

Significance of the Level of Monocyte Chemoattractant Protein-1 in Human Atherosclerosis

— Assessment in Chronic Hemodialysis Patients —

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Background Monocyte chemoattractant protein-1 (MCP-1), a potent chemoattractant for monocytes, plays an important role in the earliest events of atherogenesis. However, direct evidence of the effects of MCP-1 on atherosclerosis in chronic hemodialysis (HD) patients has not been reported.

Methods and Results The serum MCP-1 concentrations and the intimal–medial thickness (IMT) in the carotid arteries were measured in 42 non-diabetic chronic HD patients and 20 age-matched controls. The expression of MCP-1 was examined immunohistochemically in radial arterial tissues obtained from the HD patients. IMT and the serum concentration of MCP-1 in the HD patients were both significantly greater than in controls. Multiple regression analysis revealed that the serum concentration of MCP-1 was an independent factor influencing IMT. Tissue immunostaining showed that MCP-1 is expressed in both endothelial and smooth muscle cells and that its level of expression correlates with the serum concentration of MCP-1.

Conclusions An increase in MCP-1 may be an important factor in the progression of atherosclerosis in non-diabetic HD patients. (*Circ J* 2004; 68: 671–676)

Key Words: Atherosclerosis; Cytokine; Hemodialysis

The rates of cardiovascular or cerebrovascular mortality related to accelerated atherosclerosis are high in patients undergoing chronic hemodialysis (HD) treatment.^{1,2} The overall cardiovascular mortality rate in patients undergoing chronic HD treatment is estimated to be approximately 30%, of which 10% are deaths because of myocardial infarction.³

Recently, it has been shown that monocyte chemoattractant protein-1 (MCP-1) is a potent monocyte chemoattractant and largely responsible for the recruitment of monocytes/macrophages to the vessel wall in the early stage of atherogenesis.^{4–12} In the clinical setting, it has been reported that the plasma concentrations of several cytokines and chemokines, including MCP-1, increased during long-term HD treatment.¹³ However, there have been few studies of the relationship between the severity of human atherosclerosis and the serum concentration of MCP-1 in HD patients.^{13,14}

High-resolution ultrasound imaging is now a reliable means of directly examining and quantifying pre-clinical

atherosclerotic lesions in peripheral arteries.¹⁵ This method is noninvasive and has high reproducibility.¹⁶ Ultrasound measurements of intimal–medial thickness (IMT) in the carotid artery have been used as indicators of coronary atherosclerosis.¹⁷ Tabara et al reported that the plasma concentration of MCP-1 was significantly associated with IMT in community-based subjects, aged 50 years or older and free from any cardiovascular complications.¹⁸ In the present study, we measured IMT and investigated the effects of MCP-1 and other factors on the severity of IMT in HD patients. We also used immunohistochemistry to investigate the expression of MCP-1 in arterial tissue samples.

Methods

Subjects

After obtaining informed consent, 52 Japanese uremic patients undergoing chronic HD (29 men and 23 women; mean age, 56±10 years: HD group) and 20 age-matched control subjects (9 men and 11 women; mean age, 57±13 years: control group) were enrolled. In the patients undergoing chronic HD, the diagnosed kidney diseases were chronic glomerulonephritis in 47 patients and polycystic kidney disease in 5 patients. To avoid the influence of the cause of the disease, patients with diabetic nephropathy and hypertensive nephrosclerosis were excluded. Patients had been treated by HD 3 times a week for a mean duration of 10.1±7.4 years. The clinical and biochemical characteristics of the patients are summarized in Table 1. In the HD group,

(Received September 16, 2003; revised manuscript received April 19, 2004; accepted April 30, 2004)

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Table 1 Clinical Characteristics

	HD (n=52)	Control (n=20)	p value
Age (years)	56±10	57±13	NS
M/F	29/23	11/9	NS
Smoking habit			NS
Never	32 (62%)	13 (65%)	
Previous	6 (12%)	3 (15%)	
Active	14 (27%)	4 (20%)	
Existence of left ventricular hypertrophy			<0.01
Within normal limits	26 (50%)	18 (90%)	
High voltage without ST-T change	15 (29%)	2 (10%)	
High voltage with ST-T change	11 (21%)	0	
Total cholesterol (mg/dl)	189.4±37.6	209.0±39.1	NS
HDL-cholesterol (mg/dl)	48.6±13.1	59.0±30.8	NS
LDL-cholesterol (mg/dl)	118.4±30.9	129.8±25.6	NS
Triglycerides (mg/dl)	112.1±58.3	95.8±66.6	NS
Lipoprotein (a) (mg/dl)	20.2±16.3	14.1±10.5	<0.05
Uric acid (mg/dl)	6.4±1.8	4.6±0.8	<0.05
Fibrinogen (mg/dl)	371.7±80.3	280.6±53.7	<0.01
Homocystein (μmol/L)	38.8±35.9	8.4±1.0	<0.01
Apo A-I (mg/dl)	119.7±15.6	156.5±26.5	<0.05
Apo B (mg/dl)	94.2±18.1	95.5±29.4	NS
Apo E (mg/dl)	4.3±1.8	3.9±0.6	NS
Apo B/A-I	0.8±0.2	0.6±0.3	<0.05
Serum MCP-1 (pg/ml)	457.3±152.8	246.2±96.3	<0.01

Data are presented as mean±SD.

HD, hemodialysis; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Apo, apolipoprotein; MCP-1, monocyte chemoattractant protein.

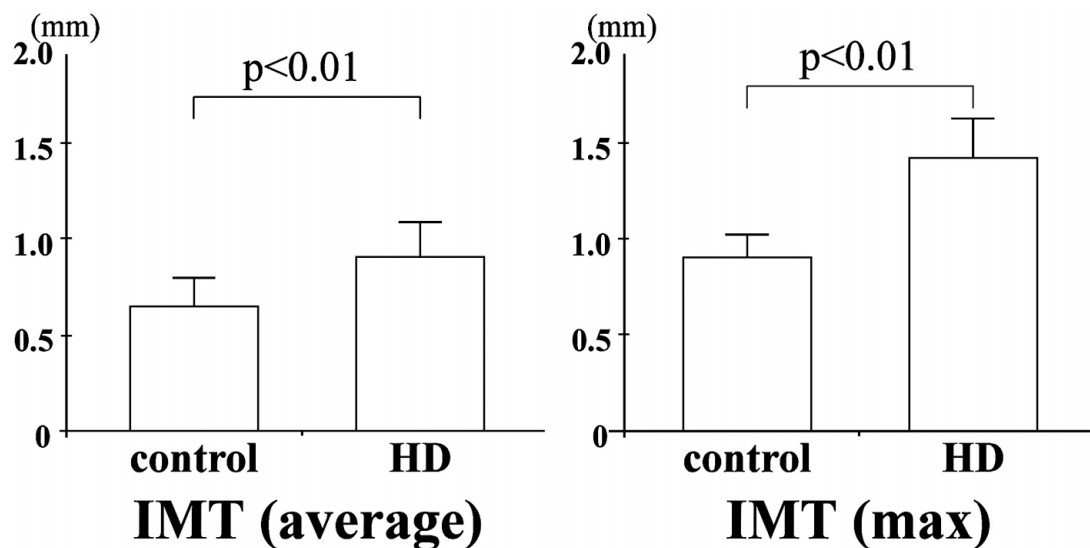


Fig 1. Carotid intimal–medial thickness (IMT). The average and maximal values of IMT of the carotid artery in the hemodialysis (HD) group were significantly greater than those in the control group.

27 patients were taking anti-hypertensive drugs: 18 patients were taking Ca antagonists, 5 were taking β -blockers, 2 were taking angiotensin-converting enzyme inhibitors, and 1 was taking a α -blocker. Six patients received hMG CoA reductase inhibitors were administered to 6 patients.

Ultrasound in the Carotid Arteries

Ultrasonic carotid arteriography was performed in all subjects with high-resolution B-mode real-time ultrasonography using an 8.0-MHz in-line linear transducer (SSA260ACE, Toshiba Co Ltd, Japan). Each subject was examined while supine, and each carotid wall and segment

was interrogated independently from continuous angles to identify the thickest intima–media site. Each scan of the common carotid artery began just above the clavicle, and the transducer was moved in the cephalad direction through the bifurcation and along the internal carotid artery. Four segments were identified on each side: a segment 15–30 mm distal and a segment 0–15 mm distal to the common carotid proximal to the bifurcation, the bifurcation itself, and the proximal 10-mm segment of the internal carotid artery. To assess the severity of atherosclerosis in the carotid artery, we calculated the mean value of 16 measurements of maximum IMT obtained independently from the

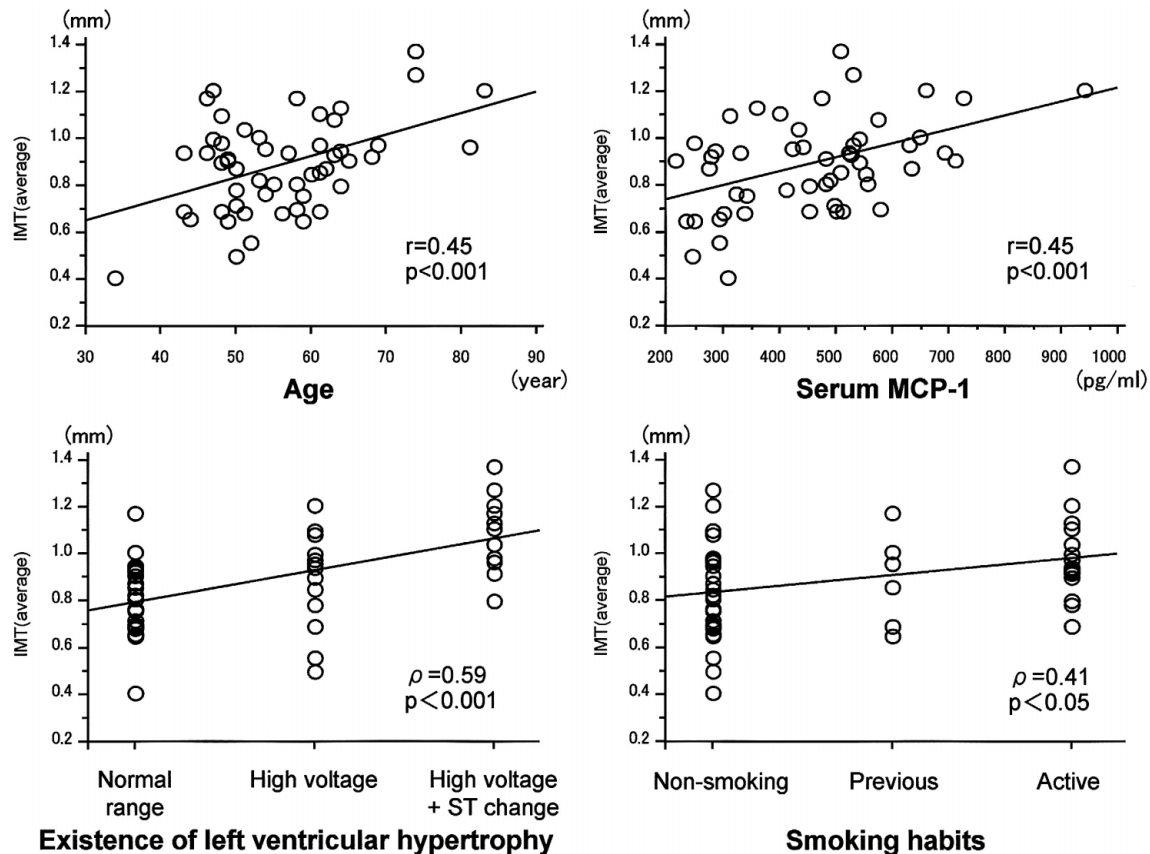


Fig 2. Relationships between carotid intimal–medial thickness (IMT) and risk factors. Univariate analysis revealed that average IMT correlated with age, serum concentration of MCP-1, smoking habits and the existence of left ventricular hypertrophy.

Table 2 Risk Factors Affecting IMT of the Carotid Arteries in Hemodialysis Patients

Risk factor	–	t value	F value	p value
Left ventricular hypertrophy	0.395	3.564	21.584	<0.001
Serum MCP-1	0.224	2.120	12.056	0.040
Age	0.323	3.147	7.552	0.003
Apo A-1	-0.291	-2.607	3.009	0.012
Smoking habit	0.122	1.052	2.480	0.299
LDL-cholesterol	0.155	1.332	1.773	0.190

IMT, intimal-medial thickness; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Apo, apolipoprotein; MCP-1, monocyte chemoattractant protein.

Dependent value: IMT average.

Independent values: age, duration of hemodialysis, smoking, left ventricular hypertrophy, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, Apo A-1, B, E, fibrinogen, homocystein, serum MCP-1.

Multiplex $R=0.78$, $p<0.001$.

near and far walls of both carotid arterial segments.

Assessment of Risk Factors of Atherosclerosis

The following possible risk factors of atherosclerosis were examined: age, duration of HD, existence of left ventricular hypertrophy, history of smoking, lipid profiles [total cholesterol, high density lipoprotein-cholesterol (HDL-C), triglycerides, apolipoprotein A-1 (Apo A-1), apolipoprotein B (Apo B), apolipoprotein E (Apo E)], lipoprotein (a) (LP(a)), uric acid, fibrinogen, homocystein and the serum concentration of MCP-1. Blood samples for the measurements were obtained after an overnight fast. Low density lipoprotein-cholesterol (LDL-C) was calcu-

lated from the Friedewald equation.¹⁹ Serum concentrations of MCP-1 were measured using a human MCP-1 ELISA system (Amersham Pharmacia Biotech, UK) as previously described.²⁰

A 12-lead ECG was used to estimate the degree of left ventricular hypertrophy (LVH): normal range, high voltage without ST segment change, and high voltage with ST segment change. The criterion of high voltage was $SV_1+RV_5 >3.5$ mV or R in lead II, III, aVF >2.0 mV.

Information on smoking habits was obtained by a self-administered questionnaire and patients were divided into 3 groups: non-smoking, previous smoking and active smoking.

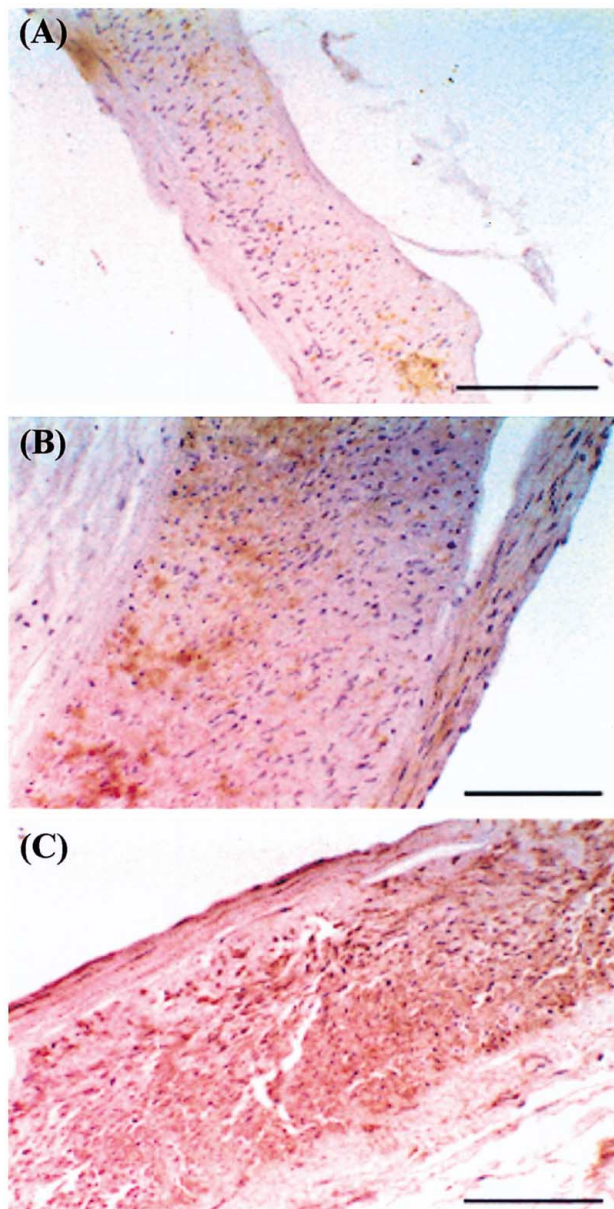


Fig 3. Tissue expression of MCP-1 in the radial artery. MCP-1 was constitutively expressed in both the vascular endothelial cells and the smooth muscle cells in the radial artery. The immunostaining of arterial tissue was graded on a 4-point scale: (A) grade 1, spotty positive MCP-1 expression observed in less than 25% of the cells; (B) grade 2, moderate positive MCP-1 expression observed in 25–50% of the cells; (C) grade 3, severe positive MCP-1 expression observed in more than 50% of the cells. Scale bar=100 μ m.

Immunohistochemistry

Eighteen arterial samples were obtained from the radial artery of patients who had undergone an arterio-venous shunt reoperation for chronic HD. To avoid the influence of the primary operation, all sample tissues were obtained far from the primary operation site. Sample tissues were immersion-fixed overnight at 4°C in formal/sucrose [4% (wt/vol) paraformaldehyde/ 5% (wt/vol) sucrose/1 mmol/L WDTA/50 μ mol/L butylated hydroxytoluene, pH 7.4] and embedded in paraffin.

Each tissue samples was serially cut into 5- μ m-thick sections, stained with hematoxylin and eosin solution, and

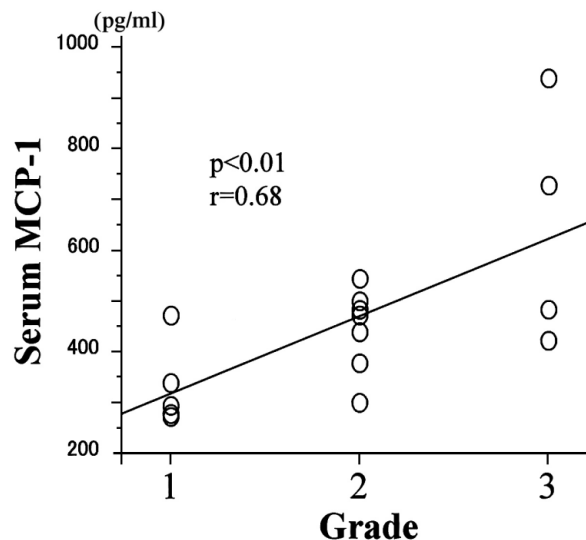


Fig 4. The correlation between the serum concentration and tissue expression of MCP-1 was positive ($r=0.68$, $p<0.01$).

examined under a light microscope. Immunostaining was performed using a DAKO LSAB System (Dako) according to the manufacturer's instructions. Briefly, the sections were preincubated with 1.5% hydrogen peroxide and normal bovine serum albumin to block nonspecific reactions. A mouse monoclonal anti-MCP-1 antibody (1:1,000 dilution American Research Products, Inc, MA, USA) was added, and the sections were incubated for 60 min at room temperature. Each section was then incubated with biotinylated anti-mouse immunoglobulin for 20 min and subsequently with horseradish peroxidase-labeled streptavidin solution for 20 min. Peroxidase activity was visualized with diaminobenzidine tetrahydrochloride solution.

The intensity of the immunostaining of MCP-1 was graded semiquantitatively on a 4-point scale from 0 to 3: grade 0, no staining; grade 1, positive immunostaining in <25% of the cells; grade 2, positivity of 25–50% of the cells; and grade 3, positivity of >50% of the cells. Grading was performed independently by 2 of the investigators who had no knowledge of the data. Grades assigned were agreed to within one grade, with any differences resolved by consensus.

Statistical Analysis

Results are presented as mean \pm SD. Differences between groups were compared using the Kruskal-Wallis test. Univariate analysis between IMT values and risk factors and between serum concentration and tissue intensity of immunostaining of MCP-1 were examined linear regression analysis using Pearson's correlation coefficient (age, duration of hemodialysis, total cholesterol, HDL-C, LDL-C, triglycerides, LP(a), uric acid, fibrinogen, Apo A-1, B, E, homocysteine and serum level of MCP-1) or Spearman's correlation coefficient (LVH, smoking, intensity of immunostaining of MCP-1). Multiple regression analysis with a forward elimination procedure was used to assess the combined influence of risk factors on IMT values. A value of $p<0.05$ was considered statistically significant. These procedures were performed on a Macintosh computer using Statistica 4.1.

Results

Patient Characteristics

Table 1 shows the clinical variables of the study groups. There were no significant differences between the groups in the serum concentrations of total cholesterol, HDL-C, LDL-C, triglyceride, Apo B and Apo E. However, LP(a), uric acid, fibrinogen, homocysteine, and Apo A-1 were significantly higher in the HD group than in the control group. The serum concentration of MCP-1 (pg/ml) was also significantly higher in the HD group than in the control group (457.3 ± 152.8 vs 246.2 ± 96.3 , $p < 0.01$; Table 1). LVH (high voltage with ST-T change) was more common in HD patients. There was no difference in smoking habits between the groups.

Carotid IMT

The average and maximal IMT values (mm) of the carotid artery in the HD group were significantly greater than those in the control group (0.89 ± 0.20 vs 0.63 ± 0.14 , $p < 0.01$; Fig 1).

Relationship Between IMT and Risk Factors for Atherosclerosis

In the HD group, univariate analysis revealed that the average IMT value correlated with age, serum concentration of MCP-1, smoking habits and LVH, but not with other parameters (Fig 2). Multiple linear regression analysis showed that LVH, serum concentration of MCP-1, age and the serum concentration of Apo A-1 to be independent factors influencing the average value of IMT (Table 2).

Immunohistochemical Analysis of Human Arterial Samples

Fig 3 shows that MCP-1 was constitutively expressed in both vascular endothelial cells and smooth muscle cells in the radial artery. When the expression of MCP-1 in the arterial tissue was graded semiquantitatively on a 4-point scale from 0 to 3, semiquantitative immunostaining demonstrated a significant positive correlation with the serum concentration of MCP-1 (Fig 4).

Discussion

The salient findings of this study were that the serum concentration of MCP-1, a potent chemoattractant, was one of the independent factors responsible for the acceleration of the progression of atherosclerosis in chronic HD patients.

Several studies have shown associations of risk factors and of changes in risk factor levels with carotid IMT²¹⁻²³ It has also been reported that the degree of stenosis of carotid arteries, determined by using B-mode or Doppler ultrasound, correlates with the incidence of coronary heart disease.^{24,25} Thus, the mean value of IMT has been used as an index of atherosclerosis in many epidemiological studies.²⁶ It is well known that chronic HD causes high cardiovascular and cerebrovascular mortality rates related to accelerated atherosclerosis.^{1,2} A previous study also demonstrated that the average IMT value in uremic patients was greater than that in age-matched controls.²⁷ Consistent with the results of the previous studies²¹⁻²³ we showed that the extent of IMT in the carotid artery significantly correlated with age, smoking habits and the existence of LVH. Furthermore, we showed that the serum MCP-1 concentration was one of the independent risk factors that cause

acceleration of the progression of atherosclerosis in chronic HD patients.

Over the past several years, circulating monocyte infiltration into the subendothelial space has been shown to be a characteristic feature of the early stage of atherosclerosis. Among the various cytokines and other factors that have chemotactic activity for monocytes, MCP-1 may be particularly important in the pathogenesis of atherosclerosis, especially for plaque formation.⁴⁻¹² It has also been reported that MCP-1 is important for smooth muscle cells in the functional switch from contractile to synthetic phenotype during the course of atherogenesis.²⁸ A recent study has suggested that MCP-1 increases serotonin-induced vascular smooth muscle cell proliferation.²⁹ Furthermore, MCP-1 has been implicated in the mechanism of vascular inflammation induced by long-term inhibition of nitric oxide synthesis¹² and restenosis after either vascular injury^{9,10} or percutaneous transluminal coronary angioplasty.³⁰ Thus, there is increasing evidence that MCP-1 is involved in many of the pathophysiologies associated with accelerated atherosclerosis. Because recent clinical studies have demonstrated that the plasma concentration of MCP-1 is elevated in patients with myocardial infarction,³¹ unstable angina³² and in-stent restenosis,³³ it is reasonable to conclude that the concentration of circulating MCP-1 reflects the clinical state of atherosclerosis. However, there have been few reports on the relationship between the severity of atherosclerosis and the serum concentration of MCP-1. Our data showed that the serum concentration of MCP-1 was one of the independent risk factors for acceleration of the progression of atherosclerosis. Our data also showed that the tissue expression of MCP-1 correlated with the serum concentration of MCP-1. Thus, the serum concentration of MCP-1 may be an important marker for the progression of atherosclerosis in HD patients.

It has been reported that endothelial cells and smooth muscle cells can secrete MCP-1 in response to several factors such as LDL,^{34,35} angiotensin II,³⁶ C-reactive protein³⁷ and tumor necrosis factor-³⁸ and that increased MCP-1 secretion from these cells leads to infiltration of monocytes into the arterial wall. In this study, we showed that MCP-1 was expressed in the endothelial cells and smooth muscle cells of the radial artery and that the level of arterial expression of MCP-1 correlated with the serum concentration. These results indicate that serum MCP-1 came from the arterial wall. Further studies that elucidate the cause of increased MCP-1 expression in HD patients are needed.

In conclusion, an increase in the concentration of MCP-1 may be an important factor in the progression of atherosclerosis in non-diabetic HD patients.

Acknowledgments

We thank Kaoru Kobayashi, Miyuki Fujiwara, Masayo Ohmori and the staff of Fukushima Clinic for their excellent technical assistance.

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