Monocyte Chemoattractant Protein-1 and Recurrent Cardiovascular Events in Patients with Stable Coronary Heart Disease

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Summary

Background: Monocyte chemoattractant protein-1 (MCP-1) participates in the recruitment of mononuclear cells to the vessel wall.

Hypothesis: The aim of the study was to evaluate the potential association between serum concentration of MCP-1 and risk of future cardiovascular events in patients with chronic coronary heart disease.

Methods: A nested case control design was used. Baseline serum samples were taken from patients with coronary heart disease who were enrolled in a secondary prevention study. The MCP-1 levels were measured in those patients who had recurrent cardiovascular events during follow-up ($n = 233$) and compared with levels in age- and gender-matched controls.

Results: There were no differences in serum MCP-1 levels between cases and controls. The relative odds of a recurrent cardiovascular event for each 1 standard deviation difference in MCP-1 serum concentration (160 pg/ml) was 1.19 (95% confidence interval, 0.95–1.45). No increase in the relative odds for recurrent cardiovascular events was observed per increasing tertiles of MCP-1 concentrations.

Conclusion: Elevated MCP-1 levels are not associated with long-term risk of cardiovascular events in patients with stable coronary disease.

Key words: inflammation, monocyte chemoattractant protein-1, coronary heart disease

Introduction

Mononuclear cells play an essential role in atherosclerosis.^{1–3} Monocyte recruitment to the vessel wall is regulated by chemokines, particularly monocyte chemoattractant protein-1 (MCP-1),⁴ and by cell surface adhesion molecules, which attach the monocytes to the endothelium and mediate their migration into the vessel wall. In cross-sectional studies, the serum concentration of MCP-1 was increased in patients with acute coronary syndromes,^{5–8} patients after percutaneous coronary interventions who developed restenosis,^{9,10} and patients after ischemic stroke.¹¹ In one study of patients with acute coronary syndromes, elevated MCP-1 levels were associated with an increased risk of myocardial infarction (MI) and death over a follow-up period of 10 months.¹²

The role of MCP-1 in predicting future long-term risk of cardiovascular events in patients with stable atherosclerotic disease has not been tested so far.

Methods

Study Sample

The sample for the present study was selected from the Bezafibrate Infarction Prevention (BIP) study, a clinical trial that evaluated the efficacy of bezafibrate in patients ($n = 3,090$) with coronary heart disease.¹³ Mean length of follow-up was 6.2 years (range 4.7–7.6 years). Coronary heart disease (CHD)

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was defined as a history of MI for >6 months but <5 years before enrollment into the study, or a history of angina pectoris confirmed by coronary angiography, nuclear scintigraphy, or positive exercise test. Primary endpoints of the study were fatal MI, nonfatal MI, or sudden death.¹³ Data on the occurrence of new cerebrovascular events were obtained during routine evaluations. Clinical data related to the new cerebrovascular event, results of brain computed tomography (CT) scan, and other available ancillary tests for assessment of stroke classification were recorded. Stroke was differentiated by the results of the CT scan into ischemic or hemorrhagic type.

For the present study, we used a prospective, nested, case-control design. The study group consisted of 233 patients in the BIP study in whom recurrent MI, sudden cardiac death, or ischemic stroke developed during follow-up; in all cases, an adequate blood sample was provided before randomization to the study. All patients who had had an ischemic stroke ($n = 123$) were included in addition to a random sample of patients with one of the coronary endpoints ($n = 122$) (12 patients had both an ischemic stroke and a coronary event during follow-up). The control group consisted of 233 patients with CHD who remained free of any recurrent coronary events or stroke throughout the follow-up period and were alive at the end of study. The control patients were pair matched to cases according to age, gender, and allocation to bezafibrate or placebo. The total study sample included case-control pairs matched for age, gender, and bezafibrate versus placebo allocation in the BIP study.

Laboratory Procedures

Blood samples for measurement of serum lipids and fibrinogen, blood chemistry, and other laboratory tests were collected at randomization for the BIP study. For the purpose of the present study, serum samples taken at baseline from each study participant and stored at -70°C were assayed for MCP-1 using a commercial enzyme-linked immunosorbent assay (ELISA) kit (R&D Diagnostics, Minneapolis, Minn., USA). All samples were processed in duplicate. A single laboratory technician blinded to the case/control status of the patients performed all the tests.

Statistical Analysis

Statistical analyses were done with the SAS statistical software (SAS, Cary, N.C., USA). The paired t -test was used to assess the significance of differences in means of continuous variables between the matched pairs, and McNemar's test for paired samples was used to assess differences in rates. Logistic regression analyses conditioned on the matching variables (age, gender, and bezafibrate allocation) were used to estimate the relative odds (and 95% confidence intervals) of experiencing all cardiovascular events (ischemic stroke and coronary endpoints of MI or sudden cardiac death), or only ischemic stroke or coronary events, according to tertiles of serum MCP-1 levels in the control group. We adjusted for the following confounding variables: smoking, serum lipoprotein concen-

trations at baseline, diabetes mellitus, hypertension, and history of MI. In separate models, we added plasma fibrinogen and soluble intercellular adhesion molecule-1 (ICAM-1) levels to the multivariate models. Similar models were used to assess the odds of recurrent cardiovascular events for each 1 standard deviation (SD) increase in serum MCP-1 level (160 ng/ml).

Results

Baseline Characteristics (Table 1)

Patients and controls were well matched in terms of age, gender, and treatment with bezafibrate. Patients had a higher rate of diabetes mellitus, smoking, previous MI, higher systolic blood pressure, and higher serum levels of ICAM-1 and fibrinogen.

Monocyte Chemoattractant Protein-1 Concentration

The mean serum concentration of MCP-1 was not significantly different between patients (328 pg/ml, SD = 170), and controls (309 pg/ml, SD = 160) ($p = 0.14$).

Monocyte Chemoattractant Protein-1 and Risk of Recurrent Cardiovascular Events

The relative odds of recurrent cardiovascular events over 6 years of follow-up, associated with an increase in 1 SD (160 ng/ml) in serum MCP-1 concentration, was 1.19 ($p = 0.13$, Table II). The risk was similar when analyzed separately for coronary and cerebrovascular events (Table II).

Compared with the lowest tertile of serum MCP-1 concentration, there was no increase in the risk of all future cardiovascular events, or of ischemic stroke or coronary events, associated with the second and third tertiles (Table III). Furthermore, there was no graded elevation in risk with increasing MCP-1 concentrations.

Discussion

Main Findings

In the present study of patients with chronic stable CHD, an elevation in MCP-1 concentration was not associated with an increase in the future risk of recurrent cardiovascular events over a mean follow-up time of 6 years.

Monocyte Chemoattractant Protein-1 Concentration

Serum concentrations of MCP-1 in the present study were similar to those reported in previous studies of patients with stable CHD,¹⁰ but higher than the reported concentrations in patients with acute coronary syndromes.^{12, 14} These differences probably reflect differences in the study populations, but they may also be related to regional differences and differ-

TABLE I Baseline characteristics of study participants

	Controls (n = 233)	Cases (n = 233)	p Value
Age (years)	61.0 ± 6.2	61.2 ± 6.4	—
Men (%)	220 (94)	220 (94)	—
Bezafibrate treatment (%)	119 (51)	119 (51)	—
Diabetes mellitus (%)	18 (8)	33 (15)	0.03
Hypertension (%)	78 (33)	81 (36)	0.48
Current smoking (%)	17 (7)	38 (16)	0.002
Previous myocardial infarction (%)	179 (77)	195 (84)	0.05
Angina pectoris (%)	139 (60)	143 (62)	0.54
NYHA class ≥ 2 (%)	58 (25)	70 (30)	0.22
Diastolic blood pressure (mmHg)	81.1 ± 8.9	81.4 ± 9.4	0.72
Systolic blood pressure (mmHg)	133.3 ± 16.4	136.7 ± 18.6	0.04
Body mass index (kg/m ²)	26.4 ± 3.2	26.9 ± 3.3	0.08
Total cholesterol (mg/dl)	212.8 ± 16.8	211.8 ± 17.3	0.54
HDL cholesterol (mg/dl)	33.8 ± 5.7	33.2 ± 5.7	0.23
LDL cholesterol (mg/dl)	149.7 ± 16.6	148.7 ± 16.3	0.60
Triglycerides (mg/dl)	145.6 ± 51.3	148.7 ± 16.3	0.35
Fibrinogen (mg/dl)	347.6 ± 67.0	367.9 ± 71.1	0.004
Soluble ICAM-1 (ng/ml)	346.3 ± 96.9	376.2 ± 109.2	0.002

Continuous variables are expressed as mean ± standard deviation, categorical variables as number (%).

Abbreviations: NYHA = New York Heart Association, HDL = high-density lipoprotein, LDL = low-density lipoprotein, ICAM-1 = intercellular adhesion molecule 1.

ences in medical treatment in studies conducted years apart. Both of these factors may also affect serum MCP-1 levels.^{15–17}

Monocyte Chemoattractant Protein-1 and Risk of Recurrent Coronary Events

In our study of patients with chronic stable CHD, we found no association between an increase in MCP-1 concentration and future risk of coronary events (MI or sudden cardiac death). These results stand in contrast with a recent study in patients with acute coronary syndrome,¹² wherein MCP-1 levels > 196 pg/ml (75th percentile of MCP-1 level in that study) were associated with an increased risk (relative risk: 1.42) of MI and death over a 10-month follow-up period, compared with concentrations < 196 pg/ml. This discrepancy is of interest. In patients with acute coronary events, the increase in MCP-1 may occur secondary to the ischemic insult as a con-

sequence of the role of MCP-1 as an acute phase reactant, or it may be pathophysiologically related to the acute atherothrombotic event. Increased MCP-1¹² as well as increased levels of B-type natriuretic peptide,^{18,19} C-reactive protein,^{18,20} cardiac troponin,¹⁸ and soluble CD40 ligand²¹ have been shown to be independent predictors of future risk in patients with acute coronary syndromes. C-reactive protein was shown to predict both long-term and short-term risk in both stable and unstable patients.²² In contrast to studies in patients with acute coronary

TABLE II Risk of recurrent cardiovascular events associated with each increase of 1 standard deviation (160 pg/ml) increase in MCP-1 serum concentration

	Odds ratio (95% CI)	p Value
All cardiovascular events	1.17 (0.95–1.45)	0.14
Multivariate adjusted	1.19 (0.95–1.49)	0.13
Ischemic stroke	1.24 (0.92–1.69)	0.17
Multivariate adjusted	1.32 (0.94–1.89)	0.11
Coronary events	1.13 (0.87–1.50)	0.36
Multivariate adjusted	1.16 (0.88–1.56)	0.30

TABLE III Crude and multivariate adjusted relative odds of incident. Recurrent cardiovascular events according to MCP-1 concentrations, divided by tertiles

	II	III
All cardiovascular events		
Model A	1.23 (0.76–1.99)	1.33 (0.80–2.23)
Model B	1.20 (0.72–2.02)	1.38 (0.80–2.34)
Ischemic stroke		
Model A	1.11 (0.58–2.16)	1.33 (0.66–2.71)
Model B	1.13 (0.53–2.41)	1.43 (0.65–3.22)
Coronary events		
Model A	1.43 (0.73–2.88)	1.34 (0.65–2.78)
Model B	1.42 (0.71–2.87)	1.34 (0.65–2.84)

Model A matched for age, gender, and BIP study medication (bezafibrate vs. placebo). Model B matched for age, gender, and BIP study medication and adjusted for diabetes mellitus, current smoking, hypertension, and previous myocardial infarction.

syndrome, in our study patients with stable chronic CHD MCP-1 was not helpful for risk prediction of recurrent cardiovascular events. We found no association with either early or late events during the follow-up. This finding may have implications for the future use of MCP-1 as a marker of risk in different CHD subgroups (patients with acute events vs. stable patients).

Monocyte chemoattractant protein-1 plays a central role in the activation of monocytes and endothelial cells and the production of tissue factor.^{23, 24} Furthermore, MCP-1 levels increase rapidly during acute coronary events, reaching a peak at 24 h and decreasing thereafter.⁶ Thus, the levels measured during the acute event (but not those measured during chronic stages) may reflect the stage of plaque vulnerability. These findings require confirmation in further studies.

Monocyte Chemoattractant Protein-1 and Ischemic Stroke

There is an increase in the production and expression of MCP-1 in brain tissue and cerebrospinal fluid after ischemic stroke.^{11, 25} Moreover, MCP-1 inhibition in an experimental model of ischemic stroke reduced brain infarct size.²⁶ Ours is the first prospective study to evaluate the association between MCP-1 concentration and long-term risk of ischemic stroke. We found that elevated levels of MCP-1 were not associated with higher odds of future ischemic stroke.

Limitations

Several aspects of the methodology of the present study may have affected the results and the interpretation of the findings. First, we used only a single baseline measure of MCP-1; therefore, we could not evaluate the significance of changes in concentration over time on risk of subsequent cardiovascular events. Second, the samples were kept frozen for 10 years, which could raise concerns about protein stability over time. However, the serum concentrations of MCP-1 in the present study were similar (and even higher) than those reported in previous studies, and there was no reason to believe that such protein degradation was different between patients and controls. Therefore, the misclassification bias is unlikely. Our case-control design is a potential limitation, which might affect our results. The choice of including patients with different clinical manifestation of coronary disease (previous MI, previous unstable angina, stable angina) may influence the results as well. It is possible that the natural history of the disease is not the same in the different subgroups, and thus differently affected by MCP-1. Despite these limitations, the study provides information into the use of MCP-1 for prediction of long-term risk of both coronary and cerebrovascular events in patients with established atherosclerotic heart disease.

Conclusion

In patients with chronic coronary heart disease in stable state, elevated MCP-1 levels were not associated with long-term risk of recurrent cardiovascular events.

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