Association between endogenous complement inhibitor and myocardial salvage in patients with myocardial infarction

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Abstract

Background: Many pathogenic processes and diseases are the result of an erroneous activation of the complement cascade and a number of inhibitors of complement have thus been examined for anti-inflammatory actions. It was recently demonstrated that supraphysiological concentrations of the endogenous complement inhibitor MASP-1 (also denoted MAPI) protect against myocardial reperfusion injury. In the present study, we examined the association between outcome after acute myocardial infarction (MI) and the plasma levels of MASP-1 and its related proteins MASP-3 in patients with first-time MI. In addition, we compared plasma levels of MASP-1, and MASP-3 in MI patients to levels in a healthy control group.

Methods: A total of 192 MI patients and 140 control persons were included. Plasma samples were obtained and analysed with time-resolved immunofluorometric assays determining the plasma levels of MASP-1, and MASP-3. The myocardial outcomes (salvage index and final infarct size) were measured by gated single-photon emission CT.

Results: MI patients had 18 % higher plasma levels of MASP-1 (IQR 11–25%) as compared to the healthy control group ($p < 0.001$). However, neither salvage index (Spearman rho -0.1 , $p = 0.28$) nor final infarct size (Spearman rho 0.02 , $p = 0.83$) correlated with plasma levels of MASP-1. Likewise, MASP-3 were elevated in MI patients ($p = 0.002$ and $p < 0.001$), but the levels were not correlated to outcome.

Conclusions: Plasma levels of MASP-1, and MASP-3 are significantly higher in patients with MI compared to healthy control persons, but are not associated with short-term outcome measured as salvage index and final infarct.

Keywords

Complement system, immunology, myocardial infarction, reperfusion

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Introduction

Inflammation is a key factor in the pathogenesis of thrombosis and acute coronary syndrome.^{1,2} Growing attention is being paid to the specific association between the complement system and ischaemia/reperfusion injury.³ Elements of the complement system and the coagulation cascade are cross-talking, which may contribute to the amplification of complement activation and thrombogenicity and eventually worsening of the outcome of acute coronary syndrome.^{1,4,5}

The complement system is a part of the innate immune system that may be activated through three pathways: the classic, the alternative, and the lectin pathways. The present study focuses on the latter. At least five soluble molecules

[mannan-binding lectin (MBL), H-ficolin, L-ficolin, M-ficolin, and collectin-K1] are capable of activating this pathway.^{6–8} The associated enzyme, MBL associated serine

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protease (MASP-1), is activated and cleaves MASP-2 and starts an accelerating complement cascade.⁹ Eventually, this leads to the formation of membrane-attack complexes causing cell lyses. The balance between activation and inhibition of this system is critical in the light of controlling the level of inflammation generated, thus keeping potential self-harming inflammation under control.

High levels of MBL and activation of the lectin pathway have been associated with an increased risk of acute coronary syndromes.¹⁰ In animal studies, MBL knockout mice showed less injury, and anti-MBL antibody treatment of rats had protective effects in myocardial ischaemia.^{11–13} Moreover an association was found between deficiency of MBL and protection after stroke.¹⁴ Inhibition of other parts of the complement system has been studied in more detail.¹⁵ In man, downstream blockade with an anti-C5 antibody was associated with reduced mortality.¹⁶

The inhibitor potential of MBL-associated protein of 44 kDa (MAp44)¹⁷ was recently tested in an in-vivo experiment on mice with induced myocardial ischaemia. High doses of MAp44 was found to attenuate thrombogenesis and myocardial ischaemia/reperfusion injury.^{18,19} In addition MASP-1 has been found to play a possible role in thrombogenesis in mice.²⁰

The present study focuses on three proteins from the lectin pathway, MAp44, MASP-1, and MASP-3, as very little is known about the impact of these proteins in acute coronary disease in humans. The aim of our study was to investigate whether physiological variations in the concentrations of MAp44, MASP-1, and MASP-3 had an impact on the extent of myocardial ischaemia/reperfusion injury in patients with first-time myocardial infarction (MI). We also tested whether the protein concentrations in patients with MI differed from the levels in healthy control persons.

Methods

MI patients

This study includes patients originally enrolled in the 'Remote Ischaemic Conditioning in STEMI' trial, which was a randomized controlled trial at Aarhus University Hospital, Denmark.²¹ In brief, 333 consecutive patients with a suspected first acute MI were included from February 2007 to November 2008. They were randomly allocated to either standard primary percutaneous coronary intervention (pPCI, $n=167$) or pPCI treatment plus remote ischaemic conditioning entailing intermittent arm ischaemia ($n=166$). A total of 251 patients met the inclusion criteria, which implied pathological factors confirming a diagnosis of first-time MI. Peripheral arterial blood was drawn in EDTA tubes from the femoral sheath at the beginning of the PCI procedure after arrival to the hospital ($n=192$). The samples were centrifuged (2000 g, 4°C, 10 min), and the plasma was removed and stored at -80°C until analysis. The primary endpoint was salvage index: the area at risk was estimated

before reperfusion therapy and the final infarct size was estimated 30 days after PCI by single-photon emission CT; final infarct size and area at risk were calculated as the area of the left ventricle containing counts lower than a mean normal limit for pixels according to the MIBIMIBI rest database. Due to practical difficulties, it was not possible to obtain salvage index on all patients (122 patients were measured). The protocol and further characterization of the study cohort have previously been described in details.²¹

Healthy controls

Blood collected at a blood bank from 140 healthy blood donors were used as control. Blood donors in Denmark must state their health status before every blood donation. The blood is screened for transmission markers and the quality of their blood including haemoglobin level is determined. In particular, the donors cannot give blood if they have ongoing infections. The peripheral blood samples from the donors were collected in EDTA tubes. The samples were centrifuged (2000 g, 4°C, 10 min), and the plasma was removed and stored at -80°C until analysis.²²

Quantification of MAp44, MASP-1, and MASP-3

The proteins were measured by in-house assays as previously described in details.^{17,23} In short the MAp44 and MASP-3 assays use the antibody sandwich-type principle, in which antibody-coated microtiter wells specifically bind the target protein. Biotinylated antibodies are subsequently added as detecting antibodies followed by addition of europium-labelled streptavidin. The amount of bound europium is detected by fluometry, i.e. time-resolved immunofluorometric assays (TRIFMAs) are conducted.¹⁷

The MASP-1 concentration was measured in a competitive binding assay by TRIFMA as previously described.²³ MASP-1 in plasma is competing with the interaction between anti-MASP-1 antibody and MASP-1 coated onto microtitre wells. The amount of antibody bound in the wells is subsequently detected by a biotinylated secondary antibody and then addition of europium-labelled streptavidin and detection by time-resolved fluometry.

The intraassay coefficients of variations were 3% for MAp44, 4% for MASP-1, and 3% for MASP-3. The interassay coefficients of variations were 7% for MAp44, 9% for MASP-1, and 7% for MASP-3.

We have previously reported on levels of MAp44, MASP-1, and MASP-3 in serum from blood donors.^{17,23} The use of EDTA plasma has been validated for all three assays and gives similar results as in serum for MAp44 and MASP-1, while the MASP-3 assay yields higher concentration in EDTA plasma compared to serum.

A study of Schlapbach et al.²⁴ shows an excellent agreement between capillary and venous samplings, which entitles our comparison of MI patients with blood donors.

Statistical analysis

All statistical analysis was performed by STATA 12.0 for Mac OS. A two-group mean comparison test was used to compare the levels of MAP44, MASP-1, and MASP-3 in myocardial patients with the levels in the control group. All three proteins are normally distributed on a logarithmic scale and there were no difference in standard deviation between the groups. A parametric t-test was used to test the hypothesis of no difference in mean between the groups. Spearman rank correlation test and one-way ANOVA was used to test the association between the concentrations of MAP44, MASP-1, and MASP-3 and the amount of myocardial injury (salvage index and final infarct size).

Results

The baseline characteristics of the MI patients are given in Table 1. The control group had a median age of 49.5 years (IQR 40–57) and 64 % were men ($n=90$).

Levels of MAP44, MASP-1, and MASP-3 in the MI and control groups

There were no correlation between age and MAP44, MASP-1, and MASP-3 concentration in neither the control group nor the patient group, and there were no differences in protein concentration between men and women in either group (data not shown).

In the MI group, the levels of MAP44, MASP-1, and MASP-3 did not differ between patients treated with remote ischaemic conditioning before PCI and those treated with PCI alone (Table 2), and the patients were thus combined into one group for subsequent analyses. The protein levels did not differ among MI patients with different TIMI flow either before or after PCI (data not shown).

The plasma levels of MAP44, MASP-1, and MASP-3 in the MI and control groups are presented in Table 3 and Figure 1. MI patients had significantly higher plasma levels of all three proteins compared to healthy controls. Patients with MI had 18% (IQR 11–25%) higher MAP44 plasma concentrations compared to the control group ($p<0.001$). The plasma levels of MASP-1 and MASP-3 were also higher in MI patients (10%, IQR 4–18%, $p=0.02$; and 16%, IQR 9–23%, $p<0.001$, respectively) compared to healthy controls.

Association between MAP44, MASP-1, and MASP-3 and outcome after MI

In correlation analyses, there were no significant correlation between MAP44, MASP-1, and MASP-3-plasma levels and the extent of ischaemia/reperfusion injury estimated as salvage index and final infarct size (Table 4). Nor did the plasma levels correlate with area at risk at admission to the hospital.

Table 1. Baseline characteristics of the myocardial infarction group.

Myocardial infarction ($n=192$)	
Age (years)	63 (56–72)
Sex (male)	147 (77)
Infarct-related artery	
Left anterior descendens	78 (41)
Right coronary or circumflex coronary	112 (58)
TIMI flow grade at admission	
0–1	107 (56)
2–3	74 (38)
Not measured	11 (6)
TIMI flow grade after pPCI[Q]	
0–1	8 (4)
2–3	174 (91)
Not measured	10 (5)

Data presented are medians (interquartile ranges) or n (%). pPCI, primary percutaneous coronary intervention; TIMI, Thrombolysis in Myocardial Infarction.

Table 2. Plasma concentrations of MAP44, MASP-1, and MASP-3 in the myocardial infarction group divided into either standard PCI or PCI and intervention.

	PCI + intervention ($n=96$)	PCI ($n=96$)	p -value
MAP44 ($\mu\text{g/ml}$)	2.0 (1.7–2.4)	1.9 (1.6–2.3)	0.216
MASP-1 ($\mu\text{g/ml}$)	12.7 (10.2–15.7)	11.7 (9.8–14.4)	0.229
MASP-3 ($\mu\text{g/ml}$)	4.3 (3.5–5.1)	4.2 (3.4–5.3)	0.783

Data presented are medians (interquartile ranges). PCI, percutaneous coronary intervention.

Table 3. Plasma concentrations of MAP44, MASP-1, and MASP-3 in the myocardial infarction and healthy control groups.

	Myocardial infarction ($n=192$)	Healthy ($n=140$)	p -value
MAP44 ($\mu\text{g/ml}$)	2.0 (1.7–2.4)	1.7 (1.4–2.0)	<0.001
MASP-1 ($\mu\text{g/ml}$)	12.3 (9.9–14.9)	11.2 (9.6–13.4)	0.002
MASP-3 ($\mu\text{g/ml}$)	4.2 (3.5–5.2)	3.6 (3.0–4.6)	<0.001

Data presented are medians (interquartile ranges).

Similarly, when salvage index or final infarct size were divided into quartiles, there were no statistical difference in the levels of MAP44, MASP-1, or MASP-3 between the quartiles, as illustrated in Figures 2 and 3.

There were no significant correlations between left-ventricular ejection fraction at 30 days and levels of MAP44, MASP-1, or MASP-3, and when patients were divided according to an ejection fraction above or below 40% at 30 days, there were no significant differences in protein levels between the two groups. In addition, correlation analyses of the protein levels and ischaemic time expressed as the time from start of symptoms to completed PCI were also non-significant (data not shown).

Figure 1. Plasma levels of MAp44 (a), MASP-1 (b), and MASP-3 (c) in patients with acute myocardial infarction (MI) and in healthy controls.

The broad horizontal lines indicate the median.

Table 4. Correlation coefficients describing the correlation between MAp44, MASP-1, MASP-3, and the myocardial outcome (salvage index and final infarct size).

	Salvage index		Final infarct size	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
MAp44	−0.1	0.28	0.02	0.83
MASP-1	−0.08	0.40	−0.07	0.36
MASP-3	0.02	0.81	−0.12	0.12

Discussion

It was recently shown that pharmacological doses of MAp44 improves outcome after myocardial ischaemia/reperfusion in an animal model of MI.¹⁸ In the present study, we demonstrate that plasma concentrations of

MAp44 and the two related proteins, MASP-1 and MASP-3, are significantly higher in patients with acute MI compared to healthy controls. Between-subject variations in the protein levels, however, were not associated with short-term outcome following acute MI as assessed by salvage index and final infarct size. Because of the case-control design of our study, we cannot conclude whether genetically determined high levels of MAp44, MASP-1, and MASP-3 are risk factors for developing acute MI or whether the higher levels are due to an upregulated synthesis secondary to the MI. However, our data clearly indicate that there is no association between naturally occurring variations in MAp44, MASP-1, and MASP-3 and outcome in patients with acute MI, although pharmacological doses of MAp44 may reduce ischaemia/reperfusion injury in an experimental set up.

An exaggerated activation of the complement system plays a role in many pathological processes,¹⁵ and it is

Figure 2. Plasma levels of MAp44 (a), MASP-1 (b), and MASP-3 (c) according to salvage index groups. Data presented are medians (lines within boxes), interquartile ranges (boxes), and 95% CIs (whiskers).

Figure 3. Plasma levels of MAp44 (a), MASP-1 (b), and MASP-3 (c) according to final infarction size. Data presented are medians (lines within boxes), interquartile ranges (boxes), and 95% CIs (whiskers).

important to keep this system of proteases depressed under normal conditions. It has been known for many years, that activation of the complement system exacerbates the tissue injury following ischaemia in different tissues, including the heart and the brain,²⁵ and markers of complement activation may predict outcome following acute MI.^{26,27}

The beneficial impact of complement inhibition has initiated the pursuit of an effective complement therapy. Complement inhibition by C3 depletion or soluble CR1 infusion is shown to reduce myocardial infarct size in rats.^{25,28} Furthermore, the terminal complement components C5a and C5b-9 are proposed to be key mediators of myocardial reperfusion injury.²⁹ Mortality was reduced by inhibition of C5 (pexelizumab) in patients with MI undergoing PCI.¹⁶ In a large clinical trial this effect of pexelizumab was not detectable, which may be due to very low mortality in patients undergoing PCI.³⁰ Classical pathway inhibition with CRP inhibitor and C1-esterase inhibitor preserves ischaemic myocardium from ischaemia/reperfusion injury^{31,32} and, thus, demonstrate this pathway's importance. The lectin pathway is less examined. Monoclonal antibodies against rat MBL protects rats against myocardial damage,¹³ but no human studies find reduced myocardial ischaemia/reperfusion injury by lectin pathway inhibition. The endogenous inhibitors are here of particular interest, given that control of the inflammation generated is essential for preventing progression of the pathological processes.

A number of inhibitors prevent the complement cascade from uncontrolled acceleration. The three proteins MASP-1, MASP-3, and MASP-4 are all part of the lectin pathway and are expressed from a common MASP1-gene. MASP-1 and MASP-3 share five structural domains but differ by having two different serine protease domains.³³ MASP-4 share the first four structural domains with MASP-1 and MASP-3 but have in addition a unique 17-amino-acid C-terminal end. MASP-1 and MASP-2 are inhibited by the binding of C1 inhibitor or by higher amounts of MASP-4 and MASP-3 competing for binding to MBL and ficolins. This knowledge

contributes to the elucidation of the complement complexity and thus might become target for complement therapy.

We find that the levels of MASP-1, and MASP-3 are higher in MI patients. Whether this is a result of pathological changes seen following an acute MI or whether the infarction is a consequence of an abnormal stoichiometry of these components of the lectin pathway is still unclear. Because of the case-control design of our study, we have no plasma samples from the patients before the MI indicating their plasma level under normal conditions. It may be that prolonged alterations in the delicate balance of complement components may trigger the MI by causing coronary inflammation. On the other hand, MI may in itself cause instant changes in the levels of complement factors. Ischaemic infarction is a potent stimulus for the acute-phase response, in which a wide range of plasma proteins, mostly produced in the liver, increase dramatically in concentration to values that usually reflect closely the extent of tissue damage. We have previously studied the impact of an acute phase response on the levels of proteins studied and have not found this to have any influence.^{17,23} Very recently a pilot study showed an association between MASP-1 and subacute MI, but this finding requires validation in a larger study.³⁴

A limitation to the present study is that the control group was not matched one-on-one with our patient group. However, no correlation with either age or gender within the groups was found, reducing the risk of such bias. The relatively small number of patients limits the study and might induce type 2 errors. A strong point of our study is the thorough characterization of patients with detailed infarct localization, early diagnosis, clear-cut inclusion criteria, and the well-defined outcome measures.

Another limitation is the fact that this is a novel research area, where possible and undiscovered confounders may influence the results: for example, atherosclerosis is accepted to be an inflammatory disease and might be a connecting factor between complement activation and MI.

In summary, we found no correlation between the plasma levels of the endogenous complement inhibitor MASP-1 and MASP-3 did not have any effect on these outcomes. However, patients with MI were found to have significantly higher plasma levels of MASP-1 and MASP-3 compared to healthy control persons, and the possible causative role of these molecules in acute MI merits further investigation.

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Conflict of interest

The authors declare that there is no conflict of interest.

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