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Fibrocytes and neutrophil extracellular traps at the culprit lesion site in myocardial infarction: a role for monocyte chemoattractant protein 1

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Aim

Leukocyte-mediated inflammation is crucial in ST-segment elevation myocardial infarction (STEMI). We recently observed that neutrophil extracellular traps (NETs) are increased at the culprit lesion site (CLS), promoting the activation and differentiation of fibrocytes, cells with mesenchymal and leukocytic properties. Fibrocyte migration is mediated by monocyte chemoattractant protein (MCP)-1 recognized via C-C chemokine receptor type 2 (CCR2). We investigated the interplay between fibrocyte function, NETs and MCP-1 in STEMI.

Results

Fibrocytes accumulated at the CLS in STEMI. Fibrocyte CCR2 expression was decreased compared with femoral control (Fig. 1a). MCP-1 (Fig. 1b) and NET marker citrullinated histone H3 (citH3) were increased at the CLS. CLS MCP-1 was correlated positively with fibrocyte accumulation (Fig. 2), and negatively correlated with CCR2 expression (Fig. 3a). *In vitro*, MCP-1 decreased fibrocyte CCR2 (Fig. 3b). NET stimulation of human coronary arterial endothelial cells induced MCP-1 (Fig. 4). MCP-1 attenuated ionomycin-induced NETosis (Fig. 5).

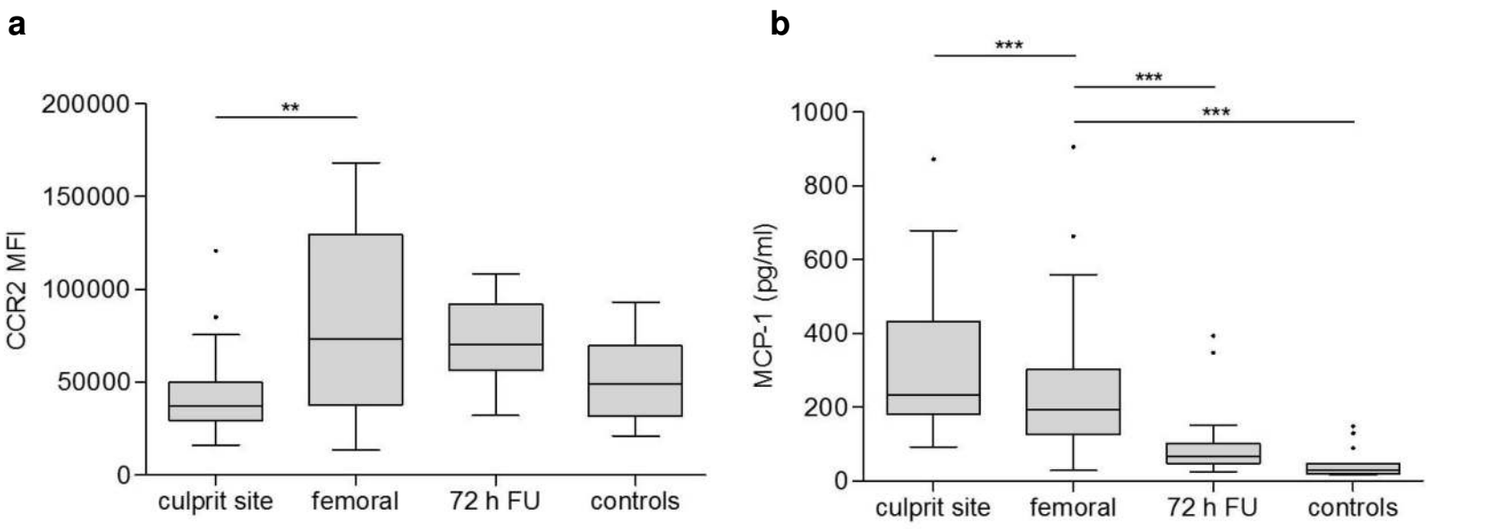

Figure 1: a, Characterization of circulating fibrocytes in patients with ST-elevation myocardial infarction and healthy controls. Cell surface receptor expression of fibrocytes was measured via flow cytometry. Fibrocytes were defined as cells positive for CD45, CD34 and Collagen I. **b**, Measurement of MCP-1 in patients with ST-elevation myocardial infarction and healthy controls. An enzyme-linked immunosorbent assay was used to determine MCP-1 concentration. Data are given as a, mean fluorescence intensity (MFI) of CCR2, or b, pg/ml. Whiskers are defined according to Tukey's method. ** p<0.01, *** p<0.001.

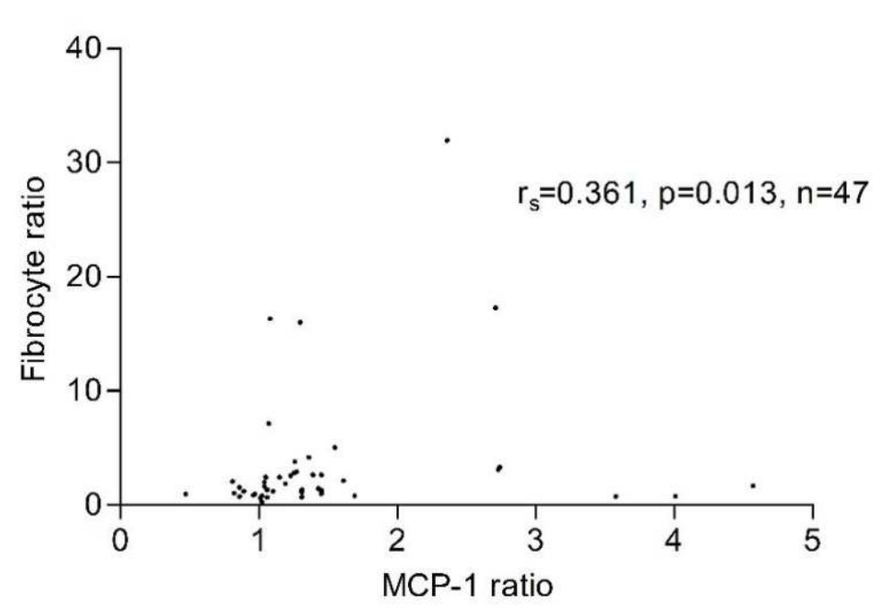

Figure 2: Correlation between fibrocyte and MCP-1 ratios. Fibrocyte frequency was measured using flow cytometry; MCP-1 was measured using ELISA. Ratios are defined as fibrocyte and MCP-1 concentration at the culprit lesion site divided by concentration of the femoral, intra-patient control.

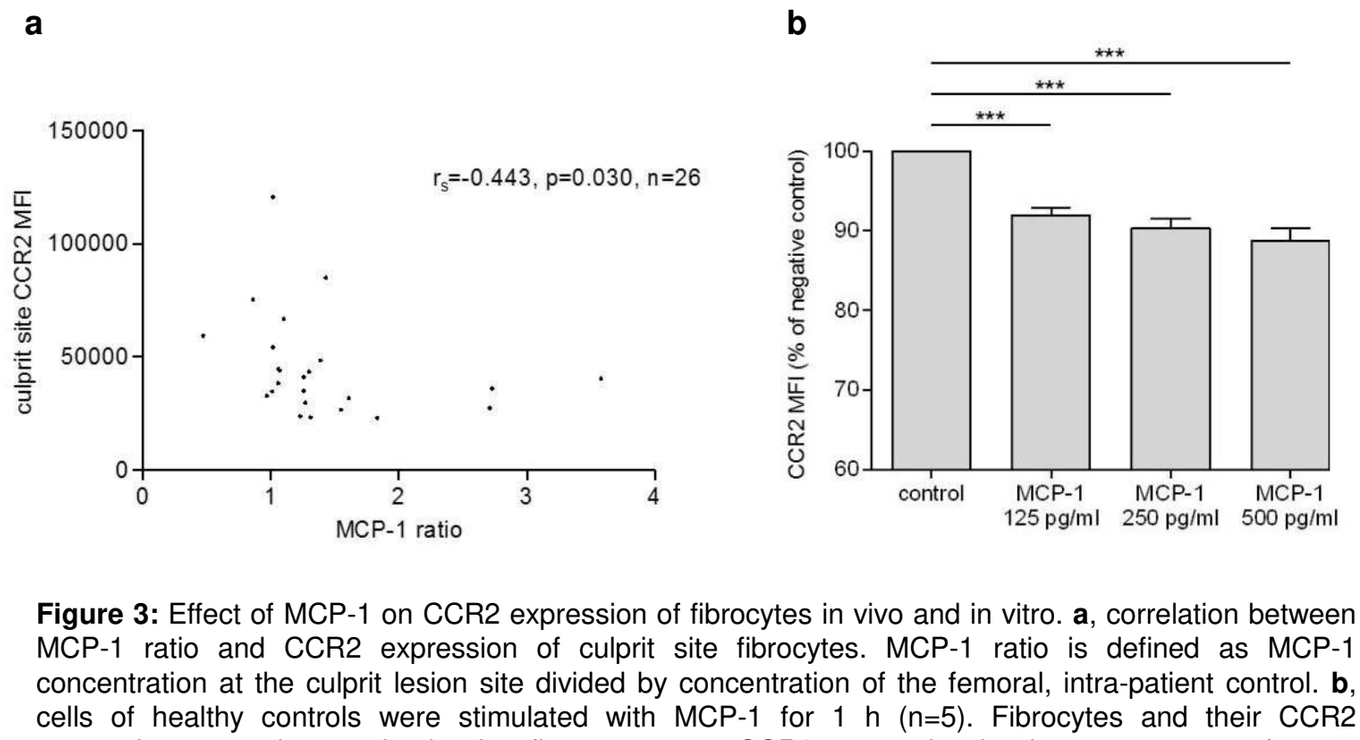

Figure 3: Effect of MCP-1 on CCR2 expression of fibrocytes in vivo and in vitro. **a**, correlation between MCP-1 ratio and CCR2 expression of culprit site fibrocytes. MCP-1 ratio is defined as MCP-1 concentration at the culprit lesion site divided by concentration of the femoral, intra-patient control. **b**, cells of healthy controls were stimulated with MCP-1 for 1 h (n=5). Fibrocytes and their CCR2 expression were characterized using flow cytometry. CCR2 expression is given as percent of mean fluorescence intensity (MFI) of negative control and presented as mean ± SEM, *** p<0.001.

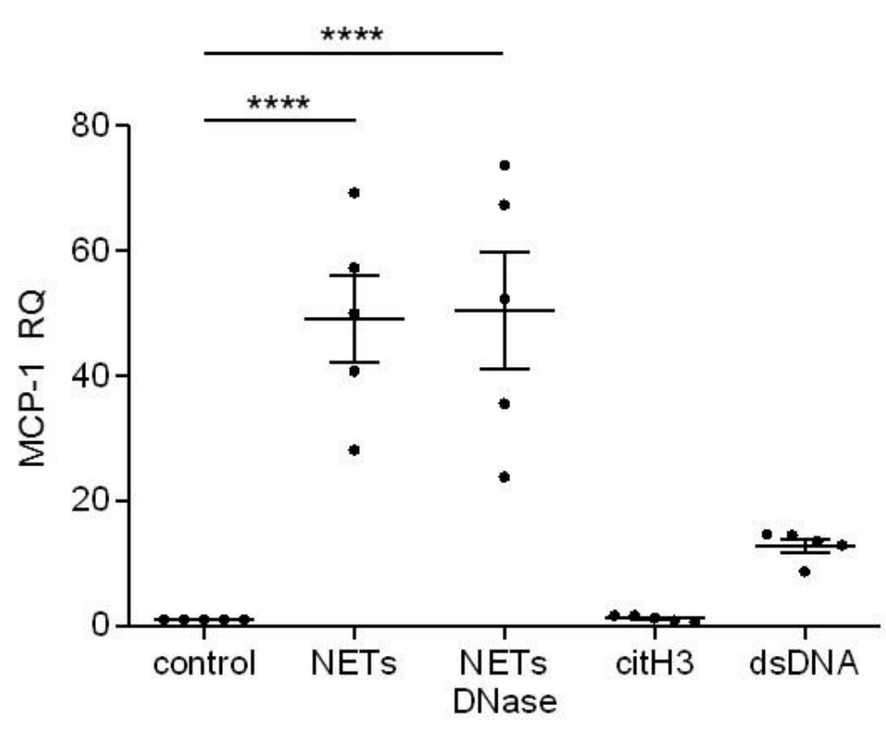

Figure 4: Influence of NETs on MCP-1 release of human coronary arterial endothelial cells (hCAECs). hCAECs were stimulated with NETs, NETs with DNase, citH3 or dsDNA for 6h. mRNA transcription of MCP-1 was assessed using qPCR. Data are presented as mean ± SEM. **** p<0.0001.

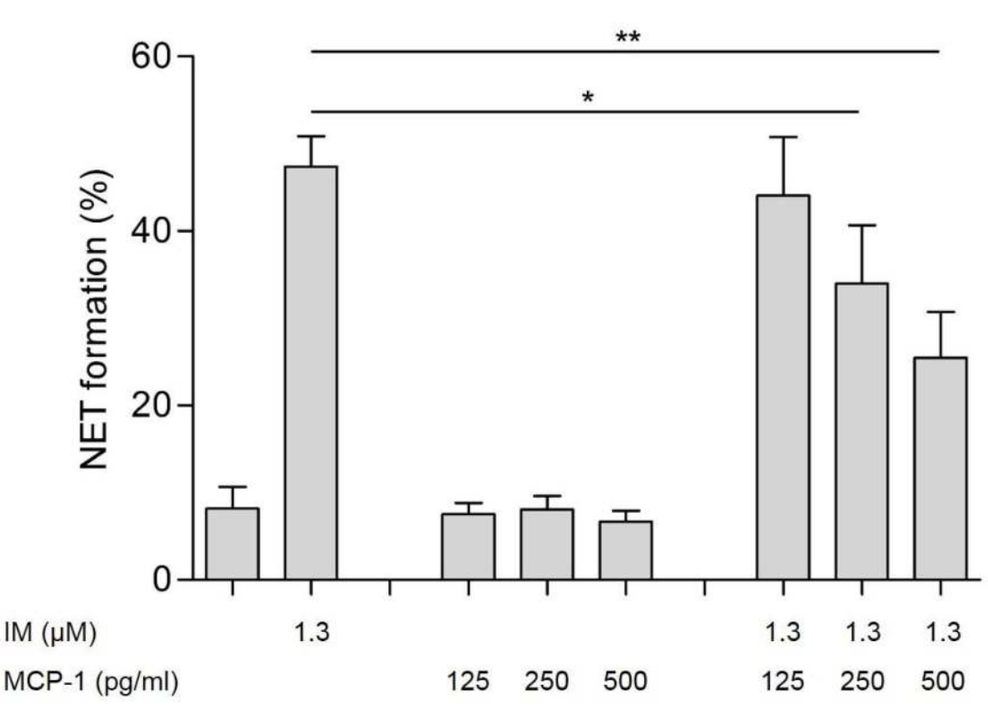

Figure 5: Effect of MCP-1 on NET formation *in vitro*. Neutrophils isolated from healthy controls were stimulated with ionomycin to induce NETosis (n=5). Prior to stimulation, neutrophils were pre-treated with increasing concentrations of MCP-1 for 20min. Results are computed as percentage of Triton X-100 positive control and presented as mean ± SEM. * p<0.05, ** p<0.01.

Conclusions

Fibrocyte accumulation at the CLS appears to be mediated by MCP-1. NETs induce endothelial MCP-1, promoting a chemotactic gradient for fibrocyte migration. The inhibitory effect of MCP-1 on NETosis might serve as a negative feedback loop, limiting inflammation.