet's receptor. We used pairs of primers and TaqMan probes previously reported.[19] Single nucleotide polymorphisms in genomic DNA were performed using TaqMan assays and analyzed 5'exonucleasa by a 7900HT Fast Real-Time PCR System (Applied Biosystem, Foster City, CA, USA).

TissueFactor. The concentration was determined by enzyme-linked immunosorbent assay (ELISA) (IMUBIND, American Diagnostica, USA) following manufacture directions and using plasma samples frozen at -70°C.

All patients signed an informed consent letter, and the protocol was approved by the Institutional Ethics Committee.

Statistics. We used descriptive statistics using the mean ± SD and the median with minimum and maximum values in accordance with their distribution.

For genetic analysis, Hardy-Weinberg equilibrium was assessed using c^2 analysis. All probability values are two-tailed with values less than 0.05 considered to be statistically significant. All analyses were performed with SPSS 15.0 for Windows. (SPSS, Chicago, IL, USA).

RESULTS

The platelet aggregation results with the different agonist concentrations are shown in the table 2. Five of the studied patients show a satisfactory response to prasugrel except one. His platelet aggregation results fulfill the diagnostic criteria for the sticky platelet syndrome and for prasugrel and clopidogrel resistance as well.

Allelic and genotypic frequencies of ABCB1, CYP2C19*2, CYP2C19*3, CYP3A5*3 gene polymorphisms and the three polymorphic sites (P2RY12 G→A, P2RY12 G52T, P2RY12 C34T) are shown in table 3. The prasugrel resistance patient, did not show any polymorphism, but resulted heterozygous for the variant CYP3A5*3, that has been described associated to clopidogrel resistance.

The median of the tissue factor concentration in patients without resistance was 106.91 pg/mL P_{25-75}(74.32-139.29), in contrast with the prasugrel resistance patient 362.55 pg/mL.

DISCUSSION

Despite the small number of patients included in our study, we could identify a case resistant to prasugrel.

The patient was a 45-year-old man, admitted in April 2011 for a ST-elevation myocardial infarction. His symptoms appear as a severe retrosternal chest pain 14 hour until his admittance. He had only few risk factors, dyslipidemia and overweight without treatment and a family history of coronary artery disease. Electrocardiogram revealed an acute myocardial infarction with ST-segment elevation in an anterolateral location. Killip class I and a TIMI Risk Score of 2 points. The laboratories' tests showed an elevated troponin. I (30.4ng/mL), CPK-MB (29.83ng/ml) DHL (2150 U/l). Management was initiated with aspirin 300 mg, prasugrel 60 mg and unfractionated heparin 5000 IU. Coronary angiography was performed via the right femoral artery; it was found the left main, left anterior descending artery (LAD) and the right coronary artery (RCA) with moderate ectasia and a "slow-flow" pattern, whereas the left circumflex artery (LCX) showed a significant ectasia and a thrombus in it proximal segment with a TIMI 0 flow. It was also performed a cardiac MRI that showed a lateral myocardial infarction with microvascular obstruction and an ejection fraction of 48%.

Given these findings, it was decided to do not perform a coronary angioplasty and starting antiplatelet therapy with prasugrel. Based on laboratory platelet aggregation tests criteria for prasugrel and clopidogrel resistance, the

Table 2. Platelet aggregation results of the drug-responsive patients and the resistance case.

Agonist Agent	*Drug-responsive patients N=5 % aggregation 24 hours post prasugrel*,***	*Resistance patient N=1 % aggregation 24 hours post prasugrel**	*Resistance patient N=1 % aggregation 1.5 hours post clopidogrel****	*Resistance patient N=1 % aggregation 24 hours post clopidogrel****
ADP 0.58µM	42.5% ± 14.2	125 %	100 %	100 %
ADP 1.17µM	33.88% ± 6.8		97 %	107 %
ADP 2.34µM	32.41% ± 5.09		68 %	68 %
ADP 10µM	26.27% ± 12.6	82 %	51 %	61 %
EPI 0.55µM	61.64% ± 37.6	149 %	25 %	50 %
EPI 1.1µM	100.89%		47 %	101 %
EPI 11µM	63.08% ± 15.7	230 %	130 %	26 %

*Prasugrel dose was 60mg, ** Variable expressed as the mean ± standard deviation ***Clopidogrel dose was 300mg.

Table 3. Allelic and genotypic frequencies of gene polymorphisms.

Nucleotide Change and Genotype	*Drug-responsive patients n = 6*	*Resistance patient*	*Hardy–Weinberg equilibrium, χ2 analysis*
ABCB1 C3435T (rs1045642)			0.66
CC- No (%)	3 (50)		
CT- No (%)	3 (50)	CT	
TT- No (%)	0 (0)		
CYP2C19*2 G681A (rs4244285)			0.66
GG- No (%)	3 (50)	GG	
AG- No (%)	3 (50)		
AA- No (%)	0 (0)		
CYP2C19*3 G636A (rs4986893)			-
GG- No (%)	6 (100)	GG	
AG- No (%)	0 (0)		
AA- No (%)	0 (0)		
CYP3A5*3 A6986G (rs776746)			0.24
GG- No (%)	4 (66.7)		
AG- No (%)	2 (33.3)	AG	
AA- No (%)	0 (0)		
P2RY12 G→A (rs16846673)			-
AA- No (%)	6 (100)	AA	
AG- No (%)	0 (0)		
GG- No (%)	0 (0)		
P2RY12 G52T (rs6809699)			0.24
GG- No (%)	4 (66.7)	GG	
GT- No (%)	2 (33.3)		
TT- No (%)	0 (0)		
P2RY12 C34T (rs6785930)			0.24
CC- No (%)	4 (66.7)	CC	
CT- No (%)	2 (33.3)		
TT- No (%)	0 (0)		

patient was treated with aspirin, three days of 60 mg, b.i.d., s.c. enoxaparin and long term anticoagulation with acenocoumarin.

The only case reported before of dual resistance to thienopyridines was in a patient homozygous to CYP2C19 that confers a poor response to the drugs.[20]

The present prasugrel/clopidogrel resistant patient, did not show any polymorphism directly associated with thienopyridines resistance, fulfilled the diagnostic criteria for the sticky platelet syndrome, in addition to the high concentration of tissue factor.

The presence of tissue factor in platelet's microvesicles should be studied in these patients in order to identify platelet procoagulant activity.

These data show the possibility of low response to two thienopyridines without an associated drug-resistance polymorphism**.**

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