

# Comparison of Differential Expression of P2Y<sub>12</sub> Receptor in Culprit Coronary Plaques in Patients With Acute Myocardial Infarction Versus Stable Angina Pectoris

Cheol Whan Lee, MD<sup>a,†</sup>, Ilseon Hwang, MD, PhD<sup>d,†</sup>, Chan-Sik Park, MD, PhD<sup>b</sup>, Hyangsin Lee, MS<sup>c</sup>, Duk-Woo Park, MD<sup>a</sup>, Su-Jin Kang, MD<sup>a</sup>, Seung-Whan Lee, MD<sup>a</sup>, Young-Hak Kim, MD<sup>a</sup>, Seong-Wook Park, MD, PhD<sup>a</sup>, and Seung-Jung Park, MD, PhD<sup>a,\*</sup>

P2Y<sub>12</sub> receptor antagonists may have pleiotropic benefits. Little is known, however, about the expression of P2Y<sub>12</sub> receptors in coronary atherosclerotic plaques. We investigated the expression of P2Y<sub>12</sub> receptor in coronary atherectomy tissues retrieved from patients with acute myocardial infarction (AMI) or stable angina pectoris (SAP). Tissue specimens were collected from 35 patients with AMI and 19 with SAP who underwent directional coronary atherectomy. Specimens were analyzed immunohistochemically using antibodies specific to P2Y<sub>12</sub> receptor and to markers of endothelial cells, macrophages, and smooth muscle cells. The 2 groups had similar baseline clinical characteristics. Plaque types were more likely to be cellular in the AMI group. The proportion of areas immunopositive for  $\alpha$ -smooth muscle actin was smaller but those positive for CD31 and CD68 were larger in the AMI than in the SAP group. In addition, the relative area immunopositive for P2Y<sub>12</sub> receptor was significantly larger for AMI than SAP ( $1.1 \pm 0.9\%$  vs  $0.5 \pm 0.4\%$ , respectively,  $p < 0.001$ ). P2Y<sub>12</sub> receptor positivity coincided with areas positive for CD31 and  $\alpha$ -smooth muscle actin. In conclusion, P2Y<sub>12</sub> receptor is present in coronary atherosclerotic plaques and is increased in culprit plaques of patients with AMI. P2Y<sub>12</sub> receptor may play a role in plaque destabilization. © 2011 Elsevier Inc. All rights reserved. (Am J Cardiol 2011;108:799–803)

P2Y<sub>12</sub> receptors are expressed on endothelial cells, vascular smooth muscle cells, and platelets.<sup>1,2</sup> Clopidogrel can decrease markers of vascular inflammation and improve endothelial function.<sup>3–7</sup> Recent results from the Platelet Inhibition and Patient Outcomes (PLATO) trial showed that the cardiovascular benefits of ticagrelor outweighed those of clopidogrel despite being associated with more spontaneous bleeding events.<sup>8,9</sup> The vascular benefits of ticagrelor could not be explained by its faster and more potent platelet inhibition alone, suggesting that ticagrelor has platelet-independent pleiotropic effects coupled with P2Y<sub>12</sub> receptor antagonism. To date, however, little is known about the expression of P2Y<sub>12</sub> receptors in coronary atherosclerotic plaques and its relation to plaque instability. We therefore compared the expression of P2Y<sub>12</sub> receptors in coronary atherectomy tissues retrieved from patients with acute myocardial infarction (AMI) to those from patients with stable angina pectoris (SAP).

## Methods

Atherectomy specimens were obtained from a biobank at our institution, which had collected tissues from 35 consecutive patients with AMI and 19 with SAP, defined as typical exertional angina with no change in symptoms within 1 month before the procedure. Patients were suitable for directional coronary atherectomy if they had a significant stenotic lesion with a large plaque burden but lacked heavy thrombi in a nontortuous epicardial coronary artery  $>3$  mm in diameter.<sup>10,11</sup> Each specimen corresponded to the de novo lesion responsible for the clinical presentation in a single patient. Directional coronary atherectomy was performed using a Flexi-Cut catheter (Abbott Laboratories/Guidant Vascular Interventions, Santa Clara, California) under intravascular ultrasound guidance. The study protocol was approved by our institutional review committee, and all patients provided written informed consent.

Tissue specimens were fixed in formalin and embedded in donor paraffin blocks. Tissue microarrays were produced by re-embedding tissues from these paraffin blocks into arrays on recipient paraffin blocks. Sections from the master block were cut using a microtome, mounted on a microscope slide, and used for subsequent staining.

Samples were stained with hematoxylin and eosin to determine cellularity and general morphologic features. The area of each plaque was measured using a microscopic image analysis system (Motic Images Advanced 3.2, Motic, Xiamen, China). Plaques were classified as atheromatous (i.e., with necrotic cores and cholesterol clefts but without connective tissue matrix) or fibrocellular and graded as

Departments of <sup>a</sup>Medicine and <sup>b</sup>Pathology, Asan Medical Center and <sup>c</sup>Asan Institute of Life Science, University of Ulsan, Seoul, Korea; <sup>d</sup>Department of Pathology, School of Medicine, Keimyung University, Daegu, Korea. Manuscript received April 6, 2011; revised manuscript received and accepted May 6, 2011.

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\*Corresponding author: Tel: 82-2-3010-3150; fax: 82-2-486-5918.

E-mail address: [sjpark@amc.seoul.kr](mailto:sjpark@amc.seoul.kr) (S.-J. Park).

<sup>†</sup> Drs. Lee and Hwang contributed equally to this work.

Table 1  
Clinical characteristics of study subjects

Characteristics	AMI (n = 35)	SAP (n = 19)	P Value
Age (years)	56.4 ± 11.2	61.0 ± 6.9	0.107
Men/women	29/6	12/7	0.181
Current smoker	16 (45.7%)	6 (31.6%)	0.391
Diabetes mellitus	7 (20%)	7 (36.8%)	0.206
Hypertension	17 (48.6%)	11 (57.9%)	0.577
Total cholesterol (mg/dl)	192.9 ± 44.6	153.0 ± 30.1	0.001
Triglycerides (mg/dl)	179.4 ± 84.2	110.5 ± 45.5	0.002
High-density lipoprotein cholesterol (mg/dl)	34.8 ± 7.9	43.0 ± 14.4	0.031
High-sensitivity C-reactive protein (mg/L)	3.3 ± 3.3	1.7 ± 1.8	0.059
Multivessel coronary disease	17 (48.6%)	7 (36.8%)	0.567
Target coronary artery			0.795
Left anterior descending	19 (54.3%)	12 (63.2%)	
Left circumflex	3 (8.6%)	1 (5.3%)	
Right	13 (37.1%)	6 (31.6%)	
Medications at time of directional coronary atherectomy			
Aspirin	35 (100%)	19 (100%)	1.0
Clopidogrel	35 (100%)	19 (100%)	1.0
Angiotensin-converting enzyme inhibitor/angiotensin receptor blocker	2 (5.7%)	2 (10.5%)	0.607
β Blockers	7 (20%)	9 (4.7%)	0.060
Calcium channel antagonists	5 (14.3%)	11 (57.9%)	0.001
Statins	11 (31.4%)	13 (68.4%)	0.012

sparingly cellular (<30 spindle cells per high-power field), moderately cellular (30 to 100 spindle cells per field), or hypercellular (≥100 spindle cells per field). All slides were graded by 2 pathologists (C.-S.P. and I.H.) blinded to patients' clinical status. Any discrepancies between their findings were resolved by consensus.

Sections of each tissue specimen were stained with rabbit polyclonal antibodies against P2Y<sub>12</sub> receptor (1:100; Novus Biologicals, Littleton, Colorado) and monoclonal antibodies against α-smooth muscle actin (1:200; mouse antihuman macrophage antibody clone 1A4; DAKO, Carpinteria, California), CD31 (1:100; mouse antihuman CD31 [platelet endothelial cell adhesion molecule-1] antibody clone 1A10; Leica, Newcastle, United Kingdom), and CD68 (1:200; mouse antihuman macrophage antibody clone KP-1; DAKO) using an Envision-plus immunostaining kit and 3,3'-diaminobenzidine or 3-amino-9-ethylcarbazole as the chromogen as described by the manufacturer (DAKO). Briefly, samples were incubated with primary antibodies for 1 hour, washed 2 times for 5 minutes each with Tris buffered saline/Tween-20, incubated with secondary antibodies conjugated with horseradish peroxidase-labeled polymer (DAKO) for 1 hour, and washed again. As negative controls, adjacent sections were stained with species- and isotype-matched irrelevant antibodies including normal rabbit immunoglobulin G (Abcam, Cambridge, United Kingdom). A sample of platelets was used as a positive control for anti-P2Y<sub>12</sub> receptor antibodies. Cell

Table 2  
Histologic characteristics of study subjects

Variables	AMI (n = 35)	SAP (n = 19)	P Value
Histology			
Atheroma	48.7 ± 26.1	31.1 ± 30.3	0.030
Fibrocellular area			
Sparsely cellular	35.5 ± 23.5	51.6 ± 30.4	0.035
Moderately cellular	7.6 ± 9.0	15.7 ± 24.6	0.179
Hypercellular	2.5 ± 5.1	0.5 ± 1.9	0.041
Thrombus	5.2 ± 16.2	0.0 ± 0.1	<0.001
Calcium	0.5 ± 1.9	1.0 ± 3.0	0.509
Total plaque area (mm <sup>2</sup> )	704.4 ± 344.0	496.5 ± 314.9	0.034
Immunohistochemistry			
α-Smooth muscle actin	2.9 ± 2.7	12.3 ± 14.4	0.011
CD31	1.1 ± 1.6	0.2 ± 0.2	0.001
CD68	15.5 ± 13.6	7.0 ± 14.6	0.038
P2Y <sub>12</sub>	1.1 ± 0.9	0.5 ± 0.4	<0.001

Data are expressed as percent positive areas (immunostained area/total plaque area × 100).

types positive for P2Y<sub>12</sub> receptor were identified by immunostaining of serial sections with anti-P2Y<sub>12</sub> receptor antibodies. The immunopositive area was calculated as the ratio of positively stained regions to total plaque area.

For immunofluorescent staining, fixed sections were hydrated in phosphate buffered saline (PBS) for 10 minutes at room temperature, incubated with DakoCytomation Protein Block (DakoCytomation, Carpinteria, California) for 5 minutes at room temperature, and washed 3 times in PBS/Tween-20. Sections were subsequently incubated with mouse monoclonal antibody to human CD31 (Leica), CD68 (DakoCytomation), or α-smooth muscle actin (DakoCytomation) or rabbit polyclonal antibody to P2Y<sub>12</sub> receptor for 60 minutes at room temperature. After 3 additional washes in PBS/Tween-20, sections were incubated with fluorescein isothiocyanate-conjugated antirabbit immunoglobulin G or allophycocyanin-conjugated antimouse immunoglobulin G for 60 minutes at room temperature and washed 3 times with PBS/Tween-20. Coverslips were mounted onto glass slides using DAKO fluorescent mounting medium (DakoCytomation). Fluorescein isothiocyanate was excited using an argon laser at 488 nm and allophycocyanin was excited by a helium-neon laser at 633 nm. Detector slits were configured to minimize any crosstalk between channels. Images were collected on a Leica TCS-NT/SP confocal microscope (Leica Microsystems, Mannheim, Germany) equipped with a 40× objective (model NA 0.75) and a Zoom 1-4 × and processed using Leica TCS-NT/SP software (version LCS) and Adobe Photoshop 7.0 (San Jose, California).

Continuous variables are expressed as mean ± SD or median with interquartile range, whereas categorical variables are expressed as frequency. Continuous variables were compared using Student's *t* test or Mann-Whitney U test, and categorical variables were analyzed using chi-square test. Linear regression analysis was used to correlate areas positive for P2Y<sub>12</sub> receptor with those positive for markers for endothelial cells, macrophages, and smooth muscle markers. Statistical significance was defined as a 2-sided *p* value <0.05.

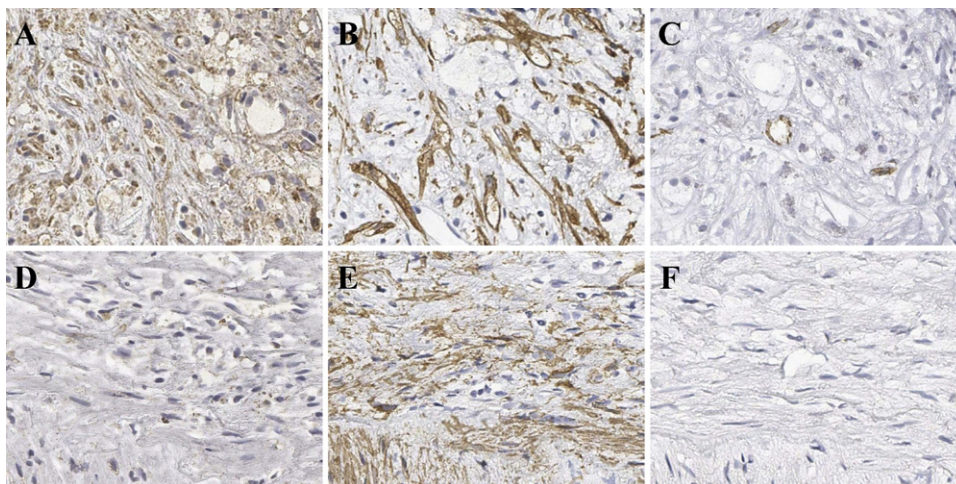


Figure 1. Representative images of immunohistochemical staining (magnification 400 $\times$ ) from patients with acute myocardial infarction (*A to C*) or stable angina pectoris (*D to F*). (*A*) P2Y<sub>12</sub> receptor was strongly stained on plaque from a patient with acute myocardial infarction (*brown*). Serial sections of immunostaining show strong positive areas of (*B*)  $\alpha$ -smooth muscle actin (*brown*) and (*C*) focal positive areas of CD31 (*brown*). (*D*) P2Y<sub>12</sub> receptor was weakly stained on plaque from a patient with stable angina pectoris. (*E*) Alpha-smooth muscle actin (*brown*) was strongly stained, (*F*) but CD31 was not stained in similar areas.

Figure 2. Immunofluorescence staining (magnification 800 $\times$ ) of coronary plaques of a patient with acute myocardial infarction using antibodies to (*A, D, G*) P2Y<sub>12</sub> receptor (*green*), (*B*)  $\alpha$ -smooth muscle actin (*red*), (*E*) CD31 (*red*), and (*H*) CD68 (*red*). P2Y<sub>12</sub> receptor-immunopositive cells colocalized with cells positive for (*C*)  $\alpha$ -smooth muscle actin (*arrows*) or (*F*) CD31 (*arrows*) but not with (*I*) CD68.

## Results

Baseline clinical characteristics were similar between the 2 groups except for their lipid profiles and medications (Table 1). Median time from symptom onset to reperfusion was 5.5 hours (interquartile range 2.5 to 7.5) for ST-segment elevation AMI ( $n = 27$ ) and 35.5 hours (interquartile range 24 to 90) for non-ST-segment elevation AMI ( $n = 8$ ). At the time of directional coronary atherectomy, larger percentages of patients in the SAP group were taking calcium channel blockers and statins than those in the AMI group.

Total plaque area was smaller, whereas atheroma area was larger, in the AMI than in the SAP group (Table 2). Plaque types were more likely to be cellular in the AMI group. Thrombi were significantly more common in the AMI than in the SAP group (68.6% vs 1.1%,  $p < 0.001$ ). Relative plaque area immunopositive for  $\alpha$ -smooth muscle actin was significantly smaller in the AMI group than in the SAP group, whereas areas immunopositive for CD31 and CD68 were significantly larger in the AMI group (Table 2). In addition, relative area immunopositive for P2Y<sub>12</sub> recep-

tor was significantly larger in plaques from patients with AMI than from those with SAP ( $1.1 \pm 0.9\%$  vs  $0.5 \pm 0.4\%$ ,  $p < 0.001$ ). In multivariate analysis, areas immunopositive for P2Y<sub>12</sub> receptor ( $r = 0.54$ ) were significantly correlated with those positive for CD31 (beta 0.30,  $p = 0.022$ ) and CD68 (beta 0.36,  $p = 0.007$ ).

Representative immunohistochemical staining of P2Y<sub>12</sub> receptor is shown in Figure 1. Immunoreactivity with the anti-P2Y<sub>12</sub> receptor antibody was stronger in the AMI than the SAP plaque. Staining of serial sections of these plaques demonstrated a similar distribution of P2Y<sub>12</sub> receptor- and  $\alpha$ -smooth muscle actin-immunopositive areas in AMI, but not in SAP. Likewise, CD31 was focally stained on the similar area in AMI but not in SAP. To determine cellular localization of the P2Y<sub>12</sub> receptor, we performed double-immunofluorescence staining on coronary atherosclerotic plaques from a patient with AMI (Figure 2). Confocal immunofluorescence staining revealed that P2Y<sub>12</sub> receptor immunoreactivity colocalized with cells positive for CD31 and  $\alpha$ -smooth muscle actin but not with those positive for CD68.

## Discussion

This study is the first to show that P2Y<sub>12</sub> receptor is present in human coronary atherosclerotic plaques, that its level of expression is higher in plaques from patients with AMI than from those with SAP, and that P2Y<sub>12</sub>-immunopositive cells are endothelial cells and vascular smooth muscle cells. These findings support the hypothesis that P2Y<sub>12</sub> receptor inhibitors may have a dual anti-ischemic effect by inhibiting platelet activation and plaque destabilization.

Clinical benefits of P2Y<sub>12</sub> receptor inhibitors are largely driven by their antiplatelet activity. More recently, however, P2Y<sub>12</sub> receptor antagonists have been shown to have platelet-independent effects on the vasculature. For example, P2Y<sub>12</sub> receptor-mediated vasoconstriction of human internal mammary arteries, as demonstrated by 2-MeSADP-induced contraction of submaximally precontracted vessels, was blocked by a selective reversible P2Y<sub>12</sub> receptor antagonist but not by clopidogrel.<sup>2</sup> Clopidogrel does not inhibit adenosine diphosphate (ADP)-mediated vasoconstriction, probably because of pharmacokinetics.<sup>12,13</sup> In contrast, ticagrelor was shown to inhibit ADP-mediated vasoconstriction of isolated human arteries.<sup>14</sup> In addition, thrombin, which is generated during vessel injury, can increase expression of P2Y<sub>12</sub> receptor in vascular smooth muscle cells, resulting in increased vasoconstriction and a greater proinflammatory response to ADP.<sup>15</sup> In our study P2Y<sub>12</sub> receptor was more strongly expressed in culprit plaques of patients with AMI than in those with SAP. Areas immunopositive for P2Y<sub>12</sub> receptor were correlated with CD68 immunopositive areas ( $r = 0.46$ ,  $p = 0.001$ ), and P2Y<sub>12</sub> receptor was more frequently found in vascular smooth muscle cells around areas of inflammatory cell infiltrations, suggesting that vascular smooth muscle cells at sites of inflammation more actively produce P2Y<sub>12</sub> receptor. ADP released by a platelet thrombus may contribute to vasospasm in a culprit lesion, worsening the ischemia caused by the thrombus. Therefore, the additional benefits of ticagrelor compared to

clopidogrel may be due, at least in part, to inhibition by ticagrelor of P2Y<sub>12</sub>-mediated vasoconstriction.

P2Y<sub>12</sub> receptor inhibitors may improve endothelial dysfunction by direct interaction with the endothelial P2Y<sub>12</sub> receptor, which is, as yet, poorly characterized.<sup>16,17</sup> Nitric oxide-dependent coronary vasodilation by clopidogrel has been shown to be independent of its antiplatelet activity in an animal study.<sup>16</sup> Clopidogrel has been found to increase endothelial nitric oxide bioavailability and decrease biomarkers of oxidant stress and inflammation in patients with coronary artery disease.<sup>5</sup> Moreover, clopidogrel, but not aspirin, has been found to block plasma-promoted endothelial activation.<sup>17</sup> We found that CD31, an endothelial cell marker, was strongly expressed in culprit plaques of patients with AMI, that there was a significant correlation between areas positive for CD31 and P2Y<sub>12</sub> receptor, and that CD31-immunopositive cells colocalized with cells positive for P2Y<sub>12</sub> receptor. These findings suggest that endothelial cells express P2Y<sub>12</sub> receptor, especially in culprit plaques of AMI. Optimal P2Y<sub>12</sub> receptor blockade has been reported to decrease endothelial injury during percutaneous coronary intervention,<sup>18</sup> and high maintenance-dose clopidogrel compared to standard-dose clopidogrel has been shown to improve endothelial function and decrease inflammation,<sup>19</sup> indicating that P2Y<sub>12</sub> receptor antagonists have a protective effect on endothelial cells. Thus P2Y<sub>12</sub> receptor is likely involved in plaque destabilization by activating endothelial cells. At present, however, very little is known about the biological functions and signaling pathways of P2Y<sub>12</sub> receptors in endothelium.<sup>1,20</sup>

One limitation of this study is that atherectomy specimens were obtained from selected lesions in large vessels because calcified, tortuous, and small vessels are not suitable for directional coronary atherectomy. Another limitation is that P2Y<sub>12</sub> receptor expression could not be confirmed by western blot analysis.

1. Shanker G, Kontos JL, Eckman DM, Wesley-Farrington D, Sane DC. Nicotine upregulates the expression of P2Y<sub>12</sub> on vascular cells and megakaryoblasts. *J Thromb Thrombolysis* 2006;22:213–220.
2. Wihlborg AK, Wang L, Braun OO, Eijolfsson A, Gustafsson R, Gudbjartsson T, Erlinge D. ADP receptor P2Y<sub>12</sub> is expressed in vascular smooth muscle cells and stimulates contraction in human blood vessels. *Arterioscler Thromb Vasc Biol* 2004;24:1810–1815.
3. Quinn MJ, Bhatt DL, Zidar F, Vivekananthan D, Chew DP, Ellis SG, Plow E, Topol EJ. Effect of clopidogrel pretreatment on inflammatory marker expression in patients undergoing percutaneous coronary intervention. *Am J Cardiol* 2004;93:679–684.
4. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Sabaté M, Jimenez-Quevedo P, Hernandez R, Moreno R, Escaned J, Alfonso F, Bañuelos C, Costa MA, Bass TA, Macaya C. Clopidogrel withdrawal is associated with proinflammatory and prothrombotic effects in patients with diabetes and coronary artery disease. *Diabetes* 2006;55:780–784.
5. Heitzer T, Rudolph V, Schwedhelm E, Karstens M, Sydow K, Ortak M, Tschentscher P, Meinertz T, Böger R, Baldus S. Clopidogrel improves systemic endothelial nitric oxide bioavailability in patients with coronary artery disease: evidence for antioxidant and antiinflammatory effects. *Arterioscler Thromb Vasc Biol* 2006;26:1648–1652.
6. Warnholtz A, Ostad MA, Velich N, Trautmann C, Schinzel R, Walter U, Munzel T. A single loading dose of clopidogrel causes dose-dependent improvement of endothelial dysfunction in patients with stable coronary artery disease: results of a double-blind, randomized study. *Atherosclerosis* 2008;196:689–695.
7. Bonello L, Harhour K, Sabatier F, Camoin-Jau L, Arnaud L, Baumstarck-Barrau K, Ait-Mokhtar O, Roubille F, Piot C, Lesavre N,

- Paganelli F, Dignat-George F. Level of adenosine diphosphate receptor P2Y<sub>12</sub> blockade during percutaneous coronary intervention predicts the extent of endothelial injury, assessed by circulating endothelial cell measurement. *J Am Coll Cardiol* 2010;56:1024–1031.
8. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, Horrow J, Husted S, James S, Katus H, Mahaffey KW, Scirica BM, Skene A, Steg PG, Storey RF, Harrington RA, Freij A, Thorsén M; PLATO Investigators. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2009;361:1045–1057.
  9. Serebruany VL, Atar D. The PLATO trial: do you believe in magic? *Eur Heart J* 2010;31:764–767.
  10. Kurisu S, Sato H, Tateishi H, Kawagoe T, Ishihara M, Shimatani Y, Sakai K, Ueda K, Matsuura H. Directional coronary atherectomy for the treatment of acute myocardial infarction. *Am Heart J* 1997;134:345–350.
  11. McKnight J, Studeny M, Roberts G, Touchon R, Wehner P. Directional coronary atherectomy in acute myocardial infarction. *W V Med J* 2001;97:109–110.
  12. Wallentin L, Varenhorst C, James S, Erlinge D, Braun OO, Jakubowski JA, Sugidachi A, Winters KJ, Siegbahn A. Prasugrel achieves greater and faster P2Y<sub>12</sub> receptor-mediated platelet inhibition than clopidogrel due to more efficient generation of its active metabolite in aspirin-treated patients with coronary artery disease. *Eur Heart J* 2008;29:21–30.
  13. Pereillo JM, Maftouh M, Andrieu A, Uzabiaga MF, Fedeli O, Savi P, Pascal M, Herbert JM, Maffrand JP, Picard C. Structure and stereochemistry of the active metabolite of clopidogrel. *Drug Metab Dispos* 2002;30:1288–1295.
  14. Högberg C, Svensson H, Gustafsson R, Eyjolfsson A, Erlinge D. The reversible oral P2Y<sub>12</sub> antagonist AZD6140 inhibits ADP-induced contractions in murine and human vasculature. *Int J Cardiol* 2010;142:187–192.
  15. Rauch BH, Rosenkranz AC, Ermler S, Böhm A, Driessen J, Fischer JW, Sugidachi A, Jakubowski JA, Schrör K. Regulation of functionally active P2Y<sub>12</sub> ADP receptors by thrombin in human smooth muscle cells and the presence of P2Y<sub>12</sub> in carotid artery lesions. *Arterioscler Thromb Vasc Biol* 2010;30:2434–2442.
  16. Jakubowski A, Chlopicki S, Olszanecki R, Jawien J, Lomnicka M, Dupin JP, Gryglewski RJ. Endothelial action of thienopyridines and thienopyrimidinones in the isolated guinea pig heart. *Prostaglandins Leukot Essent Fatty Acids* 2005;72:139–145.
  17. Dünzendorfer S, Reinisch CM, Kaneider NC, Pechlaner CH, Wiedermann CHJ. Inhibition of plasma-dependent monocyte chemokinesis and cytokine-triggered endothelial activation for neutrophil transmigration by administration of clopidogrel in man. *Acta Med Austriaca* 2002;29:100–106.
  18. Bonello L, Harhour K, Sabatier F, Camoin-Jau L, Arnaud L, Baumstarck-Barrau K, Ait-Mokhtar O, Roubille F, Piot C, Lesavre N, Paganelli F, Dignat-George F. Level of adenosine diphosphate receptor P2Y<sub>12</sub> blockade during percutaneous coronary intervention predicts the extent of endothelial injury, assessed by circulating endothelial cell measurement. *J Am Coll Cardiol* 2010;56:1024–1031.
  19. Patti G, Grieco D, Dicuonzo G, Pasceri V, Nusca A, Di Sciascio G. High versus standard clopidogrel maintenance dose after percutaneous coronary intervention and effects on platelet inhibition, endothelial function, and inflammation results of the ARMYDA-150 mg (Antiplatelet Therapy for Reduction of Myocardial Damage During Angioplasty) randomized study. *J Am Coll Cardiol* 2011;57:771–778.
  20. Simon J, Filippov AK, Göransson S, Wong YH, Frelin C, Michel AD, Brown DA, Barnard EA. Characterization and channel coupling of the P2Y(12) nucleotide receptor of brain capillary endothelial cells. *J Biol Chem* 2002;277:31390–31400.