

Preoperative platelet response to collagen is associated with myocardial injury after off-pump coronary bypass graft in patients taking aspirin

Deok Man Hong, Yun Seok Jeon, Jun Hyun Kim, Tae Wan Lim, Young Jin Lim, Jae Hyon Bahk, Ki Bong Kim*, and Il Jae Kim[†]

Departments of Anesthesiology and Pain Medicine, *Thoracic and Cardiovascular Surgery, Seoul National University Hospital, Seoul National University College of Medicine, [†]Department of Anesthesiology and Pain Medicine, National Police Hospital, Seoul, Korea

Background: We hypothesized that, even in patients taking aspirin, the variance of preoperative platelet response to collagen might be associated with myocardial injury during coronary artery bypass graft (CABG) surgery. Therefore, we evaluated the relationship between preoperative whole-blood aggregometry (WBA) by collagen and the postoperative myocardial injuries.

Methods: For 44 patients who were scheduled for elective off-pump CABG and taking aspirin, WBA was measured by the impedance method in the presence of collagen (2 mg/ml or 5 mg/ml) as stimulatory agents. After CABG, myocardial injury was evaluated by analysis of the creatine kinase (CK), creatine kinase-MB (CK-MB), and lactate dehydrogenase (LD), and by electrocardiography.

Results: High response group (n = 13) on preoperative WBA with collagen (2 and 5 mg/ml) showed significantly higher postoperative cardiac enzyme levels (CK, CK-MB and LD) than those of low response group (n = 31).

Conclusions: In patients who take aspirin and undergoing off-pump CABG, the preoperative platelet response to collagen is correlated with postoperative myocardial injury. (Korean J Anesthesiol 2010; 58: 129-135)

Key Words: Aspirin, Collagen, Coronary artery bypass grafting, Platelet.

Received: May 26, 2009. Revised: 1st, June 19, 2009; 2nd, August 24, 2009. Accepted: September 22, 2009.

Corresponding author: Yun Seok Jeon, M.D., Department of Anesthesiology and Pain Medicine, Seoul National University Hospital, Seoul National University College of Medicine, 28, Yeongeon-dong, Jongno-gu, Seoul 110-744, Korea. Tel: 82-2-2072-2469, Fax: 82-2-747-5639, E-mail: jeonyunseok@gmail.com

©This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

The endothelium is crucial to the maintenance of vascular physiology and the coagulation system. If the vessels are injured and the integrity of the endothelium is lost, platelet is exposed to the subendothelial collagen matrix, a potent platelet agonist, and become activated [1]. Although this platelet activation is basically a protective mechanism to stop bleeding, it may cause undesired consequences. The activated platelet may initiate the coagulation cascade and release potent vasoconstrictors. Consequently, tissue damage may be increased.

During coronary artery bypass graft (CABG), damage to the endothelium is difficult to avoid [2], and platelet activation in the coronary artery or graft vessels may cause fatal complication. Thus, aspirin is administered to prevent platelet activation during CABG and aspirin administration in early postoperative period is proven to reduce the morbidity and mortality in CABG patients [3]. However, 5% to 45% of the population is resistant to aspirin [4,5]. Moreover even in non resistant patients, aspirin inhibits only the thromboxane A₂ (TxA₂)-dependent platelet-activating pathway [6], and the other platelet-activating mechanism can still be activated.

We hypothesized that the variance of preoperative platelet response to collagen in patients who take aspirin might be associated with platelet activation and consequently modulates the myocardial injury during CABG. Therefore, we measured whole-blood aggregometry (WBA) with collagen preoperatively in off-pump CABG patients taking aspirin, to assess the relationship with the postoperative myocardial injuries.

Materials and Methods

Patients

This study was approved by our institutional ethics committee, and written informed consents were obtained from all patients. We enrolled adult patients who were scheduled for elective off-pump CABG and who had taken 100 mg of aspirin (Bayer Healthcare, Leverkusen, Germany) a day for more than a month. The exclusion criteria were the presence of another additional cardiac disease, such as valvular disease, requiring surgical treatment prior to heart surgery, known coagulation disorders, thrombocytopenia ($<150 \times 10^9/L$), low-molecular-weight heparin (LMWH) treatment less than 10 days before surgery, or any antiplatelet therapy other than aspirin. Patients with uncontrolled hypertension, alcoholism, heavy smoking, psychiatric illness, renal disease ($Cr > 177 \mu\text{mol/L}$), or active liver disease were also excluded.

Treatment and surgical procedures

The preoperative aspirin medication (100 mg a day in the morning) was continued until two day before surgery. From postoperative day 1, the patients were recommended to take aspirin.

For all the patients, anesthetic and surgical techniques were standardized. Anesthesia was induced with midazolam, etomidate, and sufentanil, and maintained with midazolam, sufentanil, and vecuronium by continuous intravenous infusion. During operation, intravenous fluids and dobutamine were titrated to maintain cardiac index and mean arterial pressure at a minimum of 2.2 L/min/m² and 65 mmHg, respectively. If hypotension developed with adequate cardiac index and pulmonary capillary wedge pressure (>10 mmHg), intravenous norepinephrine (0.05 $\mu\text{g/kg/min}$ to 0.2 $\mu\text{g/kg/min}$) was administered.

After the pericardium was opened, heparin was administered at a dose of 300 unit/kg, and an activated coagulation time (ACT) greater than 300 s was maintained with supplementary doses of heparin until the completion of anastomoses. The surgical technique for CABG was the off-pump technique with a stabilizer (Octopus[®]2+ Tissue Stabilization System, Medtronic Inc., Minneapolis, MN, USA) and an intracoronary shunt. After the completion of revascularization, the heparin effect was not reversed with protamine sulfate. For all the patients in our study with intraoperative bleeding, a cell separator (Cobe BRAT 2, Cobe Cardiovascular Inc., Arvada, CO, USA) was used to concentrate the lost blood, and this blood was reinfused. All patients had mediastinal and pleural drains.

Whole-blood impedance aggregometry (WBA) for evaluation of platelet aggregation

Platelet aggregation was studied in the presence of two different doses of collagen (2 $\mu\text{g/ml}$ or 5 $\mu\text{g/ml}$) as stimulatory agents with the impedance method using whole-blood aggregometry (Chrono-Log 592, Chrono-Log Corp., Havertown, PA, USA). One day before surgery, blood samples were collected by sterile venipuncture from the antecubital fossa using a 21-gauge butterfly needle. Samples were transferred into polypropylene tubes containing 0.5 ml of 3.2% (w/v) sodium citrate. Measurements of whole-blood platelet aggregation were made according to the Chrono-Log protocol [7]. Briefly, whole blood diluted 1 : 1 with 0.85% (w/v) saline was incubated with the appropriate antagonist, then supplemented with collagen (2 $\mu\text{g/ml}$ or 5 $\mu\text{g/ml}$). The impedance value reflecting the extent of platelet aggregation was read after 6 min. The patients who showed the WBA values in upper 30% were defined as high response group and the other lower 70% patients were defined

as low response group.

Cardiac enzymes

Blood was sampled for the measurement of creatine kinase (CK, reference range 20–270 IU/L), creatine kinase-MB (CK-MB, reference range 0–24 IU/L), and lactate dehydrogenase (LD, reference range 100–225 IU/L) immediately before surgical incision (T1), at the end of the operation (T2), 6 hours (T3), 1 (T4), 2 (T5), 3 (T6), and 5 days (T7) after the operation.

Electrocardiograms

Twelve-lead electrocardiograms were obtained at the end of the operation, 1, 2, 3, and 5 days after the operation. All electrocardiograms were read and agreed upon by two cardiologists blinded to the patient's clinical course and cardiac enzyme concentrations. Cardiac events measured by electrocardiographic criteria were classified as one of the following: (1) persistent new Q waves (≥ 40 ms) occurring in

at least two leads; (2) persistent new R waves ($\geq 40/50$ ms) occurring in V1/V2, in more than two leads; and (3) new-onset complete bundle branch block.

Postoperative bleeding

Chest tube outputs were assessed at 8 and 24 hours after surgery to measure postoperative bleeding. The chest tube outputs were calculated from the contents in the water-seal chest drainage system.

Statistical analysis

Continuous variables are presented as means \pm SD. Categorical variables are presented as frequencies and percentages. Unpaired *t* or Rank Sum test (if not normally distributed) were used to compare the demographic, clinical variables and the postoperative parameters (cardiac enzymes) at each time point between the 2 groups. The correlation between variables was assessed by Pearson's correlation. A

Table 1. Demographic and Clinical Characteristics

Variable	Collagen 2 $\mu\text{g/ml}$		P value	Collagen 5 $\mu\text{g/ml}$		P value
	High response group (n = 13)	Low response group (n = 31)		High response group (n = 13)	Low response group (n = 31)	
Age, yr	63.9 \pm 10.5	63.0 \pm 8.6	0.762	65.3 \pm 8.58	62.5 \pm 10.3	0.563
Male sex, %	69.2	80.6	0.561	69.2	80.6	0.561
One-vessel disease, %	0	0	0.990	0	0	0.990
Two-vessel disease, %	15.3	19.4	0.846	15.4	19.4	0.846
Three-vessel disease, %	84.6	80.6	0.846	84.6	80.6	0.846
Normal left ventricular function, %	61.5	41.9	0.471	61.5	45.2	0.842
LVEF, %	56.5 \pm 15.4	50.9 \pm 10.8	0.1	55.3 \pm 13.5	53.7 \pm 9.8	0.42
Preoperative Hb, g/dl	12.4 \pm 0.7	12.4 \pm 1.13	0.850	12.4 \pm 1.0	12.2 \pm 1.2	0.394
ASA class II, III %	100	100	0.990	100	100	0.990
CCS class I or II, %	84.6	93.5	0.211	84.6	93.5	0.211
CCS class III or IV, %	15.4	6.5	0.211	15.4	6.5	0.211
Previous MI, %	23.1	41.9	0.333	23.1	41.9	0.333
Smoking, %	38.5	54.8	0.402	30.8	58.1	0.160
Hypertension, %	38.5	35.5	0.887	53.8	35.5	0.201
Hypercholesterolemia, %	7.7	29.0	0.272	7.7	29.0	0.272
Diabetes mellitus, %	84.6	48.4	0.061	69.2	54.8	0.462
CVA history, %	15.4	0	0.433	15.4	0	0.433
COPD, %	7.7	6.4	0.154	7.7	9.6	1.000
Number of anastomoses	3.0 \pm 0.8	3.0 \pm 1.0	0.807	3.2 \pm 0.7	2.9 \pm 1.0	0.146
Postoperative Hb, g/dl	9.3 \pm 0.6	9.4 \pm 0.7	0.574	9.5 \pm 0.5	9.3 \pm 0.7	0.285
Time to extubation, hr	14.2 \pm 4.8	13.8 \pm 4.9	0.774	14.7 \pm 4.8	12.6 \pm 4.3	0.183

Data are presented as mean \pm SD or percentage of patients. Normal ventricular function means that left ventricular ejection fraction is more than 55%. MI: myocardial infarct, ASA: American Society of Anesthesiologists, CCS: Canadian Cardiovascular Society, CVA: Cerebro-vascular accident.

Table 2. The Platelet Aggregometry Data

Stimulating agent	Impedance (Ω)	
	High response group (n = 13)	Low response group (n = 31)
Collagen 2 $\mu\text{g/ml}$	18 \pm 1.32	8.84 \pm 3.87
Collagen 5 $\mu\text{g/ml}$	24.2 \pm 2.99	13.5 \pm 3.88

Values are presented as mean \pm SD.

P value of < 0.05 was considered statistically significant. In our pilot study, LD level of low response group was 250 ± 53 IU/L in 2 $\mu\text{g/ml}$ and 265 ± 58 IU/L in 5 $\mu\text{g/ml}$ at postoperative 3 day. We expected 50% increase of LD in high response group. A sample size of at least 5 patients was needed in both 2 $\mu\text{g/ml}$ and 5 $\mu\text{g/ml}$ group, to obtain a power of 0.8 with two sided alpha value of less than 0.05. Analyses were performed using SigmaStat ver.3.1 (Systat Software Inc., Chicago, IL, USA) and SPSS ver.12.0 (SPSS Inc., Chicago, IL, USA).

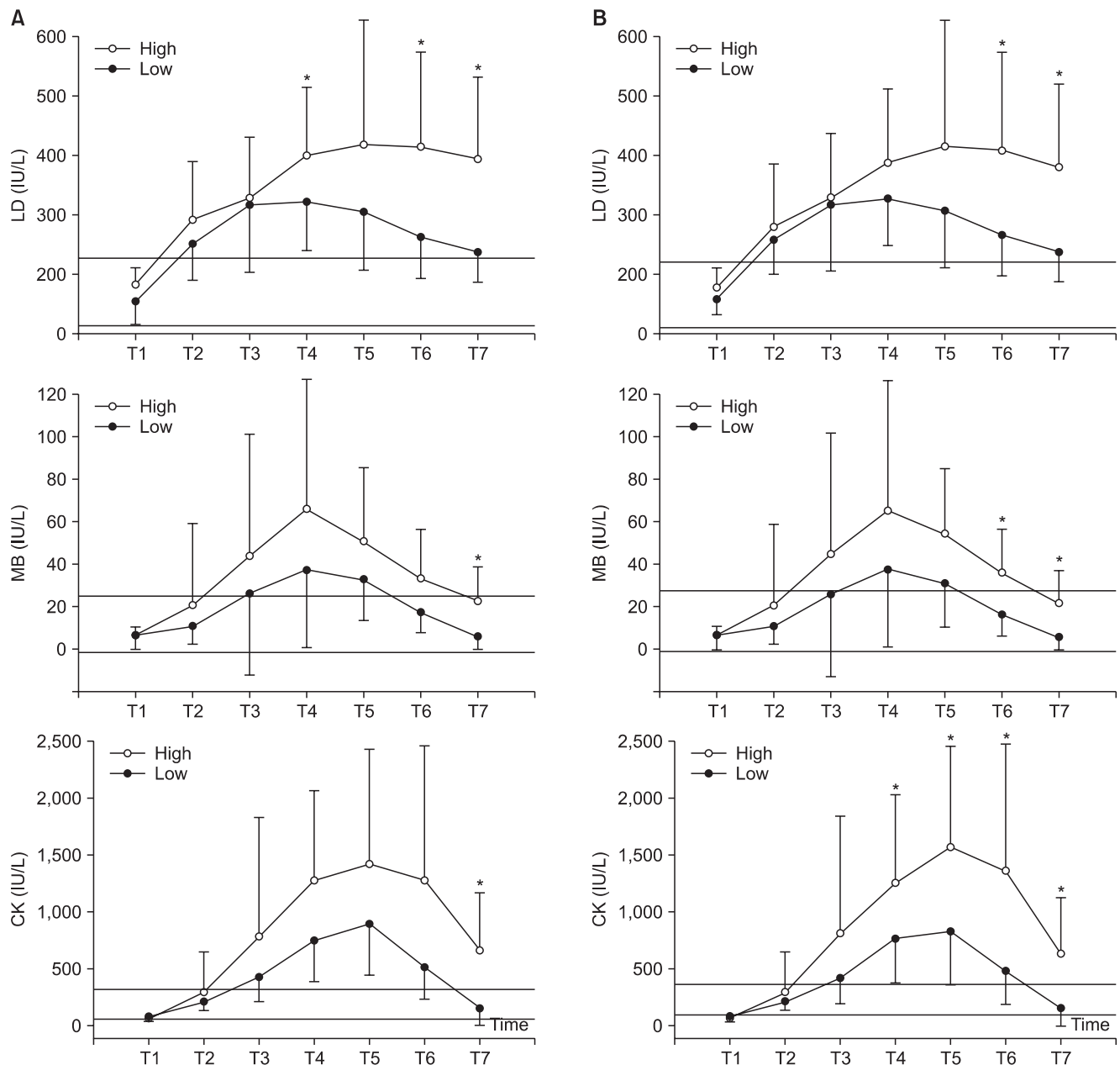


Fig. 1. Cardiac enzyme at immediately before surgical incision (T1), at the end of the operation (T2), and 6 h (T3), 1 (T4), 2 (T5), 3 (T6), and 5 day (T7) for the low and high response groups by the collagen 2 (A) and 5 (B) $\mu\text{g/ml}$ on whole blood aggregometry. LD: lactate dehydrogenase isoenzyme, CK: creatine kinase, MB: creatine kinase MB fraction, High: high response group, Low: low response group. *P < 0.05 between low and high response groups.

Results

Clinical characteristics

Forty-four patients were enrolled in this study and completed the trial. No deaths or serious adverse events were reported over the treatment period. Demographic and clinical characteristics of high and low response groups for 2 and 5 µg/ml collagen WBA are shown in Table 1. Among 44 patients, 13 and the other 31 patients were classified as high and low response groups respectively.

Whole-blood impedance aggregometry for platelet aggregation

The platelet aggregation data are presented in Table 2.

Cardiac enzymes

High response group on low dose (2 µg/ml) collagen WBA showed higher LD on postoperative 1, 3 and 5 days (P values =

0.012, <0.001 and <0.001 respectively), CK on postoperative 1, 5 days (P values = 0.027 and 0.009 respectively) and CK-MB on postoperative 5 day (P value = 0.004) than low response group. High response group on high dose (5 µg/ml) collagen WBA also showed higher LD (postoperative 3 and 5 days with P values of 0.004 and 0.002 respectively), CK (postoperative 1, 2, 3 and 5 days with P values of 0.027, 0.007, 0.005 and 0.001 respectively) and CK-MB (postoperative 2, 3 and 5 days with P values of 0.019, 0.001 and <0.001) than low response group. On high dose collagen WBA, there was no difference between low and high response groups on preoperative cardiac enzyme levels. However, on low dose collagen aggregometry, high response group showed higher LD and CK levels (P values = 0.009 and 0.028 respectively) (Fig. 1) than low response group.

Correlation between variables

Fig. 2 illustrates the relationship between CK-MB, CK and LD at 3 day after operation. A significant correlation was found only between CK-MB and CK ($r = 0.699$, $P < 0.001$).

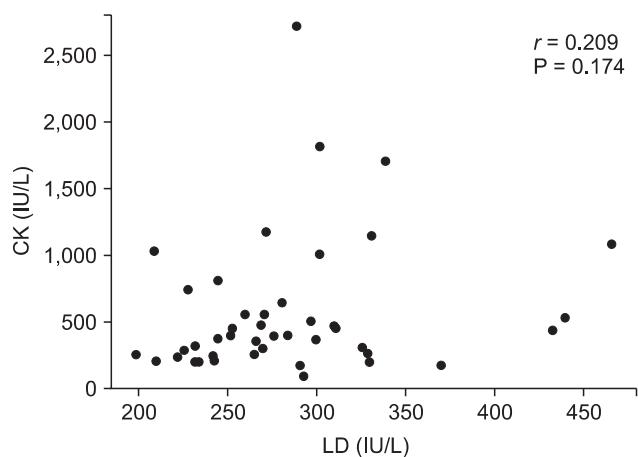
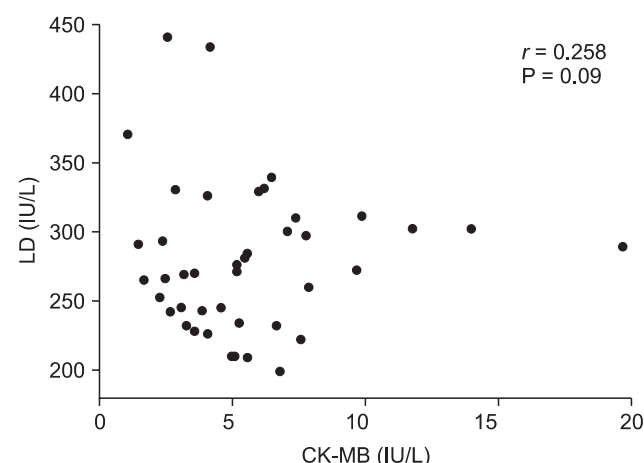
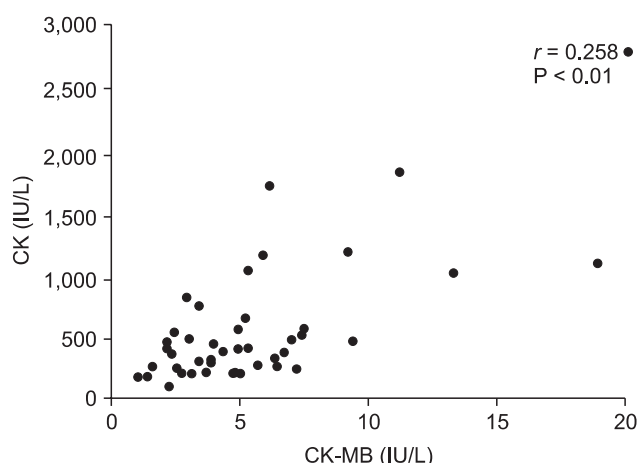


Fig. 2. Relation between CK-MB, CK and LD at 3 day after operation. r , Pearson's correlation coefficient.

Table 3. Postoperative Bleeding Assessed by Chest Tube Drain

		1–8 h	P value	1–24 h	P value
Collagen 2 ug/ml	High response group	1,031.5 ± 449.4	0.391	1,420 ± 612.9	0.169
	Low response group	852.1 ± 258.7			
Collagen 5 ug/ml	High response group	1,019.2 ± 405.6	0.205	1,355 ± 564	0.234
	Low response group	844.3 ± 294.4			

Values are chest tube output (ml) and presented as mean ± SD.

Postoperative bleeding

There was no significant difference on postoperative bleeding between two groups (Table 3).

Electrocardiographic analysis

There was no demonstrated electrocardiographic evidence of a new post-CABG cardiac event in any of the 44 patients with assessable electrocardiograms.

Discussion

This study shows that high response to collagen on preoperative WBA is associated with increased perioperative myocardial injury in the patients undergoing off-pump CABG even if they take aspirin.

The clinical importance of endothelial damage during CABG is well known, and measures are taken to prevent endothelial damage. However, even when the 'no-touch technique' is used, injury to the endothelium is difficult to avoid [2]. Damaged endothelium exposes the subendothelial collagen, a platelet agonist, and activated platelet generates microemboli, which may be an important mechanism of myocardial injury [8]. In addition, during carotid endarterectomy, preoperative platelet aggregation induced with collagen was significantly higher in patients with more microemboli on intraoperative transcranial doppler monitoring [9]. The other studies have stressed that coronary spasm induced by TxA₂ released from activated platelet is an important mechanism underlying the myocardial injury caused by activated platelet [10,11].

Aspirin inhibits platelet cyclooxygenase (COX)-1, which converts arachidonic acid to TxA₂ [4,12]. TxA₂ activates the platelet TxA₂ receptor. Thus, aspirin activity is usually reflected in the platelet response to arachidonic acid. In addition, the aspirin effect can also be detected using low and high dose collagen WBA. In low dose collagen WBA, only normal platelets aggregate, whereas in high dose collagen WBA, both normal and aspirin-inhibited platelets aggregate [13]. In this study, both low and high dose of collagens were used as platelet agonists. The result that high responders on low dose WBA

showed higher myocardial injuries can be explained by aspirin resistance. However, considering the previously reported rate of aspirin resistance [14], the number of patients in our study is not sufficient to cause the difference between groups on low dose collagen WBA by the aspirin resistance alone. Moreover, there were differences on the preoperative myocardial enzyme levels between low and high responders on low dose collagen WBA (Fig. 1). Therefore in this study the results by low dose collagen aggregometry might be influenced by not only aspirin resistance but also the difference of the preoperative disease activity expressed on both baseline myocardial enzyme and low dose collagen aggregometry.

The preoperative variance of high dose WBA values in our study could have reflected a polymorphism in platelets or been related to the variously activated state of the platelets. Either way, this study showed that the preoperative variations in the platelet response to collagen correlated with postoperative myocardial injury during CABG. Consequently, it stresses the importance of collagen-mediated platelet activation on myocardial injury during CABG and suggests the potential role of blocking the receptor for protective strategy. Although several receptors have been proposed as platelet collagen receptors, glycoprotein VI (GPVI) has recently received considerable attention. Mouse or human platelets in which GPVI has been pharmacologically blocked or immunodeleted are unresponsive to collagen [15]. Collagen signaling is conferred by the expression of GPVI in hematopoietic cell lines that express its coreceptor FcRγ [16]. This suggests that GPVI signaling is both necessary and sufficient for the activation of platelets by collagen under conditions of abundant receptors and ligands. Studies with mice have shown that GPVI-deficient mice display a nearly complete abolition of the platelet response to endothelial injury [17].

Moreover, GPVI-deficient mice do not exhibit an overt bleeding phenotype and that GPVI-deficient humans show only minor bleeding diatheses rather than major bleeding phenotypes. This is consistent with the observation in this study that collagen WBA only correlated with myocardial injury and not with blood loss after CABG. Considering the preoperative use of platelet inhibitors, such as aspirin, may increase postoperative bleeding, potential importance of collagen

receptor block during CABG is more stressed.

In conclusion, this study demonstrates that even in patients taking aspirin who undergo off-pump CABG, the preoperative platelet response to collagen correlates with postoperative myocardial injury. This emphasizes the importance of collagen-mediated platelet activation during CABG.

References

1. Kahn ML. Platelet-collagen responses: molecular basis and therapeutic promise. *Semin Thromb Hemost* 2004; 30: 419-25.
2. Svendsen E, Dalen H, Moland J, Engedal H. A quantitative study of endothelial cell injury in aortocoronary vein grafts. *J Cardiovasc Surg (Torino)* 1986; 27: 65-71.
3. Mangano DT. Multicenter Study of Perioperative Ischemia Research Group. Aspirin and mortality from coronary bypass surgery. *N Engl J Med* 2002; 347: 1309-17.
4. Patrono C, Collier B, Dalen JE, FitzGerald GA, Fuster V, Gent M, et al. Platelet-active drugs: the relationships among dose, effectiveness, and side effects. *Chest* 2001; 119: s39-63.
5. Homoncik M, Jilma B, Hergovich N, Stochlawetz P, Panzer S, Speiser W. Monitoring of aspirin (ASA) pharmacodynamics with the platelet function analyzer PFA-100. *Thromb Haemost* 2000; 83: 316-21.
6. Stein B, Fuster V, Israel DH, Cohen M, Badimon L, Badimon JJ, et al. Platelet inhibitor agents in cardiovascular disease: an update. *J Am Coll Cardiol* 1989; 14: 813-36.
7. Ray MJ, Hawson GA, Just SJ, McLachlan G, O'Brien M. Relationship of platelet aggregation to bleeding after cardiopulmonary bypass. *Ann Thorac Surg* 1994; 57: 981-6.
8. Bolli R, Marban E. Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev* 1999; 79: 609-34.
9. Hayes PD, Box H, Tull S, Bell PR, Goodall A, Naylor AR. Patients' thromboembolic potential after carotid endarterectomy is related to the platelets' sensitivity to adenosine diphosphate. *J Vasc Surg* 2003; 38: 1226-31.
10. Ellis EF, Oelz O, Roberts LJ, Payne NA, Sweetman BJ, Nies AS, et al. Coronary arterial smooth muscle contraction by a substance released from platelets: evidence that it is thromboxane A2. *Science* 1976; 193: 1135-7.
11. Needleman P, Minkes M, Raz A. Thromboxanes: selective biosynthesis and distant biological properties. *Science* 1976; 193: 163-5.
12. Roth GJ, Stanford N, Majerus PW. Acetylation of prostaglandin synthase by aspirin. *Proc Natl Acad Sci U S A* 1975; 72: 3073-6.
13. Taylor ML, Misso NL, Stewart GA, Thompson PJ. The effects of varying doses of aspirin on human platelet activation induced by PAF, Collagen and arachidonic acid. *Br J Clin Pharmacol* 1992; 33: 25-31.
14. McKee SA, Sane DC, Deliarhyris EN. Aspirin resistance in cardiovascular disease: a review of prevalence, mechanisms, and clinical significance. *Thromb Haemost* 2002; 88: 711-5.
15. Nieswandt B, Schulte V, Bergmeier W, Mokhtari-Nejad R, Rackebbrandt K, Cazenave JP, et al. Long-term antithrombotic protection by in vivo depletion of platelet glycoprotein VI in mice. *J Exp Med* 2001; 193: 459-69.
16. Chen H, Locke D, Liu Y, Liu C, Kahn ML. The platelet receptor GPVI mediates both adhesion and signaling responses to collagen in a receptor density-dependent fashion. *J Biol Chem* 2002; 277: 3011-9.
17. Massberg S, Gawaz M, Gruner S, Schulte V, Konrad I, Zohlnhofer D, et al. A crucial role of glycoprotein VI for platelet recruitment to the injured arterial wall in vivo. *J Exp Med* 2003; 197: 41-9.