Increases in Interleukin-6 and Matrix Metalloproteinase-9 in the Infarct-Related Coronary Artery of Acute Myocardial Infarction

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Background To elucidate the involvement of inflammation in coronary artery occlusion, the regional changes in cytokines and matrix metalloproteinases (MMPs) in the infarct-related coronary artery were determined in patients with acute myocardial infarction.

Methods and Results Cardiac catheterization was carried out within 24h of the onset of infarction in 36 patients. Blood samples were collected from the infarct-related coronary artery, the ascending aorta and the peripheral vein. Plasma interleukin (IL)-6 concentrations were elevated in all 3 samples. Particularly, the plasma IL-6 concentrations were 14.4 pg/ml in the infarct-related coronary artery, a value significantly greater than the respective 8.0 pg/ml and 6.5 pg/ml in the ascending aorta and peripheral vein. Plasma IL-6 concentrations in the infarct-related coronary artery had positive correlation with all of the following references measured by intravascular ultrasound: external elastic membrane cross-sectional area (EEM-CSA) ($r=0.47$, $p<0.01$), lesion EEM-CSA ($r=0.51$, $p<0.01$) and plaque area ($r=0.48$, $p<0.01$). MMP-9 was increased regionally in the infarct-related coronary artery, at 11.8 ng/ml vs 8.2 ng/ml in the ascending aorta ($p<0.001$).

Conclusions The present findings suggest that IL-6 at least is released from the ruptured vulnerable atherosclerotic plaque and that regional activation of macrophages is involved in the occlusive process of coronary artery in acute myocardial infarction. (Circ J 2004; 68: 451–454)

Key Words: Acute myocardial infarction; Interleukin-6; Matrix metalloproteinase-9

An acute occlusion of coronary artery fails into acute myocardial infarction (AMI) and it is thought that rupture of vulnerable fibrous caps of atherosclerotic plaque is involved.^{1,2} Several cytokines, including interleukin-1 (IL-1), IL-6 and tumor necrosis factor- α (TNF- α), may be released from vulnerable plaques,^{3–6} which then activates macrophages.^{7–10} Most studies have investigated the atherosclerotic plaques of experimental animals^{8–11} and there are only a few clinical reports of mild elevation of cytokines in the systemic circulation after rupture of atherosclerotic plaque.^{12–16} Only one study has reported the regional changes in cytokines in the occluded coronary artery,¹⁷ but the recent development of a coronary artery intervention technique now enables us to find any local alteration in the infarct-related coronary artery.¹⁸

In the present study we determined regional changes in cytokines and proteolytic enzymes in the infarct-related coronary artery in patients with AMI in order to elucidate the pathological role of inflammation in acute occlusion of a coronary artery.

Methods

Subjects

Thirty-six patients with AMI were admitted to the Emergency Units of Jichi Medical School Omiya Medical Center between March 2001 and July 2002 (28 males, 8 females; age range, 37–84 years (62.6 ± 11.9 years, mean $\pm$ SD)). The diagnosis of AMI was determined by the findings of continuous chest pain lasting more than 30 min, ST-segment elevation greater than 2.0 mm on at least 2 successive ECG leads, and a more than 2-fold increase in serum creatine kinase (CK) concentrations. Most of the patients had one or more coronary risk factors; that is, 19 patients had diabetes mellitus, 11 had hyperlipidemia, 26 had hypertension, 14 were obese and 25 smoked. Only one patient did not have any coronary risk. Anterior myocardial infarction was found in 26 patients, lateral myocardial infarction in 1 and inferior myocardial infarction in 9. Maximal serum concentrations of CK ranged from 379 to 8,270 mU/ml ($3,048.8\pm 1,651.6$ mU/ml, mean $\pm$ SD). Cardiac catheterization was carried out within 24h of the onset of myocardial infarction in all the patients. We excluded those with cardiogenic shock, chronic renal failure on hemodialysis, apparent infectious disease, bypass failure, history of prior use of thrombolytic therapy and those in whom it was not possible to cross the target lesion with a thrombectomy catheter because of excessive proximal vessel tortuosity.

Study Protocol

Blood samples were collected in chilled tubes containing EDTA-Na₂ (1 mg/ml blood) from a peripheral vein, and

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Fig 1. Plasma interleukin (IL)-6 concentrations in the peripheral vein (PV), ascending aorta (Ao) and infarct-related coronary artery (CA) in patients with acute myocardial infarction. Open circles () show means $\pm$ SEM, $n=36$.

then from the ascending aorta and the occluded coronary vessel by the thrombectomy catheter in order to measure the plasma concentrations of IL-6, TNF- α , and matrix metalloproteinases (MMP) -2 and -9. Briefly, coronary angiography was performed using a catheter inserted via the femoral approach. A thrombectomy catheter (RESCUE thrombectomy catheter, SciMED/BSC, Inc, Maple Grove, MN, USA) was introduced through the guiding catheter.¹⁸ Blood samples were collected by gently suction from the ascending aorta, and then from the occluded coronary artery. After thrombectomy, intravascular ultrasound (IVUS) images of the affected coronary vessels were recorded on S-VHS video for offline analysis. After the preinterventional IVUS, direct stenting was carried out. As a control group, 10 age- and sex-matched subjects (59.6 ± 11.6 years) gave blood sample from the peripheral vein for measurement of the plasma IL-6 concentrations.

The present study was approved by the Ethical Committee of Jichi Medical School for human study. We obtained written informed consent from all the patients before coronary angiography.

Analysis of the IVUS Images

A single observer unaware of the angiographic findings and clinical history analyzed the IVUS images. The cross-sectional area (CSA) was traced manually, the lumen-intimal border was also traced and the lumen CSA was calculated. The external elastic membrane (EEM)-adventitial border was traced, and the EEM CSA was determined. The reference EEM CSA is the average of the EEM CSA of the proximal and distal reference sites. The plaque CSA was calculated as (EEM CSA - lumen CSA).

Measurements

The blood samples were centrifuged at 3,000 rpm at 4°C for 15 min. The supernatants were decanted and frozen at -80°C until the time of assay for plasma IL-6, TNF- α , MMP-2 and MMP-9. IL-6 and TNF- α were determined by enzyme-linked immunosorbent assay (ELISA) using Quantikine human IL-6 and TNF- α assay kits (R&D system, Minneapolis, MN, USA). The minimal detectable concentration of IL-6 was 0.70 pg/ml. Intra- and interassay coefficients of variation were 2.6% and 4.5%, respectively, for the IL-6 assay kit and 6.7% and 13.4% for the TNF- α assay kit. MMP-2 and MMP-9 were determined by ELISA, using the respective Biotrak human assay kits (Amersham

Fig 2. Relationships between IL-6 in the infarct-related coronary artery and the reference EEM-CSA (A), the lesion EEM-CSA (B), and the plaque area (C) in patients with acute myocardial infarction.

Pharmacia Biotech, UK); the intra- and interassay coefficients of variation were 5.7% and 10.0% for the MMP-2 assay kit and 5.2% and 8.8% for the MMP-9 kit. The normal value of plasma IL-6 is ≤ 3.13 pg/ml,¹⁷ 0.7–2.1 pg/ml for plasma TNF- α ,¹⁹ 365–649 ng/ml for MMP-2,²⁰ and <0.6 ng/ml for MMP-9.²¹

Statistical Analysis

All values were expressed as means $\pm$ SEM. The data were analyzed by Student's *t*-test. Simple linear regression analysis was performed to calculate correlations. A *p*-value less than 0.05 was considered significant.

Results

Plasma IL-6 concentrations in the peripheral vein, ascending aorta and infarct-related coronary artery were all significantly greater than the 1.77 ± 0.2 pg/ml measured in the control subjects ($p < 0.05$; Fig 1). The concentration in the infarct-related coronary artery was significantly greater

than that in the ascending aorta and the peripheral vein. We confirmed that there was no relationship between the plasma IL-6 concentration and the time of measuring IL-6 after the onset of myocardial infarction in the present 36 patients. There was no correlation between the plasma IL-6 concentrations of the infarct-related coronary artery and the peripheral vein ($r=0.3$, $p=0.35$), nor did the plasma IL-6 concentration correlate with the maximal concentration of CK (data not shown).

In contrast, plasma TNF- concentrations remained normal in the patients with AMI; there was no difference among the 3 blood samples: 1.20 ± 0.13 pg/ml in the peripheral vein; 1.16 ± 0.12 pg/ml in the ascending aorta; and 1.15 ± 0.12 pg/ml in the infarct-related coronary artery.

As shown in Fig 2, the plasma concentration of IL-6 in the infarct-related coronary artery had a positive correlation with all of the reference measurements (ie, EEM-CSA ($p<0.01$), the lesion EEM-CSA ($p<0.01$), and the plaque area ($p<0.01$)), but not with the remodeling ratio (data not shown).

As shown in Fig 3A, the plasma MMP-9 concentration in both the ascending aorta and the infarct-related coronary artery was greater than normal (<0.6 ng/ml). The plasma MMP-9 concentration was significantly greater in the infarct-related coronary artery than that in the ascending aorta ($p<0.001$). Plasma MMP-2 concentrations remained normal in the ascending aorta and the infarct-related coronary artery in the patients with AMI, and there was no difference in the concentrations between the infarct-related coronary artery and the ascending aorta (Fig 3B).

Discussion

We have determined the inflammatory changes in the infarct-related coronary artery in patients with AMI. IL-6 and TNF- , which are inflammatory cytokines, were measured within 24h of the onset of infarction and the concentration of plasma IL-6 in the systemic circulation were increased. Furthermore, it was significantly greater in the infarct-related coronary artery than in the ascending aorta or the peripheral vein. Furthermore, we measured the IL-6 concentration in the culprit infarct-related coronary artery after percutaneous coronary artery intervention in 23 of 36 patients and found it was as high as 15.7 ± 2.2 pg/ml, which is persistently high after the removal of the obstructive thrombi. IL-6 may be released from macrophages in vulnerable atherosclerotic plaque by an autocrine mechanism, which activates the macrophages to further break down the plaque's fibrous cap in the lesioned artery.²² Plasma IL-6 concentrations in the infarct-related coronary artery had a positive correlation with all of the reference measurements (EEM-CSA, the lesion EEM-CSA and the plaque area) observed by intravascular ultrasound. The findings thus indicate that plasma IL-6 concentrations in the infarct-related coronary artery are closely associated with the size of the vulnerable plaque itself. However, it has been reported that increased concentration of IL-6 is associated with the stability of atherosclerotic plaque,⁵ but in the present study we could not determine how fragile the plaques were. Similarly, it has been suggested that TNF- and macrophage chemoattractant protein-1 are also secreted from vulnerable plaque,^{16,19} but in the present patients the plasma concentrations of TNF- remained normal and did not differ among the 3 blood samples. The change in the plasma concentration of TNF- is slow and subtle,²³ and

Fig 3. Plasma matrix metalloproteinase (MMP) -9 (A) and -2 concentrations (B) in the ascending aorta (Ao) and infarct-related coronary artery (CA) in patients with acute myocardial infarction. MMP-9 was measured in 36 patients, and MMP-2 in 24 patients. Open circles () show means $\pm$ SEM.

may not have been detected in the present protocol. However, the present study results are unique evidence that local alterations in the concentrations of cytokines in the infarct-related coronary artery may facilitate the breakdown of the vulnerable region of atherosclerotic plaque. Activation of macrophages is the key to the degradation of the extracellular matrix in the vulnerable regions of atherosclerotic plaque. Macrophages secrete proteolytic enzymes, proteinases, that breakdown both collagen and elastin to peptides and eventually amino acids.²⁴⁻²⁷

There are many kinds of MMPs, and we determined the concentrations of MMP-2 and MMP-9. Under normal conditions the MMPs play a pivotal role in tissue remodeling, such as tissue morphogenesis and wound healing.^{28,29} In the present study, the plasma concentration of MMP-9 was significantly increased in the infarct-related coronary artery, as compared with the ascending aorta, but that of MMP-2 was not increased. It is reported that MMP-9 is mainly synthesized in macrophages and vascular smooth muscle cells, whereas MMP-2 is mainly synthesized in vascular smooth muscle cells.³⁰ Even though we could not measure all the MMPs, the present findings indicate that MMP-9 at least is involved in the fragility of the vulnerable atherosclerotic plaque in the lesioned coronary artery. However, further study will be necessary to elucidate how many MMPs are activated locally in the infarct-related coronary artery.

In conclusion, we have demonstrated a regional elevation of IL-6 and activation of macrophages in the infarct-related coronary artery within 24h of the onset of AMI. The inflammatory changes may directly participate in the rupture of vulnerable atherosclerotic plaques and the subsequent occlusion of the coronary artery.

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References

- Ross R. Atherosclerosis: An inflammatory disease. *N Engl J Med* 1999; **340**: 115–126.
- Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002; **105**: 1135–1143.
- Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. *Circ Res* 2001; **89**: 763–771.
- Ikeda U, Ito T, Shimada K. Interleukin-6 and acute coronary syndrome. *Clin Cardiol* 2001; **24**: 701–704.
- Schieffer B, Schieffer E, Hifiker-Kleiner D, Hilfiker A, Kovanen PT, Kaartnen M, et al. Expression of angiotensin II and interleukin-6 in human coronary atherosclerotic plaques. *Circulation* 2000; **101**: 1372–1378.
- Seino Y, Ikeda U, Ikeda M, Yamamoto K, Misawa Y, Hasegawa T, et al. Interleukin 6 gene transcripts are expressed in human atherosclerotic lesions. *Cytokine* 1994; **6**: 87–91.
- Libby P. Molecular bases of the acute coronary syndrome. *Circulation* 1995; **91**: 2844–2850.
- Libby P, Geng YJ, Aikawa M, Schoenbeck U, Mach F, Clinton SK, et al. Macrophages and atherosclerotic plaque stability. *Curr Opin Lipidol* 1996; **7**: 330–335.
- Lippy P. Current concepts of the pathogenesis of acute coronary syndrome. *Circulation* 2001; **104**: 365–375.
- Tashiro H, Shimokawa H, Yamamoto K, Nagano M, Momohara M, Muramatsu K, et al. Monocyte-related cytokine in acute myocardial infarction. *Am Heart J* 1995; **130**: 446–452.
- Ikeda U, Ikeda M, Seino Y, Takahashi M, Kano S, Shimada K. Interleukin 6 gene transcripts are expressed in atherosclerotic lesions of genetically hyperlipidemic rabbits. *Atherosclerosis* 1992; **92**: 213–218.
- Ikeda U, Ohkawa F, Seino Y, Yamamoto K, Hidaka Y, Kasahara T, et al. Serum interleukin 6 levels become elevated in acute myocardial infarction. *J Mol Cell Cardiol* 1992; **24**: 579–584.
- Sturk A, Hack CE, Aarden LA, Brouwer M, Koster RR, Sanders GT. Interleukin-6 release and the acute-phase reaction in patients with acute myocardial infarction: A pilot study. *J Lab Clin Med* 1992; **119**: 574–579.
- Pannitteri G, Marino B, Paolo C, Martucci R, Teata U, Peschle C. Interleukin-6 and -8 as mediators of acute phase response in acute myocardial infarction. *Am J Cardiol* 1997; **80**: 622–625.
- Shimada Y, Yoshiyama M, Jissho S, Kamimori K, Nakamura Y, Iida H, et al. Hepatocyte growth factor production may be related to the inflammatory response in patients with acute myocardial infarction. *Circ J* 2002; **66**: 253–256.
- Hokimoto S, Oike Y, Saito T, Kiaoka M, Oshima S, Noda K, et al. Increased expression of monocyte chemoattractant protein-1 in atherectomy specimens from patients with restenosis after percutaneous transluminal coronary angioplasty. *Circ J* 2002; **66**: 114–116.
- Kato K, Matsubara T, Iida K, Suzuki O, Sato Y. Elevated levels of pro-inflammatory cytokines in coronary artery thrombi. *Int J Cardiol* 1999; **70**: 267–273.
- Kotani J, Nanto S, Mintz GS, Kitakaze M, Ohara T, Morozumi T, et al. Plaque gruel of atheromatous coronary lesion may contribute to the no-reflow phenomenon in patients with acute coronary syndrome. *Circulation* 2002; **106**: 1672–1677.
- Tsukada N, Miyagi K, Matsuda M, Yanagisawa N, Yone K. Tumor necrosis factor and interleukin-1 in the CSF and sera of patients with multiple sclerosis. *J Neurol Sci* 1991; **104**: 230–234.
- Fujioto N, Mouri N, Iwata K, Ohuchi E, Okada Y, Hayakawa T. A one-step sandwich enzyme immunoassay for human matrix metalloproteinase 2 (72-kDa gelatinase/type IV collagenase) using monoclonal antibodies. *Clin Chim Acta* 1993; **221**: 91–103.
- Fujioto N, Hosokawa N, Iwata K, Shinya T, Okada Y, Hayakawa T. A one-step sandwich enzyme immunoassay for inactive precursor and complexed forms of human matrix metalloproteinase 9 (92-kDa gelatinase/type IV collagenase, gelatinase B) using monoclonal antibodies. *Clin Chim Acta* 1994; **231**: 79–88.
- Shah PK, Falk E, Badimon JJ, Fernandez-Ortiz A, Mailhac A, Villareal-Levy G, et al. Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerosis plaque: Potential role of matrix-degrading metalloproteinases and implications for plaque rupture. *Circulation* 1995; **92**: 1565–1569.
- Guillen I, Blanes M, Gomez-Lechon MJ, Castell JA. Cytokine signaling during myocardial infarction: Sequential appearance of IL-1 and IL-6. *Am J Physiol* 1995; **269**: R229–R235.
- Galis ZS, Sukhova GK, Kranzhofer R, Clark S, Libby P. Macrophage foam cell from experimental atheroma constitutively produce matrix degrading proteinases. *Proc Natl Acad Sci USA* 1995; **92**: 402–406.
- Saren P, Welgus HG, Kovanen PT. TNF- and IL-1 selectively induce expression of 92-kDa gelatinase by human macrophages. *J Immunol* 1996; **157**: 4157–4165.
- Dollery CM, McEwan JR, Henney AM. Matrix metalloproteinases and cardiovascular disease. *Circ Res* 1995; **77**: 863–868.
- Kai H, Ikeda H, Yasukawa H, Kai M, Seki Y, Kuwahara F, et al. Peripheral blood levels of matrix metalloproteinases-2 and -9 are elevated in patients with acute coronary syndromes. *J Am Coll Cardiol* 1998; **32**: 368–372.
- Wossner JF. Matrix metalloproteinase and their inhibitors in connective tissue remodeling. *FASEB J* 1991; **5**: 2145–2154.
- Matrisian LM. Metalloproteinases and their inhibitors in matrix remodeling. *Trends Genet* 1990; **6**: 121–125.
- Galis ZS, Sukhova GK, Lark MW, Lippy P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest* 1994; **94**: 2493–2503.