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Original article:

Strenuous physical exercise induces monocyte chemoattractant protein-1 release in patients with coronary artery disease

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ABSTRACT

Infiltration of the arterial vessel wall with monocytes is one of the initial inflammatory events. Monocyte chemoattractant protein-1 (MCP-1) is the key chemokine for the recruitment of monocytes to the atherosclerotic lesion. So far, it is unknown, if strenuous exercise enhances or reduces the release of MCP-1, the key initiator of pro-atherosclerotic inflammatory events in patients at risk for atherosclerotic diseases. 15 Patients with at least three coronary risk factors (CRF) like smoking, hypertension, diabetes, hypercholesterolemia and overweight and 17 corresponding healthy controls were tested with bicycle ergometry. Additionally, 8 patients with coronary artery disease (CAD) were investigated. Before and 10 minutes after maximal exercise, venous blood was taken and MCP-1 serum levels were analyzed. Furthermore, we measured monocyte CD11b expression by flow cytometry. Independently of CRF, the MCP-1 serum level was significantly increased after exercise. In control subjects the MCP-1 serum level rose from 71 pg/ml to 94 pg/ml ($p \leq 0.05$). In patients with CRF the MCP-1 serum level went up from 154 pg/ml to 224 pg/ml ($p \leq 0.05$). Patients with coronary artery disease had elevated MCP-1 serum levels before and after exercise, too, even if they did not have CRF (143 vs. 174 pg/ml; $p \leq 0.05$). The monocyte activation parameter CD11b showed a significant raise after physical exercise (relative fluorescence intensity 31 vs. 44; $p \leq 0.05$). These data indicate, that MCP-1 serum levels are elevated after physical exercise especially in patients at risk for coronary artery disease. These effects may in part result from an increased monocyte activation following strenuous physical exercise.

Keywords: MCP-1, physical exercise, inflammation, monocyte, coronary risk factors

INTRODUCTION

Monocyte chemoattractant protein-1 (MCP-1) is a potent specific chemoattractant for monocytes without chemoattractant activity on neutrophils (Rollins 1997 for review). MCP-1 is shown to be secreted by cytokine-activated endothelial cells and vascular smooth muscle cells (Rollins et al. 1991; Poon et al. 1996) in vitro. There is evidence, that not only cytokine-activation but also interaction of activated platelets with endothelial cells or monocytes can induce MCP-1 secretion. (Weyrich et al. 1995).

Monocytes migrate into the intima due to a MCP-1 concentration gradient that is formed by endothelial and monocyte activation. Once inside the intima, the monocyte amplifies its signal by synthesizing and

secreting its own MCP-1 (Rollins et al. 1995). MCP-1 was detected in macrophage-rich areas of rabbit and human atherosclerotic plaques (Ylä-Herttuala et al. 1991). A MCP-1 antibody inhibited transmigration of monocytes into the subspatial space. The ability of minimally modified LDL to activate MCP-1 production and secretion in cultured endothelial cells in vitro (Cushing et al. 1990) and in a rabbit model in vivo (Ylä-Herttuala et al. 1991) emphasizes its important role in atherogenesis.

Several publications already investigated the effect of short term physical exercise on different cytokines and leukocyte surface molecules (Kühlwein et al. 2001; Hu et al. 2004; Rhind et al. 2001; Meksawan et al. 2004), but none of these investigated MCP-1 as the chemokine that links monocyte activation directly to vascular inflammation in patients with atherosclerotic vessel disease.

METHODS

Blood was drawn from the antecubital vein from 15 patients with CRF, from 8 patients with angiographically proven coronary artery disease and from 17 healthy age-matched volunteers (co) [mean age (years): CAD: 64,8 ±5,7; co: 59 ± 4,7; ±SEM, n.s.] before and after a maximal physical exercise testing by bicycle ergometry. In the patient group, the exercise test was terminated at the onset of angina or ST-segment depression. Cardiovascular risk factors like hypertension, hypercholesterolemia, diabetes mellitus, positive family history and nicotine abuse were evaluated in all patients and control subjects. For the diagnosis of obesity, the body-mass-index was calculated. Patients with a body mass index exceeding 25 were defined as obese. The medical history of each participant was evaluated. Routine blood tests were performed for creatinine, liver parameters, c-reactive protein, low-density lipoprotein cholesterol, hemoglobin 1C, and lipoprotein (a). Participants with elevated levels of creatinine, liver parameters and infectious parameters were excluded from the study. Serum levels of MCP-1 were measured with a commercially available ELISA-system (R&D Systems, Wiesbaden, Germany). In 4 patients, serum MCP-1 was determined 4 times the day at 8 am, noon, 4 p.m. and 8 p.m. to test, if MCP-1 expression is stable throughout the day.

The expression of the adhesion molecule L-selectin (CD62L) and CD11b were measured by a flow cytometer (FACScan, Becton-Dickinson, San Jose, CA, USA) by indirect immunofluorescence using corresponding murine monoclonal antibodies against L-selectin (Biodesign), CD11b and a FITC-labelled goat anti-mouse IgG antibody (Serotec, Immunotech). Mean fluorescence intensity was taken from the cells gated in the monocyte window. The non-specific binding was measured with the isotype control Ox 6, an IgG antibody against rat MHC class II-Antigen (a kind gift of Dr. J. Reske, Institute of Immunology, Mainz, FRG), which is not expressed on monocytes. Non-specific binding was <1% throughout all experiments. The labeling was performed as follows: 42 or 40µl blood was incubated with 8 or 10 µl of the CD11b or CD62L antibody, respectively, at 4°C for 45 minutes. 2 ml of FACS-Lysis solution (Becton-Dickenson) were added and incubated another 20 minutes at 4°C. The samples were centrifuged at 240 x g for 5 minutes and the pellet was fixed in 0.5% paraformaldehyde over 10 minutes at 4°C. The samples were centrifuged again and resuspended in FACS-buffer (Becton Dickenson) with 0.1% sodium azide.

Data are given as mean ± SEM. Student's t-test for unpaired data or ANOVA was applied where appropriate. A p-value below 0.05 was considered significant.

RESULTS AND DISCUSSION

Monocyte chemoattractant protein-1 (MCP-1) is a secreted chemokine that exhibits powerful monocyte chemoattractant properties even at an extremely low

picomolar concentration range. MCP-1 mRNA and protein was detected in rabbit and human atherosclerotic lesions (Ylä-Herttuala et al. 1991; Nelken et al. 1991). So far, the role of circulating MCP-1 and its relationship to human atherosclerotic diseases is not well-defined. So far, there do not exist many data about the MCP-1 serum level in a healthy population. However, some studies investigated patients with cardiovascular diseases, but the data are not consistent so far. One study demonstrated that circulating levels of MCP-1 and tissue factor are simultaneously increased in patients with acute coronary syndromes. Patients with stable exertional angina did not show increased MCP-1 serum levels (Nishiyama et al. 1998). Aging as a risk factor for atherosclerotic diseases was already investigated before (Inadera et al. 1999). The authors already stated, that this increase in MCP-1 serum levels with age might reflect the existence of atherosclerosis. However, they did not find increased MCP-1 levels in patients with stroke or coronary artery disease. One possible explanation is, that the authors did not differentiate healthy subjects from subjects with multiple coronary risk factors.

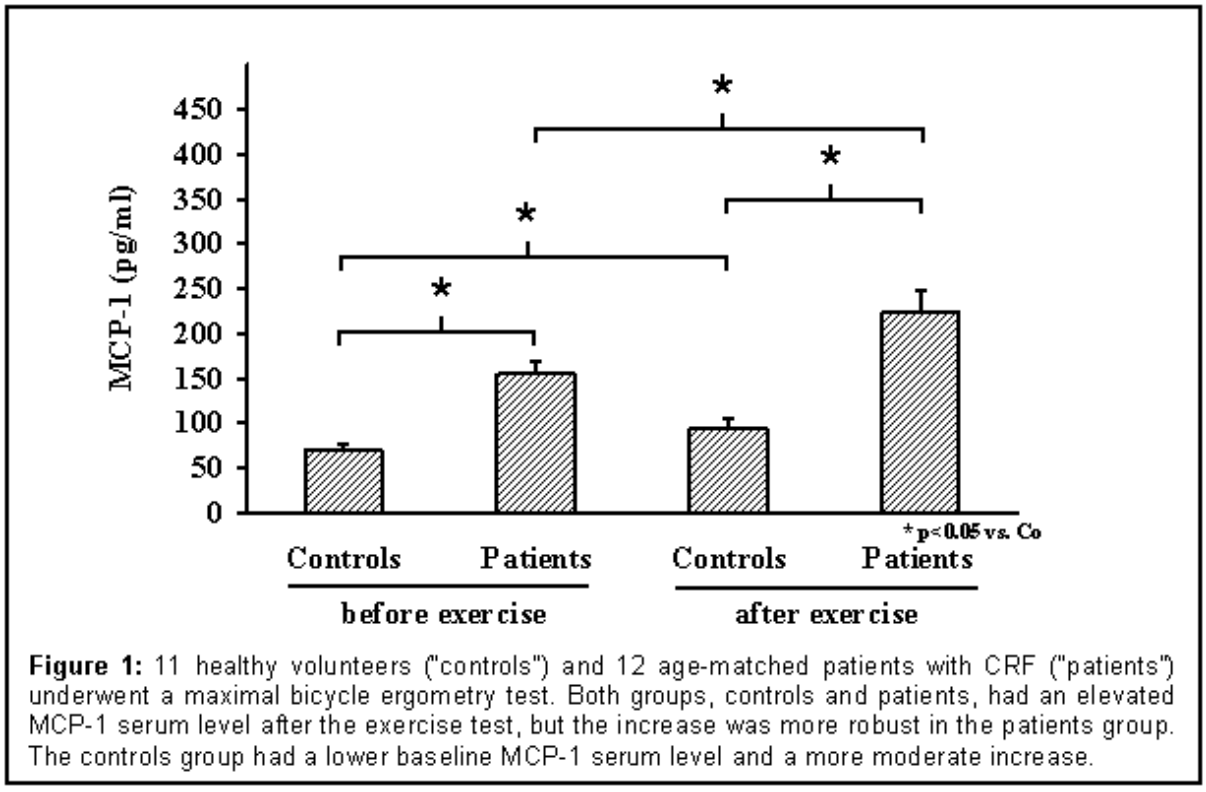

Figure 1: 11 healthy volunteers ("controls") and 12 age-matched patients with CRF ("patients") underwent a maximal bicycle ergometry test. Both groups, controls and patients, had an elevated MCP-1 serum level after the exercise test, but the increase was more robust in the patients group. The controls group had a lower baseline MCP-1 serum level and a more moderate increase.

Although not subject to this study, our data imply elevated MCP-1 serum levels in patients with atherosclerotic diseases or multiple risk factors, but this issue was investigated in detail in a further study of our group (Lindemann et al., manuscript submitted 2004). In healthy control patients we found a MCP-1 Serum level of 71pg/ml. In patients with CRF the MCP-serum level was 154pg/ml at baseline. After physical exercise the MCP-1 level increased to 94pg/ml in the healthy controls compared to 224pg/ml in the CRF-patients group (Figure 1). There are several reasons why the MCP-1 levels rise after exercise: Physical exercise increases shear stress. Shear stress activates endothelium and monocytes, both of which may release MCP-1. In both groups, the healthy controls and the patients with CRF, the MCP-1 serum level increase after physical exercise, but the increase in the patients group is much more pronounced. In patients with atherosclerotic diseases, the elasticity of the vessels is reduced. This may increase the shear stress and enhance endothelial and monocyte activation. Patients

with angiographically proven CAD had a MCP-1 baseline level of 143pg/ml. After physical exercise, the MCP-1 level raised to 174pg/ml. (Figure 2). We do not know the exact reason, why the patients with a manifest CAD do not increase to the same MCP-1 levels after exercise like the patients with CRF do.

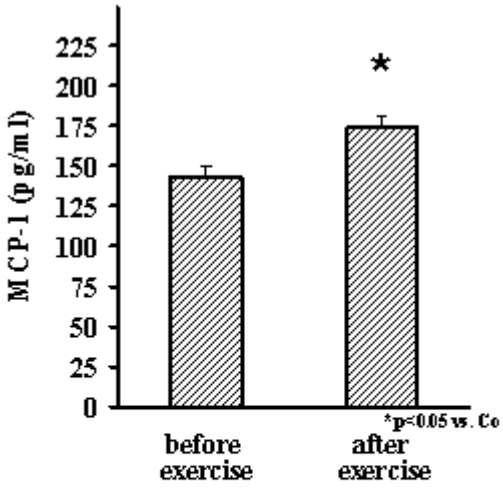

Figure 2: 12 patients with CAD were tested by submaximal bicycle ergometry. After the exercise test, the serum MCP-1 level was significantly increased, although the increase was less robust compared to the patients with solely risk factors for CAD.

One explanation is that patients with CAD receive ß-blockers that limit maximal heart rate during exercise. These patients also receive aspirin that reduces platelet-monocyte interaction. in an earlier publication, we even showed, that patients with CAD had a reduced platelet activity after exercise, most likely due to an increased inhibitory mediator release (Lindemann et al. 1999). There are a few studies that investigated the effects of strenuous exercise on leukocyte and platelet activation and on inflammatory mediators. Consistent with our data is a Japanese paper that states a systemic inflammatory response to short term exercise in a healthy population. Besides other cytokines, MCP-1 serum levels augment after strenuous exercise (Suzuki et al. 2002). Hu et al. (2004) found platelet and monocyte hyperactivity in diabetic patients at baseline, but no substantial change after exercise in well –controlled diabetic patients compared to healthy controls. Poorly-controlled diabetic patients were not studied. Their data are conflict with ours, but our patient population was the typical CAD population. Our patients were much older and had probably more vascular complications. However, they found also an increase in CD11b adhesion molecule expression. Monocyte CD11b was significantly increased in our patients, regardless of CRF or CAD (Figure 3). CD62L on the monocyte did not change significantly. One reason might be an increased CD62L shedding from the monocyte surface after maximal exercise due to enhanced shear stress exposure of the monocytes. However, an American study found an increased CD62L expression in adolescent girls after water polo practice (Nemet et al. 2003), but the study population is significantly different from ours. In two German studies, young healthy non-smokers were investigated for platelet-leukocyte aggregates after maximal exercise, and both studies were able to show increased platelet-leukocyte formation after exercise (Hilberg et al. 2003, Hilberg et al. 2004). Taken together these data show an increased monocyte activation even in healthy controls.

In conclusion, physical exercise induces an inflammatory response that is expressed by an increased MCP-1 serum level after exercise. This is in part due to an enhanced monocyte activation. Also, endothelial activation might contribute to this inflammatory response, but endothelial activation parameters were not investigated in this study. In patients with CAD this inflammatory response is enhanced compared to healthy controls, most likely due to an increased baseline inflammatory burden of the patients with endothelial dysfunction and atherosclerotic vessel diseases.

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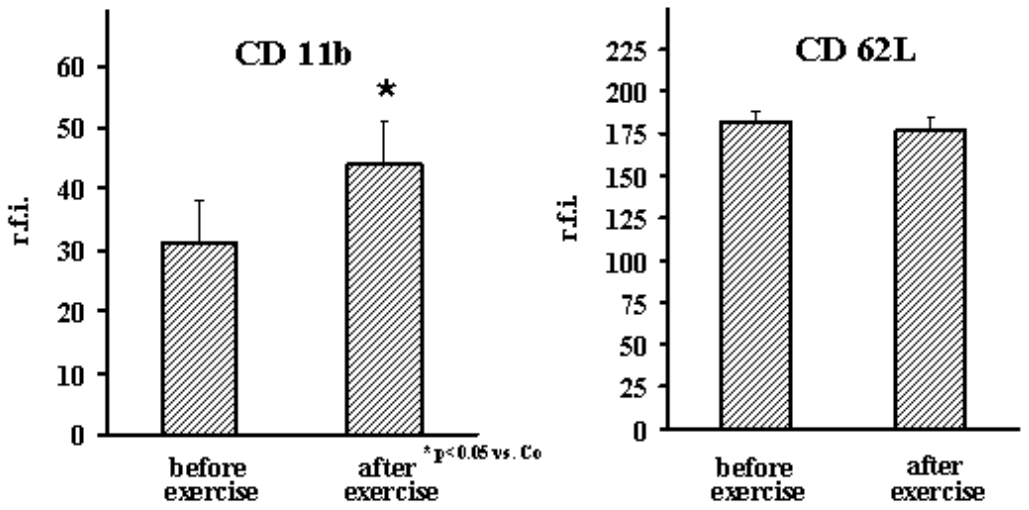

Figure 3: In 11 patients and controls monocyte CD11b and CD 62L fluorescence intensity was determined by flow cytometry. While the CD11b expression on monocytes was upregulated after physical exercise, the CD62L expression seem to remain stable after the exercise test.

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