Plasma Monocyte Chemoattractant Protein-1 Antigen Levels and the Risk of Restenosis After Coronary Stent Implantation

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Monocyte chemoattractant protein-1 (MCP-1) plays a fundamental role in monocyte recruitment and has been implicated in atherosclerosis. The present study tested the hypothesis that increased levels of MCP-1 are associated with an increased risk for restenosis post stent implantation. The plasma MCP-1 antigen levels were measured pre-stenting, and at 24 and 48 h and 6 months post stenting in 41 patients with stable exertional angina (SEA) who had undergone successful stent implantation. Nineteen patients with chest pain syndrome were selected as a control group. Initial plasma MCP-1 antigen levels (mean $\pm$ SE, pg/ml) in the patients with SEA were significantly higher than those in the control group (852.3 ± 51.4 vs 418.2 ± 26.7 , $p < 0.001$). The patients with SEA were divided into 2 groups based on follow-up angiographic findings: 17 patients with restenosis (R group); 24 patients without restenosis (N group). The lesion was significantly longer in the R group than in the N group ($p < 0.03$). Plasma MCP-1 antigen levels at pre-stenting were not significantly different between the 2 groups (820.6 ± 69.1 in the R group vs 874.7 ± 73.8 in the N group). Serial changes of plasma MCP-1 levels were plotted as percent changes from the initial levels (mean $\pm$ SE, %) and were significantly higher in the R group than in the N group at 48 h and at 6 months post stent implantation (104.6 ± 4.8 vs 89.2 ± 3.4 , $p < 0.01$, 109.6 ± 11.2 vs 98.5 ± 5.0 , $p < 0.05$). The study concludes that MCP-1 production at stented coronary arterial sites is associated with an increased risk for restenosis post stent implantation. (*Jpn Circ J* 2001; 65: 261–264)

Key Words: MCP-1; Restenosis; Stent

As compared with balloon angioplasty, intracoronary stenting has reduced rates of restenosis;^{1,2} nevertheless, in-stent restenosis continues to be an important clinical issue.^{3,4} Serial (post intervention and follow-up) intravascular ultrasound studies have shown that in-stent restenosis is almost exclusively caused by neointimal hyperplasia^{5–7} and tissue proliferation occurring inside or adjacent to the stent sites has been shown to be directly related to the aggressiveness of the stent implantation technique,⁷ with histological studies confirming that neointimal hyperplasia post stent implantation is related to vessel trauma during the procedure.^{8–11} In stented, nonatherosclerotic, balloon-injured rabbit iliac arteries, peak monocyte adherence was observable 3 days post stent implantation, with maximum proliferation observed at 7 days.¹² There was a linear correlation between monocyte adherence and neointimal hyperplasia.¹¹

Monocyte chemoattractant protein-1 (MCP-1), which has a potent chemotactic action on monocytes,¹³ can amplify the inflammatory response through the recruitment of additional monocytes.¹⁴ Macrophage activity has been demonstrated

to play an important role in the progression of atherosclerosis;^{15–17} however, the association between MCP-1 and restenosis post coronary stent implantation remains unclear.

We designed this study to test the hypothesis that increased levels of MCP-1 are associated with an increased risk for restenosis post stent implantation.

Methods

Patient Population

Forty-one patients with stable exertional angina (SEA) (ie, proven ischemia on laboratory testing) underwent angiographically successful stent implantation of a single lesion with de novo, or one previous balloon angioplasty, in a native coronary artery between June 1997 and November 1997. Exclusion criteria were unsuccessful procedures (defined as the failure to place the stent at the desired position or to achieve a final diameter stenosis $< 30\%$ by visual inspection) or the occurrence of one or more major adverse cardiac events (death, myocardial infarction, coronary bypass graft surgery, or repeat balloon angioplasty) during the first month post procedure. All the patients with SEA underwent follow-up angiography 6 months after the procedure. Nineteen patients with chest pain syndrome were selected as control subjects; their coronary angiographic findings were normal. The study protocol followed the guidelines of the ethics committee at the institution and written informed consent was obtained from each patient.

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Table 1 Clinical Characteristics of 41 Patients With Stable Exertional Angina

	Restenosis (n=17)	No restenosis (n=24)	p value
Age (years)	69.8±7.5	66.2±9.0	NS
Sex (M/F)	12/5	18/6	NS
Hypertension	8 (47.1%)	14 (58.3%)	NS
Diabetes mellitus	5 (29.4%)	8 (33.3%)	NS
Smoking	8 (47.1%)	12 (50%)	NS
Old myocardial infarction	9 (52.9%)	11 (45.8%)	NS
Cholesterol (mg/dl)	196.2±9.8	197.1±7.7	NS
Triglycerides (mg/dl)	142.1±14.9	185.5±22.9	NS
HDL-cholesterol (mg/dl)	50.0±3.1	43.8±2.5	NS

Data are presented as mean±SD. HDL, high density lipoprotein.

Table 2 Lesion and Procedural Characteristics at the Time of Intervention

	Restenosis (n=17)	No restenosis (n=24)	p value
No. of vessels			
1-VD	8	13	
Multi-VD	9	11	NS
Culprit lesion			
LAD	8	13	NS
RCA	5	4	
LCX	4	7	
Type of stent			
P-S	12	15	NS
ML	5	9	
Before stenting			
RD (mm)	3.19±0.28	3.24±0.46	NS
MLD (mm)	0.77±0.45	0.85±0.39	NS
Diameter stenosis (%)	76.3±13.2	75.0±9.8	NS
Lesion length (mm)	19.8±10.4	13.6±6.0	0.03
Procedural data			
Maximal balloon pressure (atm)	10.5±1.9	11.1±2.1	NS
Balloon-to-vessel ratio	0.99±0.08	1.00±0.09	NS
Immediately after stenting			
MLD (mm)	3.01±0.29	3.08±0.50	NS
Diameter stenosis (%)	6.5±8.1	4.8±6.5	NS
Restenosis lesion	5	3	NS

Data are presented as mean±SD. VD, vessel disease; LAD, left anterior descending coronary artery; RCA, right coronary artery; LCX, left circumflex coronary artery; P-S, Palmaz-Schatz stent; ML, multi-Link stent; RD, reference diameter; MLD, minimal lumen diameter.

Stent Implantation

Before the intervention, all patients received heparin (10,000 U) in a single intravenous bolus. Slotted-tube stents (65% Palmaz-Schatz stents, Johnson & Johnson; 35% Multi-Link stents, Advanced Cardiovascular Systems Inc) were implanted as previously described.¹⁸ All patients received intravenous heparin (15,000 U/day) for 48 h and oral ticlopidin (200 mg bid po) for 3 months post stent implantation as follow-up therapy. None of the patients were taking medications that effect restenosis, such as tranilast.^{19,20}

Quantitative Angiographic Analysis

Quantitative analyses were performed with a validate edge-detection algorithm (AWOS version 4.0, Siemens) using a contrast-filled catheter for image calibration in at least 2 projections. Minimal lumen diameter (MLD), reference diameter (RD), diameter stenosis, lesion length and diameter of the maximally inflated balloon were obtained with this analysis system. Balloon-to-vessel ratios were calculated as the diameter of the inflated balloon divided by the RD of the coronary artery. Angiographic restenosis was defined as greater than 50% diameter reduction of the mean

proximal and distal reference lumens on follow-up coronary angiography.

Blood Sampling and Assay

Venous blood samples (4.5 ml) for the measurement of plasma MCP-1 antigen levels were obtained before the sheath insertion and were each drawn into a blood collection tube containing 0.5 ml of 0.19 mol/L buffered sodium citrate and then centrifuged for 10 min at 3,000 g at 4°C; the samples were stored at -80°C until analyzed. Blood samples were also obtained at 24 and 48 h and 6 months post stent implantation. Blood samples in the control group were obtained upon admission.

Plasma MCP-1 antigen levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (HyCult Biotechnology, The Netherlands). The assay was performed by one of the investigators, who was unaware of the patients' clinical data. The intra-assay and inter-assay coefficient of variation of the kit at the MCP-1 antigen level of 630 pg/ml were 10.3% and 4.9%, respectively.

Fig 1. Plasma monocyte chemoattractant protein-1 (MCP-1) antigen levels (mean $\pm$ SE, pg/ml) in the patients with stable exertional angina (SEA; closed circles) and in the control chest pain syndrome group (open circles).

Statistical Analysis

Statistical analyses were performed with commercially available software programs. The plasma antigen levels of MCP-1 were expressed as mean $\pm$ SEM. All data except for MCP-1 levels were expressed as mean $\pm$ SD. The statistical significance of the changes during the time course in plasma MCP-1 levels was evaluated by using a 2-way analysis of variance for repeated measures and by using Duncan's test.²¹ Differences between groups were evaluated by the chi-square test or by the Fisher exact test for categorical variables; the Student t test was used for continuous variables. P values of less than 0.05 were considered significant.

Results

Patient Characteristics

There were no significant differences between the SEA patients and the controls, except for hypertension, which was more frequently observed in the SEA patients than in the control patients (66% vs 16%, $p<0.05$). Follow-up angiography was performed at a median of 5.1 months (4–6 months) post stent implantation in all patients with SEA. Angiographic restenosis was found in 17 patients, and the clinical characteristics of these patients were compared with the 24 patients free of restenosis; there were no significant differences in age, sex, history of myocardial infarction or coronary risk factors (Table 1).

Lesion and Procedural Characteristics at Time of Intervention

Table 2 lists the angiographic and procedural characteristics of the lesions for the restenosis group and the nonrestenosis group. There were no significant differences between the 2 groups except for the lesion length.

MCP-1 Levels Between the Patients With Stable Exertional Angina and the Control Group

Plasma MCP-1 antigen levels pre-stenting in the patients with SEA were significantly higher than those in the control group (852.3 ± 51.4 vs 418.2 ± 26.7 , $p<0.001$) (Fig 1).

Serial Changes of Plasma MCP-1 Antigen

Plasma MCP-1 antigen levels (pg/ml) at pre-stenting were not significantly different between the restenosis group and the nonrestenosis group (820.6 ± 69.1 vs 874.7 ± 73.8). Because of large individual variations in the initial MCP-1 values, the serial changes of MCP-1 levels were plotted as

Fig 2. Serial changes of plasma monocyte chemoattractant protein-1 (MCP-1) antigen levels pre-stenting and at 24 and 48 h and 6 months post stent implantation in patients with restenosis (circles) and without restenosis (squares).

percent changes from the initial level and were significantly higher in the restenosis group than the nonrestenosis group at 48 h (104.6 ± 4.8 vs 89.2 ± 3.4 , $p<0.01$) and at 6 months (109.5 ± 11.2 vs 98.5 ± 5.0 , $p<0.05$) after stent implantation (Fig 2). In the restenosis group, plasma MCP-1 antigen levels were greater at 48 h and at 6 months post stent implantation than at pre-stenting ($p<0.05$). On the other hand, the MCP-1 levels in the nonrestenosis group were less at 24 and 48 h post stenting than the levels pre-stenting ($p<0.01$) (Fig 2).

Discussion

We have shown that plasma MCP-1 antigen levels, a potent monocyte chemotactic factor, were significantly elevated in the patients with SEA compared with the control chest pain syndrome group. MCP-1 is reported to be upregulated in the arteries of primates and in vascular endothelial and smooth muscle cells exposed to minimally modified lipids;^{22,23} moreover, MCP-1 plays an important role in the development of early atherosclerotic lesions.^{24,25} Recent clinical studies have shown that plasma levels of MCP-1 are increased in patients with unstable angina and acute myocardial infarction;^{26,27} so it is logical to conclude that the level of circulating MCP-1 reflects the clinical state of coronary artery disease.

In-stent restenosis continues to be an important clinical issue^{3,4} and recent studies have indicated that neointimal hyperplasia post stent placement is related to vessel trauma during stent implantation^{8–10} although, importantly, angiographic measurements could not predict restenosis post stent implantation.⁵ In the present study, as well as in the previous report,⁵ the angiographic measurements did not differ between the patients with restenosis and those without restenosis; however, the current study revealed a difference in the lesion length. Restenosis was present in 17 (41%) of 41 consecutive stent-treated patients, which appears to be a rather high rate for stenting in this size coronary vessels and is thought to be a statistical quirk related to the small number of subjects in the present study.

We demonstrated that the serial changes of plasma MCP-1 antigen levels differed between the restenosis group and the non restenosis group at 48 h and at 6 months post stent implantation: the restenosis group had higher levels of MCP-1. In some inflammatory processes, MCP-1 is expressed by many kinds of cells and modifies the movement of monocytes into an inflammatory lesion.¹⁴ Our findings

indicate that immunologic and inflammatory processes are important possible causative factors of restenosis post stent implantation. Recent animal studies have shown a linear correlation between monocyte adherence and neointimal hyperplasia in stented arteries^{11,12} and seem to support our results. Furthermore, Furukawa et al demonstrated that MCP-1 promotes neointimal hyperplasia in early neointimal lesion formation and that neutralization of MCP-1 before, and immediately after, arterial injury may be effective in preventing restenosis after angioplasty.²⁸

In the restenosis group, plasma MCP-1 antigen levels at 48 h and at 6 months after stenting were increased from the levels pre-stenting. In contrast, the plasma MCP-1 antigen levels were less at 24 and 48 h post stent implantation than the levels pre-stenting in the non restenosis group. In the present study, heparin infusion therapy was performed during the 48 h post stent implantation. Heparin can bind with high affinity to a range of cytokines including interferon-gamma and members of the chemokine superfamily, and can block interferon-gamma-dependent upregulation of MCP-1 production by cultured endothelial cells.²⁹ Therefore, we speculated that heparin infusion therapy modified the levels of plasma MCP-1 antigen for 48 h post stent implantation in the present study.

In conclusion, we suggest that MCP-1 production at the stented sites of coronary arteries is associated with an increased risk for restenosis post coronary stent implantation, although histological findings of the target lesion sites and MCP-1 levels in the coronary sinus were inconclusive in the present study.

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