Ecto-nucleoside triphosphate diphosphohydrolase inhibits ATP- and ADP-induced vasoconstriction

Ecto-nucleoside triphosphate diphosphohydrolase inhibits ATP and ADP-induced vasoconstriction.

E. Furukoji[a], N. Tanaka[b], A. Yamashita[c], M. Matsumoto[d], Y. Fujimura[d]; R. Yamamoto[b], S. Tamura[a], Y. Asada[c].

[a]Department of Radiology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

[b]First Department of Pharmacology, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, Nobeoka, Japan

[c]Department of Pathology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

[d]Department of Blood Transfusion Medicine, Nara Medical University, Kashihara, Nara, Japan

Correspondence to: Yujiro Asada MD

Department of Pathology, Faculty of Medicine, University of Miyazaki

5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan.

Fax: +81-985-85-7614

Phone: +81-985-85-2810

E-mail: yasada@fc.miyazaki-u.ac.jp

Word counts of the text: 1984

To the Editor:

Occlusive thrombus formation on disrupted atherosclerotic plaque is a critical event in the onset of acute myocardial infarction and serious complication after vascular intervention. Although the exact mechanisms of occlusive thrombus formation remain unclear, it might be affected by amount of thrombogenic materials in disrupted plaques and blood flow alternation due to vasoconstriction of injured vessels or increased peripheral resistance mediated by microemboli [1, 2]. Activated platelets play a pivotal role in thrombus formation, and they release additional agonists such as adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin and thromboxane A_2, which cause further platelet recruitment to injured sites. Since ADP plays a key role in platelet aggregation [3], its metabolism in the blood is important in the regulation of platelet activation and recruitment. Ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) is a major metabolic enzyme of ADP and ATP in the vasculature, and thereby inhibits platelet aggregation [4, 5]. We previously reported that local expression of placental E-NTPDase I, an isoform of E-NTPDase, suppresses platelet aggregation and occlusive thrombus formation in rat carotid arteries [6]. In addition to platelet agonists, ATP and ADP induce vasoconstriction via its prynergic receptors on vascular smooth muscle cell (SMC)s [7, 8]. Platelet aggregation after plaque rupture, therefore, would contribute to vasoconstriction, simultaneously platelet aggregation and thrombus formation are highly modulated by the degree of vascular narrowing after rupture [2]. This suggests that the local delivery of E-NTPDase might inhibit thrombogenic vasoconstriction, and subsequently reduce thrombus growth. However, the direct evidence showing E-NTPDase affect vasoconstriction is still lacking.

We have observed that over-expression of placental E-NTPDase I in injured arteries inhibited vasoconstriction induced by ADP and ATP in rat carotid arteries. Adenovirus-mediated gene transfer of placental E-NTPDase I (AdPlac I) or bacterial β-galactosidase (AdLacZ) (each final titer,

5.0×10^8 plaque forming units) was performed into injured carotid arteries of male Sprague-Dawley rats (weighing 400 to 500 g), as described previously [6]. All procedures were carried out according to the protocol approved by The Animal Care Committee of University of Miyazaki. Five days after gene transfer, ATPase and ADPase activities in E-NTPDase infected arteries were 2.0 and 1.7 fold higher than those of control arteries, respectively (2.49+/-0.22 vs. 1.22+/-0.20; 1.50+/-0.12 vs. 0.85+/-0.10 nmol Pi/min/mg, n=6 each, $p<0.05$). Vasoconstriction of these arteries to ATP and ADP was investigated by the isometric tension of artery strips with denudation of endothelial cells as described previously [9]. Although the contraction to norepinephrine did not differ among in control, AdPlac I- and AdLacZ-infected arteries, those to ATP and ADP were significantly reduced in AdPlac I -infected arteries, but not in control and AdLacZ-infected arteries (Figure 1). The maximum concentration ratios to KCl of AdPlac I-infected arteries by ATP and ADP (10^{-2} M) were 68.1+/-13.9 and 61.1+/-9.3 %, respectively.

Thrombus-mediated vasoconstriction is a critical vascular response in the onset of cardiovascular events, because blood flow alteration after plaque disruption would promote thrombus growth [1, 2]. Nucleotides released from activated platelets are the major mediators in this process as well as serotonin and thromboxane A_2. It has been reported that nucleotides stimulate P2 receptors on vascular SMCs, and regulate vascular tone and blood pressure [7]. The present results demonstrate the local expression of E-NTPDase might prevent ATP- and ADP-induced vasoconstriction in injured vessels. The role of E-NTPDases on SMCs have not been fully defined. When vascular injuries with endothelial denudation occur, E-NTPDase on SMCs might play dual protective roles, prevention of platelet activation and vasoconstriction by the nucleotides released from activated and adhered platelets at the injured vessels, however native E-NTPDase activity of vascular SMCs is very low [6]. In addition, E-NTPDase activity is considered to be reduced in atherosclerotic lesions, because

E-NTPDase would be inactivated by exposure to oxidative stress [10]. We previously investigated E-NTPDase/CD39 expression in coronary atheromatous plaques by immunohistochemistry, and demonstrated that the protease is expressed by SMCs and endothelial cells, but not by macrophages, and that the immunopositive cell ratio was significantly lower in plaques from patients with unstable angina than stable angina [11]. It is also showed that lymphocyte ADP/ATP ectonucleotidase activity is reduced in patients with ischemic heart diseases, compared with that in healthy subjects [12]. Reduced E-NTPDase activities might contribute to thrombus growth and vasoconstriction of atherosclerotic arteries with plaque disruption. Several prynargic receptors on SMCs elicit vasoconstriction when stimulated by extracellular nucleotides [13]. It is generally accepted that ATP acts as vasoconstrictor via P_{2X1} and P_{2Y2} receptors [14, 15], and ADP receptor P_{2Y12} expressed on SMC stimulate vasoconstriction [8]. Because nucleotide induced vasoconstriction is mediated by several prynargic receptors, E-NTPDase rather than specific receptor antagonists might be useful tool for suppression of vasoconstriction. The finding in this study suggests that local expression of E-NTPDase in atherosclerotic vessels might be a novel therapeutic strategy for prevention of cardiovascular events and complications after vascular intervention.

References

[1] Topol EJ. A guide to therapeutic decision-making in patients with non-ST-segment elevation acute coronary syndromes. *J Am Coll Cardiol* 2003;**41**:S123-9.

[2] Fuster V, Moreno PR, Fayad ZA, Corti R, Badimon JJ. Atherothrombosis and high-risk plaque. Part I: Evolving concepts. *J Am Coll Cardiol* 2005;**46**:937-54.

[3] Ruggeri ZM. Platelets in atherothrombosis. *Nat Med* 2002;**8**:1227-34.

[4] Robson SC, Wu Y, Sun X, Knosalla C, Dwyer K, Enjyoji K. Ectonucleotidases of CD39 family

modulate vascular inflammation and thrombosis in transplantation. *Semin Thromb Hemost* 2005;**31**:217-33.

[5] Marcus AJ, Broekman MJ, Drosopoulos JH, Olson KE, Islam N, Pinsky DJ, Levi R. Role of CD39 (NTPDase-1) in thromboregulation, cerebroprotection, and cardioprotection. *Semin Thromb Hemost* 2005;**31**:234-46.

[6] Furukoji E, Matsumoto M, Yamashita A, Yagi H, Sakurai Y, Marutsuka K, Hatakeyama K, Morishita K, Fujimura Y, Tamura S, Asada Y. Adenovirus-mediated transfer of human placental ectonucleoside triphosphate diphosphohydrolase to vascular smooth muscle cells suppresses platelet aggregation in vitro and arterial thrombus formation in vivo. *Circulation* 2005;**111**:808-15.

[7] Kunapuli SP, Daniel JL. P2 receptor subtypes in the cardiovascular system. *Biochem J* 1998;**336**:513-23.

[8] Wihlborg AK, Wang L, Braun OO, Eyjolfsson A, Gustafsson R, Gudbjartsson T, Erlinge D. ADP receptor P2Y12 is expressed in vascular smooth muscle cells and stimulates contraction in human blood vessels. *Arterioscler Thromb Vasc Biol* 2004 ;**24**:1810-5.

[9] Kihara H, Koganei H, Hirose K, Yamamoto H, Yoshimoto R. Antithrombotic activity of AT-1015, a potent 5-HT_{2A} receptor antagonist, in rat arterial thrombosis model and its effect on bleeding time. *Eur J Pharmacol*. 2001; **433**: 157-62.

[10] Candinas D, Koyamada N, Miyatake T, Siegel J, Hancock WW, Bach FH, Robson SC. Loss of rat glomerular ATP diphosphohydrolase activity during reperfusion injury is associated with oxidative stress reactions. *Thromb Haemost.* 1996; **76**: 807–812.

[11] Hatakeyama K, Hao H, Imamura T, Ishikawa T, Shibata Y, Fujimura Y, Eto T, Asada Y. Relation of CD39 to plaque instability and thrombus formation in directional atherectomy specimens from patients with stable and unstable angina pectoris. *Am J Cardiol* 2005;**95**:632-5.

[12] El-Omar MM, Islam N, Broekman MJ, Drosopoulos JH, Roa DC, Lorin JD, Sedlis SP, Olson KE, Pulte ED, Marcus AJ. The ratio of ADP- to ATP-ectonucleotidase activity is reduced in patients with coronary artery disease. *Thromb Res* 2005;**116**:199-206.

[13] Boarder MR, Hourani SM. The regulation of vascular function by P2 receptors: multiple sites and multiple receptors. *Trends Pharmacol Sci* 1998;**19**:99-107.

[14] Evans RJ, Kennedy C. Characterization of P2-purinoceptors in the smooth muscle of the rat tail artery: a comparison between contractile and electrophysiological responses. *Br J Pharmacol* 1994;**113**:853-60.

[15] Miyagi Y, Kobayashi S, Nishimura J, Fukui M, Kanaide H. Dual regulation of cerebrovascular tone by UTP: P2U receptor-mediated contraction and endothelium-dependent relaxation. *Br J Pharmacol* 1996;**118**:847-56.

Figure legend

Figure 1. Concentration–response curves for ATP and ADP in infected rat carotid arteries with endothelial denudation.

ATP induced contraction (A), ADP induced contraction (B), Non-infected carotid artery (Control, □), AdLac Z-infected carotid artery (□), AdPlac I-infected carotid artery (□). (n = 6 each, ∗P < 0.05 vs non-infected artery and AdLac Z-infected artery)

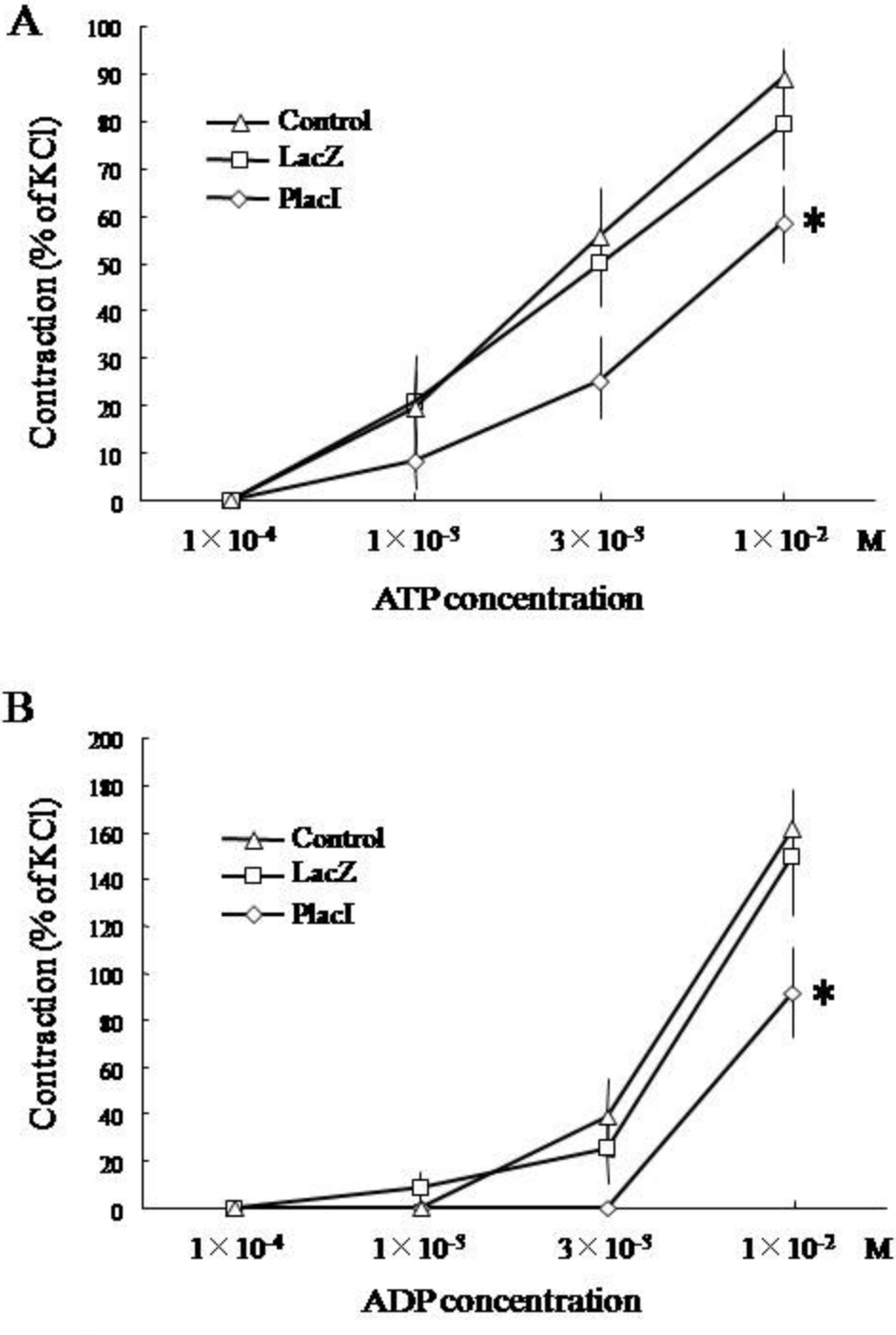
A
Contraction (% of KCl)
Control
LacZ
PlacI
*
1×10-4
1×10-3
3×10-3
1×10-2
M
ATP concentration
B
Contraction (% of KCl)
Control
LacZ
PlacI
*
1×10-4
1×10-3
3×10-3
1×10-2
M
ADP concentration

Figure 1