A single nucleotide polymorphism of macrophage migration inhibitory factor is related to inflammatory response in coronary bypass surgery using cardiopulmonary bypass

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Abstract

Objective: Cardiac surgery causes induction and release of inflammatory mediators that may be regulated by genetic background. Macrophage migration inhibitory factor (MIF) is a proinflammatory mediator that is known to be up-regulated in patients undergoing cardiac operations. Here we analyzed genotype distribution and allele frequency of the MIF-173*G/C single nucleotide polymorphism (SNP) and MIF plasma levels in patients undergoing surgical revascularization with (on-pump, n = 45) and without (off-pump, n = 34) cardiopulmonary bypass (CPB).

Methods: Genotyping was performed using a real-time PCR-based system with a hybridization probe system specific for the MIF-173*G/C SNP. In on-pump patients, blood samples were drawn before start of CPB, after termination of CPB and 12 h postoperatively. In off-pump patients, blood samples were collected before stabilizer placement, after removal of the stabilizer and 12 h postoperatively. MIF levels were measured using ELISA technique. Results: Genotype distribution and allele frequencies were comparable between on-pump and off-pump patients. When comparing patients according to MIF genotype, a significant increase of MIF plasma levels after completed coronary bypass grafting using CPB was found in patients heterozygous for the MIF-173*G/C SNP (p < 0.05). Moreover, on-pump patients showed significantly decreased MIF plasma levels after 12 h postoperatively (p < 0.05). In off-pump patients, MIF plasma levels were not significantly different over the time-course and were independent of the genotype.

Conclusions: Patients carrying the C-allele showed significantly increased levels of the proinflammatory cytokine MIF compared to G/G homozygous when revascularization was carried out using CPB. The G/C genotype may be associated with a severe inflammatory reaction and therefore preoperative screening could be beneficial for patients undergoing cardiac surgery using CPB.

Keywords: MIF; Promoter; Polymorphism; Coronary artery bypass graft; Cardiopulmonary bypass

1. Introduction

Cardiopulmonary bypass (CPB) with cardioplegic arrest induces a known proinflammatory cascade in patients undergoing coronary artery bypass grafting, resulting in an inflammatory reaction to CPB with influence on postoperative morbidity and mortality [1]. Therefore, alternative revascularization approaches on the beating heart using a cardiac stabilizer (off-pump) and thus omitting CPB have gained increasing popularity [2]. Although off-pump techniques induce a systemic inflammatory response as well, these techniques have been suggested to cause a marked reduction in postoperative inflammatory-related side effects when compared to surgery with CPB [3–6].

Macrophage migration inhibitory factor (MIF) is a critical and early mediator that initiates and influences pro- and anti-inflammatory response after surgical interventions and trauma, and is involved in a wide range of inflammatory diseases ranging from rheumatoid arthritis to septic shock [7–10]. It has been shown that MIF is released in patients undergoing cardiopulmonary bypass surgery [11]. Moreover, an emerging body of evidence suggests a regulatory function of MIF on cardiac myocytes [12,13], on myocardial function [14,15] and also a role in experimental autoimmune myocarditis [16]. Furthermore, two functionally relevant promoter polymorphisms of the MIF gene have been described. A single nucleotide polymorphism (SNP) was identified in the untranslated 5′ region of the MIF gene at position −173 consisting of a G-to-C transition (MIF-173*G/C SNP) [17]. The
mutant allele, MIF-173*G, introduces an activator protein 4 (AP-4) binding site and is associated with increased MIF protein production [18]. Moreover, a tetranucleotide (CATT)3 repeat was found mapping upstream at −794 of the MIF-173*G/C SNP [19]. Further studies revealed in vivo functional and prognostic relevance of MIF-173*G/C SNP in various inflammatory diseases, e.g. juvenile idiopathic arthritis [20].

The aim of this study was to analyze association between the MIF-173*G/C SNP and MIF protein expression and the use of CPB in patients undergoing coronary artery bypass grafting.

2. Materials and methods

After approval by the local ethics committee and obtaining written and informed consent of each patient, 79 consecutive patients with single, double, or triple vessel coronary disease were included in the study. The individual decision, whether safe and complete revascularization, with or without CPB, seemed feasible, was made preoperatively by cardio-thoracic surgeons. The genotyping of the patients was done after surgical procedure. Forty-five patients were selected for conventional myocardial revascularization with CPB (on-pump group) and 34 patients received coronary artery bypass grafts on the beating heart without extracorporeal circulation using a cardiac stabilizer (off-pump group). Median sternotomy was performed in both patient groups. The 91 control individuals were healthy, Caucasian, blood donors. They were judged healthy on the basis of medical history and physical examination. The control individuals were used to compare genotype distribution and allele frequencies with study patients.

Whole blood samples for genotyping were drawn preoperatively from patients and healthy blood donors. Blood samples for MIF plasma concentrations were drawn from patients before beginning of CPB or before stabilizer placement on the beating heart (T1, time point 1), after termination of CPB or after stabilizer removal from the beating heart (T2, time point 2), and 12 h postoperatively (T3, time point 3). MIF plasma concentration was measured using ELISA (R&D Systems, Wiesbaden, Germany) according to the manufacturer’s recommendations.

For genotyping of the MIF-173*G/C SNP 3.2 ml of whole blood were collected from each individual. DNA was prepared using the Flexi Gene DNA Kit (Qiagen, Hilden, Germany) according to the manufacturer’s recommendations. Genotyping was done using a real-time PCR-based system (LightCycler, Roche, Mannheim, Germany) with hybridization probes specific for the MIF-173*G/C SNP. The PCR primer pair comprised of forward primer: 5’ GGCTTCTCTGGAAGGTTA and reverse primer: 5’ CAGCAACCGTCGCTAAGC. The sequence for the MIF-173*G/C SNP specific hybridization anchor probe was: 5’ GGCGGCTAGAATCGCTGTTTCA. The sequence for the MIF-173*G/C SNP specific sensor probe was: 5’ GGCGGCTAGAATCGCTGTTTCA. The anchor probe was phosphorylated at the 3’ end and carried a fluorescein dye at the 5’ end. Primers and probes were designed in cooperation with TIB-MOLBIOL (Berlin, Germany) and provided by this company. In brief the PCR was done using 45 cycles of 5 s denaturation at 95 °C, 8 s annealing at 60 °C, and 8 s of primer extension at 72 °C. Subsequent melting curve analysis for determination of the MIF genotype was done with an initial 20-s denaturation at 95 °C, followed by an 60-s annealing at 50 °C, and a final ramp to 85 °C with continuous fluorescence acquisition at a transition rate of 0.1 °C/s. Additionally, individual samples representing the G/G, G/C, or C/C genotypes as analyzed by real-time PCR were also genotyped by DNA sequencing proofing the accuracy of the real-time PCR method. All controlled samples had matching results between real-time PCR and DNA sequencing.

Statistical analysis for group differences of patients undergoing coronary artery bypass grafting with or without CPB in terms of clinical characteristics was done by Mann—Whitney U-test. Genotype distribution was analyzed using chi-square test and allele frequency using Fischer’s exact test. Analysis of group differences and group trends over time for plasma MIF with regard to genotype and use of CPB were done with two-way ANOVA including post hoc comparison (Scheffe’s test). Statistical significance was assumed at \(p < 0.05\). All analyses were done in GraphPad Prism version 3.0 (GraphPad Software, San Diego, CA, USA). All data are presented as mean ± SEM.

3. Results

The general characteristics of the study population as well as the postoperative cardiac and inflammatory profiles are shown in Table 1. Age and SAPS II score were comparable in both patient groups. The SAPS II score indicates severity of illness [21] with higher scores reflecting a more critical condition. Postoperative maximal plasma levels of creatine kinase (CK), CK-isoenzyme MB (CK-MB) and troponin-I were significantly higher in patients after myocardial revascularization employing cardiopulmonary bypass when compared to patients after myocardial revascularization without CPB (\(p < 0.01\), Table 1). The inflammatory cytokine interleukin 6 (IL-6) was postoperatively significantly higher in the CPB group (\(p < 0.05\), Table 1). Inflammatory mediators C-reactive protein (CRP) and tumor necrosis factor (TNF) were comparable in both groups postoperatively. When comparing the number of sclerotic coronary arteries, multivessel disease was more prominent in the on-pump group (Table 2).

<table>
<thead>
<tr>
<th>Table 1 General characteristics of study population</th>
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<tbody>
<tr>
<td>All patients (n = 79)</td>
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<td>----------------------</td>
</tr>
<tr>
<td>Age (years)</td>
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<tr>
<td>SAPS II</td>
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<tr>
<td>Cardiac profile of study population</td>
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<tr>
<td>CK (U/l)</td>
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<td>CK-MB (µg/l)</td>
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<td>Troponin-I (ng/ml)</td>
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<td>Inflammatory profile of study population</td>
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<td>CRP (mg/ml)</td>
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<td>IL-6 (ng/ml)</td>
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<td>TNF (ng/ml)</td>
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</table>

The laboratory data indicate maximal postoperative values. Data are given as mean (range). CPB, cardiopulmonary bypass; CK, creatine kinase; CK-MB, creatine kinase isoenzyme MB; CRP, C-reactive protein; IL-6, interleukin 6; TNF, tumor necrosis factor.
multivessel revascularization was performed more often in the on-pump group as well (Table 2).

The MIF-173*G/C SNP genotype distribution and allele frequencies was comparable between patients and healthy subjects \((p > 0.05, \text{Table 3})\). The rare homozygous genotype C/C was detected in only 3 of the 91 healthy blood donors and was not found in patients undergoing myocardial revascularization. In addition, no difference in genotype distribution and allele frequencies was found between on-pump and off-pump group (Table 3).

A significant increase of MIF plasma levels after using CPB (T2) was found in patients with heterozygous genotype when compared to homozygous patients: G/C: 83.8 ng/ml \((\pm 7.47)\) versus G/G: 68.6 ng/ml \((\pm 4.32)\) \((p < 0.05, \text{Fig. 1})\). Moreover, a significant decrease in MIF plasma level between immediately after surgery completion (T2) and 12 h postoperatively (T3) was observed in all CPB patients independent of the genotype: homozygous: 68.6 ng/ml \((\pm 4.32)\) at T2 versus 48.6 ng/ml \((\pm 5.65)\) at T3 \((p < 0.05, \text{Fig. 1})\); and heterozygous: 83.8 ng/ml \((\pm 7.47)\) at T2 versus 49.9 ng/ml \((\pm 5.64)\) at T3 \((p < 0.05, \text{Fig. 1})\). Patients undergoing revascularization on the beating heart had comparable MIF plasma levels independent of the genotype \((p > 0.05, \text{Fig. 2})\).

When comparing MIF plasma levels according to the number of bypass grafts and revascularization method, no significant difference was observed (Table 4; \(p > 0.05\)). Finally, length of stay showed only a trend towards a longer stay for on-pump patients carrying the G/C genotype \((9.6 \text{ days} \pm 1.82)\) versus the G/G homozygous patients \((7.7 \text{ days} \pm 0.80; \ p > 0.05)\). In the off-pump group, the length of stay was 7.0 days \((\pm 0.27)\) for the G/G homozygous patients and 6.6 days \((\pm 0.67)\) for the G/C heterozygous patients \((p > 0.05)\).

4. Discussion

Cardiac surgery is associated with manifold disturbances in the inflammatory system leading to mediator induction and release. This is mainly attributable to tissue ischemia reperfusion injury and interaction between blood and foreign surface of the extracorporeal circuits. The released mediators and activated immune cells lead to increased capillary permeability with consecutive low cardiac output, respiratory distress, and even multiple organ failure [1,4]. Therefore, avoiding the use of CPB is suggested to be beneficial for the outcome [2,3,22]. Indeed, recent clinical trials suggested...
Table 4
MIF plasma levels by number of bypass grafts and revascularization method

<table>
<thead>
<tr>
<th>Plasma MIF (ng/ml)</th>
<th>1 + 2 bypass grafts</th>
<th>&gt;2 bypass grafts</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (n = 79)</td>
<td>91.82 (±5.7)</td>
<td>89.81 (±7.4)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>On-pump (n = 45)</td>
<td>82.22 (±6.8)</td>
<td>80.04 (±8.0)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Off-pump (n = 34)</td>
<td>97.04 (±8.1)</td>
<td>96.70 (±19.3)</td>
<td>&gt;0.05</td>
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</table>

Data are given as mean (±SEM).

that coronary artery bypass grafting without CPB is associated with reduction of the inflammatory response, and with decreased postoperative morbidity and mortality [2,3,23]. In these trials, cytokine concentrations were significantly higher in on-pump patients when compared to off-pump patients. Moreover, post-CPB concentration of inflammatory mediators was significantly higher than before CPB, indicating the proinflammatory effect of the extracorporeal circuit.

In contrast, in our study MIF plasma levels were already elevated before CPB in all patients studied when compared to baseline levels reported in the literature [7–10]. Similarly, in a previous study by Gando and co-workers [11], investigating the effect of CPB on MIF secretion in 30 patients, elevated MIF plasma levels were present before initiation of CPB as well. Of note, the pre-CPB MIF levels were higher in our study compared to the study by Gando and co-workers. In their study, the stimulatory effect of CPB on MIF release was evaluated in 30 patients with only 10 patients having coronary artery disease. In our study, we focused on coronary artery disease patients exclusively. MIF is known to be up-regulated in coronary artery disease patients and unlike other cardiac enzymes, which decrease in the course after myocardial injury, MIF expression remains elevated over several days [24]. This fact may explain the increased preoperative MIF levels in both patients groups in our study.

The influence of the individual genetic background on the extent of inflammatory disease has been an evolving concept over the past years. Polymorphic sites within the promoter region or the coding sequence have been identified in many genes related to inflammatory disease. Recently, functional promoter polymorphisms of the MIF gene have been described as well. An SNP was identified in the MIF gene at position –173 consisting of a G-to-C transition (MIF-173*G/C) [17]. The mutant allele, MIF-173*C, introduces an activator protein 4 (AP-4) binding site and is associated with increased MIF protein production [18]. In our study, all patients had high MIF plasma levels comparable to previously reported data [11]. But when comparing our patients according to their genotype, we found a significant increase in MIF plasma levels at T2 in heterozygous patients carrying the mutant C-allele versus patients being G-homozygous (p < 0.05, Fig. 2). This important effect of the mutant C-allele is reflected by previous studies investigating the MIF-173*G/C SNP in a variety of inflammatory diseases. Donn and co-workers reported a significantly increased risk of systemic-onset juvenile idiopathic arthritis (JIA) in individuals carrying the C-allele [17]. Moreover, De Benedetti and co-workers suggested the MIF-173*C allele as a predictor for poor outcome in JIA [20]. In inflammatory polyarthritis (IP), the MIF-173*C allele is associated with an approximate twofold increase risk of IP susceptibility [25]. In light of all these data, the prolonged length of stay in the on-pump group with G/C genotype, compared to all other groups even though not being significant, may suggest an increased susceptibility of these patients for the increased inflammatory response resulting in prolonged hospitalization. But the low incidence of complications in our study makes our assumption somewhat speculative and points out small patient number as the major limitation of our study.

We would also like to address other limitations of our study. Patient inclusion was not matched for the number of coronary vessel disease and the number of grafts. Accordingly, multivessel disease was significantly more prominent in the on-pump group with higher graft number per patient in this group as well (Table 2). Therefore, the results of our study are somewhat limited as far as extent of the coronary artery disease and accompanying revascularization procedure is concerned. Furthermore, MIF induces and controls several downstream immunomodulatory cascades. The present study did not investigate the influence of the revascularization procedure on these MIF-driven cascades. Further studies are needed to address the potentially important extent of changes in these downstream cascades in relation to the use of CPB or beating heart procedures.

In conclusion, as MIF has proven to be involved in the systemic inflammatory response to CPB in our study and by others [11], patients with a genetic determination for high MIF levels may have an increased risk for development of severe systemic inflammation and even organ failure upon revascularization using CPB. Therefore, preoperative genotyping may be beneficial to identify patients scheduled for coronary revascularization that are probably at higher risk for developing severe inflammatory responses, especially in those presenting with multiple comorbidities.

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References


