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SHORT COMMUNICATION

Serum Levels of the MCP-1 Chemokine in Patients With Ischemic Stroke and Myocardial Infarction

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Chemokine-driven migration of inflammatory cells has been implicated in pathogenesis of atherosclerosis-associated conditions such as ischemic stroke and myocardial infarction. In this study, a candidate chemokine, monocyte chemoattractant protein (MCP)-1, was investigated in patients with both aforementioned manifestations of atherosclerotic inflammation. MCP-1 levels in serum were determined by ELISA in 40 healthy, control subjects (C), 40 patients with ischemic stroke (IS), and in 64 patients with myocardial infarction (MI). Statistical analysis utilised Mann-Whitney test, Fisher's exact test, and Spearman's rank correlation ($P < .05$). In comparison to control subjects (C; median/interquartile range: 239/126 pg/mL), MCP-1 serum levels were increased in both investigated patient cohorts (IS: 384/370, $P < .001$; MI: 360/200, $P < .002$). There was a substantial variability of MCP-1 serum levels, especially in the IS group. No relationship was observed between chemokine levels and atherosclerosis risk factors (hypertension, diabetes, smoking, and alcohol consumption), and MCP-1 was also not related to age or gender. Elevation of MCP-1 in circulation of patients with atherosclerosis-associated complications implicates this CC chemokine ligand (CCL)2 in inflammatory processes, which contribute to pathogenesis of myocardial infarction and ischemic stroke. Further investigations, including patient stratification, are however necessary to evaluate if MCP-1 can be utilised for clinical management of patients with these diseases.

INTRODUCTION

Ischemic stroke (IS) and myocardial infarction (MI) are atherosclerosis-associated complications that are the leading causes of mortality and disability all over the world [1]. IS and MI share common features, but at the same time there are differences in many risk factors, at least quantitative, if not qualitative. Compared with myocardial infarction, stroke patients are at least 10 years older, the incidence in middle-aged men compared with women is smaller, and increasing blood pressure is more strongly associated with stroke, whereas increasing plasma cholesterol is less strongly associated with stroke [2].

Recent studies showed that aberrant immune response evolves during both IS and MI that is deleterious and contributes to cell death after the insults [3, 4, 5]. Both pathologies are characterized by expression of proinflammatory cytokines, adhesion molecules, and importantly, chemokines that orchestrate the infiltration of leukocytes into infarct area, expression of damaging agents and activation of complement system (for review see [6, 7]).

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Chemokines are low molecular weight polypeptides that, besides other functions, exert potent chemotactic and activating effects on specific leukocyte populations [8]. Recently, there has been growing interest in applications of chemokine biology into clinic, including atherosclerosis [9]. CC chemokine ligand (CCL)2, more widely known as monocyte chemoattractant protein-1 (MCP-1), is a potent mononuclear cell attractant [10]. It has been previously implicated in the development of both IS and MI. For example, in animal experiments, high levels of MCP-1 mRNA have been found in the brain 6 hours after cerebral ischemia. The maximal expression of this chemokine was observed between 12 hours and 2 days [11, 12]. MCP-1 mRNA expression was upregulated in infarcted area after MI in the same manner like in IS [13, 14].

Most of the data about cytokine and chemokine involvement in pathogenesis of IS and MI has been derived from animal studies. Nowadays, more attention is being paid to studies utilising human subjects. In this context, blood becomes an invaluable target for such studies, due to its relative availability. Recently, it has been shown that the levels of several cytokines are elevated in the blood of patients with IS and MI [15, 16, 17, 18]. However, data on MCP-1 are scarce, especially in strokes. We, therefore, performed the present study to provide additional data on involvement of MCP-1 in MI, and namely in IS.

Doing so, we wished to complement existing studies targeted at evaluation of chemokine inflammatory response in pathogenesis of these two atherosclerosis-associated complications.

SUBJECTS AND METHODS

Study population

Forty patients with IS (17 female, age [years, mean ± SD]: 67.5 ±10.5) and 64 patients with MI (8 female, age 55 ±8.8) were entered in the study. Patients with concurrent diseases or conditions interfering with the aim of the study, such as infections, malignancies and those on immunosuppressive drugs were excluded from study.

Diagnosis of IS was based on clinical history and neurological examination and was confirmed by brain CT. On CT examination, all stroke patients presented anatomically relevant hypodense areas in subcortical parts of cerebral hemispheres.

Diagnosis of MI was determined from the presence of > 30 minutes of continuous chest pain, ST-segment elevation > 2.0 mm on at least 2 contiguous ECG leads, and more than a 3-fold increase in serum creatine kinase levels.

Regarding risk factors, 44% of MI and 50% of IS patients had hypertension, 18% of MI and 9% of IS patients had diabetes mellitus, 54% of MI and 18% of IS patients reported themselves as current smokers, and 8% of MI and 5% of IS patients reported themselves as alcohol consumers. In addition 7% of IS patients previously had ischemic heart disease.

Forty healthy subjects (7 female, age 52 ± 3) free of clinical signs of infection, other systemic diseases, and negative family history of IS or MI served as control population.

Venous blood samples were obtained from IS and MI patients within 24 hours after disease onset and once from controls. Serum samples were stored at −70° C until further analysis.

IS patients were recruited at Erebouni Medical Center, Yerevan, Armenia; MI patients were diagnosed at the Faculty Hospital Olomouc, Czech Republic. All subjects gave their informed consent to be involved in this study. Local Ethical Committees approved the study.

Determination of MCP-1 protein

MCP-1 protein levels in serum obtained from study participants were measured using a solid-phase sandwich enzyme-linked immunosorbent assay (MCP-1 Quantikine ELISA kit, R&D systems, Abingdon, UK). Briefly, 100 μL of duplicated samples or standards (reeombinant human MCP-1) were incubated (2 hours at room temperature) in the wells precoated with primary antihuman MCP-1 antibody. After incubation, wells were washed three times and horseradish peroxidase-conjugated polyclonal antibodies against MCP-1 were added (for 2 hours at room temperature). Finally, tetramethylbenzidine substrate solution was applied for 30 minutes and, after stopping the reactions by 2 M sulfuric acid, the absorbance was measured at 450 nm (with correction at 540 nm). The data were evaluated with KIM-E software (USOL, Prague, Czech Republic); the detection limit of the MCP-1 assay was 5.0 pg/mL.

Statistical analysis

SPSS 11 (SPSS Inc, Chicago, Ill) software was used to calculate basic statistical parameters and to perform the Mann-Whitney U-test to test for differences in chemokine protein levels between the MI, IS, and control groups as well as for further subanalysis. Fisher's exact test was used to compare differences in qualitative parameters between IS and MI groups. Spearman's rank correlation was used to evaluate relationship between age and MCP-1 levels. Data on MCP-1 levels was presented as median [interquartile range (IQR)]. $P < .05$ was considered statistically significant.

RESULTS

In this study, MCP-1 immunoreactive protein was determined in peripheral blood of patients with MI and IS.

Prior to chemokine analysis, the differences in risk factors among our study groups were estimated. MI patients were in average 12 years younger ($P < .0001$). Also, there were differences in male/female ratio (IS, 42% versus MI, 12%, $P < .0001$) and in number of smokers (MI, 54% versus IS, 18%, $P < .0001$). Number of sufferers from diabetes was 2-fold higher in MI group compared with IS, but this difference did not reach significance (MI, 18% versus IS, 9%, $P > .05$). The study groups did not differ with regard to hypertension and alcohol consumption.

In comparison to control subjects (median [IQR]: 239 [126] pg/mL), the levels of MCP-1 protein were elevated in patients from both study groups (Figure 1). This increase was more apparent in the IS group (384 [370] pg/mL), for which also a greater degree of interindividual variability was observed than among patients with MI (360 [200] pg/mL). While only 10/40 (25%) of control subjects had MCP-1 level higher than 314 pg/ml (75th percentile of the control group), in patients with IS this proportion reached 68% (27 /40) and in MI group it was 61% (39/64). The differences between both investigated patient groups and controls, therefore, reached high significance (IS versus C, $P < .001$; MI versus C, $P < .002$), while there was no difference between patients' groups themselves (IS versus MI, $P > .05$).

Further, we investigated whether MCP-1 protein is influenced by risk factors of MI and IS. Serum chemokine levels were compared between patients with and without diabetes, hypertension; smokers and nonsmokers; and also groups divided based on alcohol consumption. Data analysis showed that none of the above risk factors had an influence at circulating MCP-1 levels in patients with MI and/or IS (Table 1). Furthermore no gender effect MCP-1

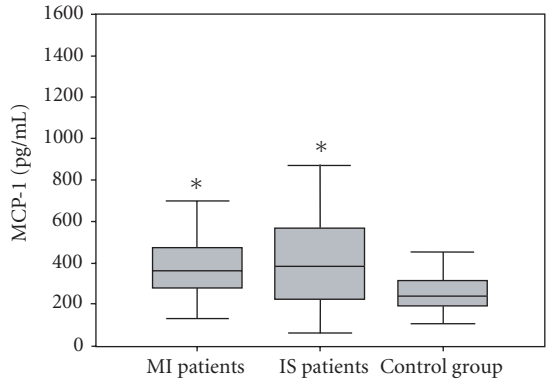

Figure 1. Monocyte chemoattractant protein (MCP)-1 levels (pg/mL) in serum of patients with myocardial infarction (MI, $n = 64$), ischemic stroke (IS, $n = 40$), and healthy, control subjects ($n = 40$). The data are expressed as whisker box plots; the box represents the 25–75th percentiles, the median is indicated by a bar across the box, the whiskers on each box represent the 10–90th percentiles. * $P < .002$ when comparing with the control group.

levels was apparent in both studied groups as well as in control group (IS males versus females: 417 [400] versus 253 [296], $P > .05$; MI males versus females: 343 [203] versus 397 [300], $P > .05$; C males versus females: 244 [125] versus 234 [93], $P > .05$), and there was no correlation between age and the chemokine levels (IS: $r_s = -0.009$, $P > .05$, MI: $r_s = -0.06$, $P > .05$, C: $r_s = 0.168$, $P > .05$).

DISCUSSION

This study aimed at the evaluation of the MCP-1 chemokine in the circulation of patients with myocardial infarction and ischemic stroke. These two atherosclerosis-associated complications share many similarities, but at the same time they have at least quantitative if not qualitative differences in their risk factors [2]. This trend is preserved in our patients' characteristics: our IS patients were older and the different incidence in males and females is less pronounced in IS compared to MI. Also, IS patients were more prone to hypertension than MI patients. Presence of these features, therefore, confirms that our patients' groups were properly created and reflect the actual situation with IS and MI phenotypes. This fact is important for interpretation of chemokine data.

Our data showed almost 2-fold increase of serum MCP-1 levels in patients with MI and IS compared with control group of healthy subjects. We also found that serum MCP-1 levels did not differ between IS and MI patients. Finally, the levels of this chemokine were not influenced by known risk factors for these diseases such as hypertension, diabetes, smoking, or alcohol consumption.

In myocardial infarction, elevation of circulating MCP-1 has been already reported [19, 20, 21] and, therefore, our findings of increased MCP-1 in our patients with MI are of a confirmatory nature. However, infrequent reports regarding this chemokine in ischemic stroke are contradictory [22, 23]. Losy and Zaremba [22] reported that blood MCP-1 levels were not increased in his group of 23 patients with IS. By contrast and in line with our results, Reynolds et al [23] demonstrated elevation of the MCP-1 chemokine in the blood from a big group of more than 200 stroke patients. This discrepancy may occur due to low number of subjects included in the study by Losy and Zaremba [22] and possibly also by marked variability of circulating MCP-1 levels.

There are at least two mechanisms by which the observed upregulation of MCP-1 protein levels in circulation of patients with IS and MI can be achieved. Firstly, it may be the direct consequence of atherosclerosis development. The main sources of MCP-1 are endothelia and macrophage like cells [24], which are known to play a significant role in atherosclerosis development and plaque formation. In vitro studies have revealed that endothelial cells are able to produce MCP-1 in response to LDL an important atherosclerosis triggering factor [25, 26].

Second, increased MCP-1 levels can mirror the development of inflammatory response in heart and brain. Numerous studies on animal models of stroke showed that MCP-1 and MIP-1beta are two main chemokines that orchestrate infiltration of blood-derived monocytes and lymphocytes into ischemic area. The upregulation of MCP-1 mRNA levels is detected in both permanent and transient models of focal ischemia [11, 12]. Moreover study by Hughes et al [27] demonstrated that MCP-1 deficiency is protective in a murine stroke model. The same processes are taking place in heart during MI [13, 14]. Furthermore, it has been demonstrated that mice lacking CCR2, the primary receptor for MCP-1, showed significant impairment of monocyte infiltration, reduction of TNF-alpha, and matrix metalloproteinases expression in infarcted area in experimental MI [28].

Our data showed that elevation of MCP-1 in the blood is a common feature for MI and IS. This may suggest that in atherosclerosis-associated complications, inflammatory response, share some similarities and are not organ-specific. This interpretation fits in with other current reports [29, 30, 31], including our recent observation of approximately 2-fold increase of MCP-1 in the blood of patients with peripheral arterial disease [32].

Unexpectedly, in our study none of four investigated common risk factors for MI and IS did not influence serum MCP-1 levels. In number of previous studies in patients with myocardial infarction, the association of MCP-1 levels with several risk factors such as hypertension [33] and diabetes [20] has been reported. However, these were large-population-based studies where even slight differences may be identified. For example, in the study by de Lemos et al [20] more than 2000 subjects were enrolled and thus the authors were able to identify a rather minor difference of 6% as significant. It is, therefore, possible that in our relatively small patient population of 64 MI patients, differences of similar magnitude may not be visible. Regarding patients with ischemic stroke, there have been

Table 1. Differences in MCP-1 levels (pg/mL) in the serum of patients with IS and MI reflecting the presence (+) or absence (−) of known risk factors.

MI patients		IS patients	
Hypertension +	Hypertension −	Hypertension +	Hypertension −
365/188	379/256	384/384	394/302
Diabetes +	Diabetes −	Diabetes +	Diabetes −
380/262	371/199	396/283	380/244
Smoking +	Smoking −	Smoking +	Smoking −
353/203	400/318	396/303	380/213
Alcohol +	Alcohol −	Alcohol +	Alcohol −
576/346	365/211	387/237	302/223

no studies on influence of risk factors on MCP-1 levels. We, therefore, plan to further explore this phenomenon so that it can be assessed if monitoring MCP-1 serum levels can be included into the algorithm of management of IS patients as an independent marker.

In conclusion, our data expand previous observations showing that MCP-1 plays an important role in inflammatory response developing during myocardial infarction and ischemic stroke. Further work is, however, necessary to define more precisely the relationship between MCP-1 and clinical course of investigated diseases. Emphasis should be given to patients' stratification in order to ascertain clinical utilisation of MCP-1 measurements, namely in ischemic stroke.

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