Concentration of Soluble P-Selectin and White Blood Cell Counts in Infarct Coronary Arteries in Patients with Acute Myocardial Infarction Differ from the Systemic Circulation

Teng-Hung Yu, MD; Sarah Chua, MD; Cheng-I Cheng, MD; Wen-Hao Liu, MD;
Cheng-An Chiu, MD; Cheng-Hsu Yang, MD; Chih-Yuan Fang, MD;
Yuan-Kai Hsieh, MD; Chi-Ling Hang, MD; Wei-Chin Hung, MD; Yen-Hsun Chen, MD;
Kuo-Ho Yeh, MD; Morgan Fu, MD; Hon-Kan Yip, MD

Background: Previously, researchers have suggested that Soluble (s) P-selectin mediates the accumulation of leukocytes which in turn promotes fibril deposition. Soluble P-selectin and white blood cell (WBC) counts have been shown to be increased in the systemic circulation after acute myocardial infarction (AMI). However, whether the infarct coronary artery (ICA) and systemic circulation differs with respect to the concentration of sP-selectin and WBC counts following AMI remain unknown. In this study, we investigated whether the concentration of sP-selectin and WBC counts differed between the ICA and the systemic circulation after AMI.

Methods: Blood samples for circulating sP-selectin and WBC counts were immediately obtained after vascular puncture in 72 patients with AMI of < 12 h undergoing primary percutaneous coronary intervention (PCI). Additionally, blood samples for ICA sP-selectin and WBC counts were obtained via Export Suction Catheter during PCI. For comparison, blood samples for sP-selectin and WBC counts were obtained once in 30 healthy subjects.

Results: The results demonstrated that the circulating sP-selectin [64.7 $\pm$ 18.1 (ng/ml) vs. 29.5 $\pm$ 6.3 (ng/ml), $p < 0.0001$] and WBC counts [12.1 $\pm$ 3.6 ($\times$ 10^3/ml) vs. 5.0 $\pm$ 1.0 ($\times$ 10^3/ml), $p < 0.0001$] were significantly higher in our patients than in healthy subjects. Furthermore, the sP-selectin [72.7 $\pm$ 23.3 (ng/ml) vs. 64.7 $\pm$ 18.1 (ng/ml), $p < 0.0001$] and the WBC counts [16.2 $\pm$ 3.8 ($\times$ 10^3/ml) vs. 12.1 $\pm$ 3.6 ($\times$ 10^3/ml), $p < 0.0001$] were markedly higher in the ICA than in the systemic circulation for the patients.

Conclusions: The plasma level of sP-selectin and WBC counts were more elevated in the ICA than in the systemic circulation of patients with AMI undergoing primary PCI. These findings strengthen the role of sequestration of WBC and sP-selectin in the ICA as crucial in thrombus formation.
(Chang Gung Med J 2006;29:169-74)

Key words: soluble P-selectin, white blood cell counts, acute myocardial infarction.

The glycoprotein P-selectin, a membrane component of cell storage granules[1] is rapidly translocated from α-granules of platelets and in the Weibel-Palade bodies of endothelial cells to the cell surface following an inflammatory process or other stimulation.[2,3] P-selectin is a cell adhesion molecule of activated platelets and endothelial cells, and thus, plays an important role in modulating the interaction of these cells with neutrophils and monocytes.[3-6] Previously, researchers have suggested that P-selectin mediates the accumulation of leukocytes which in turn promotes fibril deposition.[7] Other researchers have shown that P-selectin expression on platelets determines the size and stability of platelet aggregates.[8] Surprisingly, while numerous animal and in vitro studies have stressed the important role of P-selectin in facilitating atherosclerosis lesion development,[9] mediating endothelial-leukocyte-platelet interactions,[3-6] stabilizing platelet aggregation,[8] and also in fibrin deposition within a thrombus,[7] data on the role and circulating levels of sP-selectin after acute myocardial infarction (AMI) remain limited.[10,11] Furthermore, researchers have recent shown that elevation of WBC counts during AMI was associated with reduced epicardial blood flow and myocardial reperfusion, thromboresistence, and adverse outcomes.[12-14] However, whether there are differences in sP-selectin concentrations and WBC counts between occluded arteries (ICAs) and systemic circulation remains unclear.

Therefore in this study, we investigated whether the concentrations of sP-selectin and WBC counts differed between the ICAs and the systemic circulation of patients with AMI undergoing primary percutaneous coronary intervention (PCI).

METHODS

Enrollment of patients and exclusion criteria

From May 2002 through October 2003, PercuSurge GuardWire™ devices (Medtronic AVE) were used in 100 consecutive patients presenting with AMI (< 12 hour's duration) undergoing primary PCI. The inclusion criteria and application procedure for this mechanical device were described in detail in our recent study.[15] For the purposes of this study, plasma levels of sP-selectin and WBC counts of all patients who underwent primary PCI were prospectively measured. Blood samples were drawn from arterial puncture sites before diagnostic procedure was performed. Blood samples from ICAs for levels of sP-selectin and WBC counts were obtained using the Export Suction Catheter during the procedure. To avoid the possibility of diluted concentrations of sP-selectin and WBC counts, the 3 cc of blood inside the Export Suction Catheter was first removed followed by the first 6-8 cc of removable blood from the ICAs was collected for measuring sP-selectin and WBC counts.

To avoid other variables influencing the plasma levels of sP-selectin and WBC counts, our exclusion factors included: history of renal insufficiency (creatinine > 1.5 mg/dL), liver cirrhosis, malignancy, febrile disorders (1 patient), acute or chronic inflammatory disease on study entry, history of recent infection, or gross hemolysis of blood sample, and immunosuppressive therapy. Patients were also excluded if fever (body temperature > 37.5°C) was observed in the emergency room. Therefore, 28 (28.0%) of the 100 patients were subsequently excluded due to fever (two patients), infection (two patients), malignancy (one patient), steroid use (one patient), gross hemolysis (five patients), renal function impairment (four patients), or incomplete blood sampling (13 patients). Accordingly, the remaining 72 patients constituted the study population.

Thirty age- and gender-matched healthy volunteers were also studied as control subjects. Informed consent was obtained from all subjects. The protocol was approved by the Institutional Review Committee on Human Research of our institution.

Arterial blood samples were obtained once in healthy volunteers during health clinic examinations. WBC counts and biochemical measurements were determined using standard methods.

Laboratory investigations and assays

The concentrations of sP-selectin were measured using a standard enzyme-linked immunosorbent assay (ELISA) and a commercially available kit (R and D Systems; Minneapolis, Minn). The method for the ELISA was described in detail in our recent report.[16] The assay was sufficiently sensitive to detect less than 0.5 ng/ml of P-selectin, according to the manufacturer of the assay kits. Intra-individual variability of sP-selectin levels was assessed in the study patients, risk-control subjects, and healthy subjects. The mean intra-assay coefficients of variance

were 5.10%, 4.71%, and 4.34%, respectively.

Statistical analysis

Data were expressed as mean ± SD. Categorical variables were compared using the Chi-square test. Continuous variables between the two groups were compared using the *t* test. Continuous variables of sP-selectin between ICA and systemic circulation were compared using the Wilcoxon rank-sum test for paired data. Statistical analysis was performed using SAS statistical software for Windows version 8.2 (SAS institute, Cary, NC). A probability value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics and laboratory findings of our study patients and healthy control subjects

The relevant baseline characteristics and laboratory findings of both our study patients and healthy control subjects are listed in Tables 1 and 2. The healthy control subjects did not have any coronary risk factors. More than 60% of the study patients were current smokers. Additionally, over 50% of the study patients had a history of hypertension or hypercholesterolemia. On the other hand, less than 35% of our patients had history of diabetes mellitus.

Nearby 45% of the study patients experienced an anterior wall MI. More than 15% of the patients had cardiogenic shock upon presentation. The mean reperfusion time was 289 minutes. Coronary angiographic results demonstrated that the incidence of multi-vessel disease was 54.2%. The procedural successful rate was 94.4%.

Table 2 demonstrated that plasma levels of the sP-selectin [72.7 ± 23.3 (ng/ml) vs. 64.7 ± 18.1 (ng/ml), *p* < 0.0001] and the WBC counts [16.2 ± 3.8 (x 10^3/ml) vs. 12.1 ± 3.6 (x 10^3/ml), *p* < 0.0001] were significantly higher in the ICAs than in the systemic circulation of our study patients.

No significant differences were noted between the patients and healthy control subjects with regard to age and gender (Table 1). However, the circulating level of sP-selectin [64.7 ± 18.1 (ng/ml) vs. 29.5 ± 6.3 (ng/ml), *p* < 0.0001] and WBC counts [12.1 ± 3.6 (x 10^3/ml) vs. 5.0 ± 1.0 (x 10^3/ml), *p* < 0.0001] were significantly higher in our study patients than in the healthy control subjects.

Table 1. Baseline Characteristics of Study Patients and Healthy Control Subjects

Variables	Study patients (n = 72)	Healthy control (n = 30)	*p*
Age (years)	58.7 ± 11.2	59.1 ± 8.0	0.712
Men	84.7% (61)	80.0% (24)	0.560
Currently smoking	69.4% (50)	0% (0)	
Hypertension	55.6% (40)	0% (0)	
Diabetes mellitus	33.3% (24)	0% (0)	
Hypercholesterolemia	51.4% (37)	0% (0)	
Previous MI	5.6% (4)	0% (0)	
Previous stroke	1.4% (1)	0% (0)	
WBC counts (x 10^3/ml)	12.1 ± 3.6	5.0 ± 1.0	< 0.0001
Soluble P-selectin (ng/ml)	64.7 ± 18.1	29.5 ± 6.3	< 0.0001
Mean reperfusion time (min)*	289 ± 168	—	
Anterior infarction	44.4% (32)	—	
Cardiogenic shock	15.3% (11)	—	
Multi-vessel disease†	54.2% (39)	—	
Pre-PCI TIMI flow ≤ 1	91.7% (66)	—	
Post-PCI TIMI flow = 3	94.4% (68)	—	

Abbreviations: MI: myocardial infarction; PCI: percutaneous coronary intervention; TIMI: thrombolysis in myocardial infarction; WBC: white blood cell.

* Defined from chest pain to first balloon inflation.

† Multi-vessel disease was defined by stenoses of > 50% in ≥ 2 major epicardial coronary arteries.

Data are expressed as mean value ± SD or number (percent) of patients.

Table 2. Comparison of Serum Levels of Soluble P-Selectin and WBC Counts between Intra-Coronary Artery and Systemic Circulation

Variables	ICA (n = 72)	Circulation (n = 72)	*p*
Soluble P-selectin (ng/ml)	72.7 ± 23.3	64.7 ± 18.1	< 0.0001
WBC counts (x 10^3/ml)	16.2 ± 3.8	12.1 ± 3.6	< 0.0001

Abbreviations: ICA: intra-coronary artery; WBC: white blood cell.
Data expressed as mean ± SD.

DISCUSSION

While the circulating level of sP-selectin has been shown to be increased in patients following AMI,[10,11] no data is available on the levels of sP-selectin in the infarct arteries of patients following AMI. In the present study, the most important finding was that plasma levels of sP-selectin were significantly higher in the ICAs than in the systemic circulation.

The reason this phenomenon was found in the

present study remains uncertain. However, we hypothesize that P-selectin is rapidly expressed on the surface of activated platelets and endothelial cells after plaque rupture and thrombus formation in coronary arteries.[4] Consequently, both enzymatic cleavage of expressed P-selectin and alternative splicing of P-selectin messenger ribonucleic acid occur, giving rise to sP-selectin, detectable in the peripheral blood.[17-19] We suggest that the rigorous response of both expression and enzymatic cleavage of sP-selectin was present more in the ICAs primarily from the stimulations of the endothelial-platelet-leukocyte interaction and thrombus formation, as well as the acute and inflammatory process than in the systemic circulation, which resulted primarily from acute stress stimulation and inflammatory process. Additionally, most P-selectin was retrieved and accumulated in the ICAs after it was cleaved into the soluble form rather then relieved into the systemic circulation. Furthermore, the present results demonstrated that over 91% pre-intervention TIMI flow ≤ 1 in infarct-related arteries was observed in our patients. This observation suggested that few sP-selectin would be washed out from the ICA and into the systemic circulation. Therefore, these reasons could explain why the plasma level of sP-selectin was markedly higher in the ICAs than in the systemic circulation during AMI.

The link between endothelial-platelet-leukocyte interaction and thrombus formation is well recognized.[4,7,20] Additionally, the pivotal role of P-selectin on interactions of these cells has also been extensively debated.[4,5] Furthermore, P-selectin expression on platelets in determination of ability and size of platelet aggregation has been proved using in vitro study.[8] In the present study, we suggest that the ICA levels of sP-selectin which were markedly elevated following AMI may be crucial in thrombus formation and organized-thrombus maintenance in the infarct artery. These findings further support our recent clinical observations that high-burden thrombus formation is usually maintained and no re-flow phenomenon more frequently occur during late reperfusion time (> 240 minutes ≤ 3 days).[21,22] Studies on animal models have demonstrated that the infarction size of myocardium can be reduced when administering monoclonal antibodies against P-selectin.[23,24] Those study results imply that P-selectin

may be an attractive target for adjunctive therapy of MI.[25] The present findings of markedly elevated sP-selectin in ICA may stimulate seriously considerations of the suggestion of monoclonal antibodies against P-selectin according to the results of previous investigations.[23-25]

Recently, researchers have demonstrated that increased WBC count in patients with AMI is strongly associated with reduced epicardial blood flow and myocardial reperfusion, thromboresistence, and adverse clinical outcomes.[12-14] In the present study, we found that the WBC count was substantially increased in our study patients than in healthy control subjects. Of importance was the fact that the WBC count was significantly higher in the ICAs than in the systemic circulation. We suggest that sequestration of WBC and sP-selectin in the ICA could play a key role in thrombus formation in our patients. Our suggestion which is based on clinical observation and laboratory findings is supported by the results of a previous study which has demonstrated that leukocyte accumulation promoting fibrin deposition was mediated in vivo by P-selectin on adherent platelets.[7] Accordingly, our findings reinforce the recent report that elevation in WBC counts was associated with thromboresistence and reduced myocardial reperfusion.[13]

There were two limitations on this study. First, without serial measurements of circulating sP-selectin levels, we did not provide information regarding how early the circulating sP-selectin elevated and how long the elevation of sP-selectin persisted in our patients. Second, the effects of circulating sP-selectin and WBC counts on short-term and long-term clinical outcomes were not part of the design protocol, therefore, we did not provide information regarding to the links between these two inflammatory biomarkers and untoward clinical outcomes.

The plasma levels of sP-selectin and WBC counts were markedly raised in both the systemic circulation and the ICA of patients with AMI undergoing primary PCI. Additionally, these two inflammatory biomarkers were significantly higher in the ICA than in the systemic circulation. Our findings provide a crucial insight into consideration of new strategic management for reducing ischemic insults in patients following AMI.

REFERENCES

1. Berman CL, Yeo EL, Wencel-Drake JD, Furie BC, Grinsberg MH, Furie B. A platelet alpha-granule membrane protein that is associated with the plasma membrane after activation: characterization and subcellular localization of platelet activation-dependent granule-external membrane protein. J Clin Invest 1986;78:130-7.

2. McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF. GMP-140, a platelet α-glanule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. J Clin Invest 1989;84:92-9.

3. Hsu-Lin SL, Berman CL, Furie BC, August D, Furie B. A platelet membrane protein expressed during platelet activation and secretion: studies using a monoclonal antibody specific for thrombin-activated platelets. J Biol Chem 1984;259:9121-6.

4. Carlos TM, Harlan JM. Leukocyte-endothelial cell adhesion molecules. Blood 1998;84:2069-101.

5. Frenette PS, Johnson RC, Hynes RO, Wagner DD. Platelets roll on stimulated endothelium in vivo: an interaction mediated by endothelial p-selectin. Proc Natl Acad Sci USA 1995;92:7450-4.

6. Gamble JR, Skinner MP, Berndt MC, Vadas MA. Prevention of activated neutrophil adhesion to endothelium by soluble adhesion protein GMP 140. Science 1990;249:414-7.

7. Palabrica T, Lobb R, Furie BC, Aronovitz M, Benjamin C, Hsu YM, Sajer SA, Furie B. Leukocyte accumulation promoting fibrin deposition is mediated in vivo by p-selectin on adherent platelets. Nature 1992;359:848-51.

8. Merten M, Thiagarajan P. P-selectin expression on platelets determines size and stability of platelet aggregates. Circulation 2000;102:1931-6.

9. Burger PC, Wagner DD. Platelet P-selectin facilitates atherosclerotic lesion development. Blood 2003;101:2661-6.

10. Shimomura H, Ogawa H, Arai H, Moriyama Y, Takazoe K, Hirai N, Kaikita K, Hirashima O, Misumi K, Soejima H, Nishiyama K, Yasue H. Serial changes in plasma levels of soluble p-selectin in patients with acute myocardial infarction. Am J Cardiol 1998;81:397-400.

11. Gurbel PA, O'Connor CM, Dalesandro MR, Serebruany VL. Relation of soluble and platelet p-selectin to early outcome in patients with acute myocardial infarction after thrombolytic therapy. Am J Cardiol 2001;87:774-7.

12. Pellizzon GG, Dixon SR, Stone GW, Cox DA, Mattos L, Boura JA, Grines LL, Addala S, O'Neill WW, Grines CL. Relation of admission white blood cell count to long-term outcomes after primary coronary angioplasty for acute myocardial infarction (the stent PAMI trial). Am J Cardiol 2003;91:729-31.

13. Barron HV, Cannon CP, Murphy SA, Braunwald E, Gibson M. Association between white blood cell count, epicardial blood flow, myocardial perfusion, and clinical outcomes in the setting of acute myocardial infarction: a thrombolysis in myocardial infarction 10 substudy. Circulation 2000;102:2329-34.

14. Barron HV, Harr SD, Radfort MJ, Wang Y, Krumholz HM. The association between white blood cell count and acute myocardial infarction mortality in patients ≥ 65 years of age: findings from the cooperative cardiovascular project. J Am Coll Cardiol 2001;38:1654-61.

15. Yip HK, Wu CJ, Chang HW, Fang CY, Yang CH, Chen MC, Hung WC, Chen CJ, Cheng CI, Hsieh YK. Effect of PercuSurge GuardWire device on the integrity of microvasculature and clinical outcomes during primary transradial coronary intervention in acute myocardial infarction. Am J Cardiol 2003;92:1331-5.

16. Cheng MC, Wu CJ, Chang HW, Juang SS, Yip HK, Wu CJ. Increased plasma levels of soluble P-selectin in rheumatic mitral stenosis. Chest 2004;126:54-8.

17. Ishiwata N, Takio K, Katayama M, Watanabe K, Titani K. Alternative spliced isoform of P-selectin is present in vivo as a soluble molecule. J Biol Chem 1994;269:23708-15.

18. Johnston GI, Bliss GA, Newman PJ, McEver RP. Structure of the human gene encoding granule membrane protein-140, a member of the selectin family of adhesion receptors for leukocytes. J Biol Chem 1990;265:21381-5.

19. Michelson A, Bernard M, Hechtman H. In vivo trafficking of platelets: circulating degranulated platelets rapidly loose surface but continue to circulate and function. Proc Natl Acad Sci 1996;93:11877-82.

20. Berger G, Hartwell DW, Wangner DD. P-selectin and platelet clearance. Am Soci Hematol 1998;92:4446-52.

21. Yip HK, Chen MC, Chang HW, Hang CL, Hsieh YK, Fang CY, Wu CJ. Angiographic morphologic features of infarct-related arteries and timely reperfusion in acute myocardial infarction: predictors of slow-flow and no-reflow. Chest 2002;122:1322-32.

22. Yip HK, Wu CJ, Yang CH, Chang HW, Chen SM, Hung WC, Hang CL. Delayed post myocardial infarction invasive measures, helpful or harmful? a subgroup analysis. Chest 2004;126:38-46.

23. Weyrich AS, Ma XL, Lefer DJ, Albertine KH, Lefer AM. In vivo neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. J Clin Invest 1993;91:2620-9.

24. Ueyama T, Ikeda H, Haramaki N, Kuwano K, Imaizumi T. Effects of monoclonal antibody to P-selectin and analogue of sialyl Lewis X on cyclic flow variations in stenosed and endothelium-injured canine coronary arteries. Circulation 1997;95:1554-9.

25. Murphy RT, Foley B, Mulvihill N, Crean P, Walsh MJ. Impact of preexisting statin use on adhesion molecule expression in patients presenting with acute coronary syndromes. Am J Cardiol 2001;87:446-8.

心肌梗塞急性期病患之冠狀動脈和體循環中可溶性黏著因子 (sP-Selection) 濃度及白血球數目之差異

尤登弘 蔡慧玲 鄭正一 劉文浩 邱正安 楊正旭 方志元 謝元凱 洪志凌
洪尉欽 陳彥勳 葉國和 傅懋洋 葉漢根

背　景： 以前的研究認為可溶性黏著因子 [soluble (s) P-selectin] 可使白血球 (white blood cell) 數目在形成血栓處堆積。此過程因此，會進一步導致凝血纖維 (fibrin deposition) 的累積。因此，使形成的血塊 (thrombus) 更穩固。另一些研究報告指出，急性心肌梗塞後血液循環中的可溶性黏著因子的濃度及白血球的數目會增加。但是，在急性心肌梗塞時病人血液循環中的這兩種發炎指標 (inflammatory biomarkers) 的濃度是否和梗塞的冠狀動脈內 (intra-coronary artery) 有所不同則仍不清楚。因此，這項研究的目的就是要探討仍未知的這兩項存在的問題。

方　法： 72位急性心肌梗塞在 12 小時內到院的病人，在進行緊急心導管手術的同時，接受了末端保護裝置 (PercuSurge distal balloon protection device) 的處置。測量體循環的可溶性黏著因子的濃度及白血球數目的血液樣本都在心導管手術前取得。另外，測量梗塞冠狀動脈血管內的可溶性黏著因子的濃度及白血球數目的血液樣本則利用 Export Suction catheter 在手術中取得。再者，在健康門診中也取得 30 位健康者的血液樣本測量體循環的可溶性黏著因子的濃度及白血球的數目血作為對照組。

結　果： 急性心肌梗塞的病人體循環的可溶性黏著因子的濃度及白血球的數目比正常人有統計意義的升高。研究結果同時顯示梗塞血管內的可溶性黏著因子濃度及白血球的數目在統計上有非常顯著差異的高於體循環中的可溶性黏著因子濃度及白血球的數目。

結　論： 急性心肌梗塞的病人體循環的可溶性黏著因子的濃度及白血球的數目比正常人有統計意義的升高。另外，這兩項的發炎生物標記 (inflammatory biomarkers) 在梗塞冠狀動脈內比體循環中高出更多。因此，這項研究結果我們使推測累積可溶性黏著因子及白血球於梗塞血管內可能扮演導致血栓形成的重要角色。
(長庚醫誌 2006;29:169-74)

關鍵字： 可溶性黏著因子，白血球數目，急性心肌梗塞。