The Inflammatory response in myocarditis and acute myocardial infarction

Emmens, R.W.

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Chapter 5

On the value of therapeutic interventions targeting the complement system in acute myocardial infarction

Reindert Emmens, Diana Wouters, Sacha Zeerleder, Marieke van Ham, Hans Niessen, Paul Krijnen

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SUMMARY

The complement system plays an important role in the inflammatory response subsequent to acute myocardial infarction (AMI), by actively increasing cell death of cardiomyocytes beyond the primary infarced area. The aim of this study is to create a systemic overview of studies that investigated therapeutic administration of complement inhibitors in both AMI animal models and human clinical trials. *Ex vivo* studies on isolated hearts and proof-of-principle studies on inhibitor administration before experimental AMI induction were excluded, to enable extrapolation of observations from included animal studies towards post-AMI clinical trials. Positive therapeutic effects in AMI animal models have been described for cobra venom factor, soluble complement receptor 1, C1-inhibitor (C1-inh), FUT-175, C1s-inhibitor, anti-C5 and clusterin. Two types of complement inhibitors have been tested in clinical trials, being C1-inh and anti-C5. Pexelizumab (anti-C5) did not result in reproducible beneficial effects for AMI patients. Beneficial effects were reported in AMI patients for C1-inhibitor, albeit in small patient groups. In general, despite absence of consistent positive effects in clinical trials thus far, the complement system remains a potentially interesting target for therapy in AMI patients. Based on the study designs of previous animal studies and clinical trials we have provided several issues which require attention in the design of future studies: adjustment of clinical trial design to precise mechanism of action of administered inhibitor, optimizing the duration of therapy and optimization of time point(s) on which therapeutic effects will be evaluated.
Introduction
Acute myocardial infarction (AMI) is an important cause of morbidity and mortality worldwide, forming 7% and 5% of the global disease burden in males and females respectively.\textsuperscript{1} An important factor contributing to AMI-related morbidity and mortality is inflammation. The inflammatory response post-AMI is necessary for clearance of the jeopardized infarcted tissue but at the same time enhances cell death of at that moment still viable cardiomyocytes, resulting in infarct expansion.\textsuperscript{2} The complement system plays an important role herein, both by stimulating inflammation and by directly causing death to reversibly damaged cardiomyocytes (summarized in figure 1).

In the 1950s, an increase in serum complement was already recognized as a serological marker for AMI.\textsuperscript{3} Later, in 1971, a hallmark study by Hill and Ward demonstrated for the first time that cleavage of complement factor C3 occurs in infarcted myocardium following AMI in rats, and that depletion of C3 using cobra venom factor (CVF) inhibited polymorphonuclear leukocyte (PMN, neutrophils) infiltration of the infarcted myocardium.\textsuperscript{4}

This finding triggered a wide interest in the complement system as potential therapeutic target for AMI. Between 1990 and 2005, a large number of animal studies were published describing positive effects of various complement inhibitors on the outcome of AMI, and complement was considered a promising target for therapy.\textsuperscript{5,8} Disappointing results from several clinical trials in the 2000s however, dampened the general enthusiasm for complement inhibitors as therapy for AMI patients.

The main objective of this review is to provide a detailed overview on the animal studies and clinical trials that studied complement inhibitors as therapeutic option for AMI. This objective is in contrast to that of other recently published reviews on complement inhibitors in both animal models and clinical trials,\textsuperscript{9,10} as these focussed on ischemia-reperfusion injury in general. Based on our overview, we aim to elucidate whether or not the concept of complement inhibition as therapy in AMI has been abandoned prematurely, and to provide directions for future research in this field.

Structure and function of the complement system
The complement system is part of the humoral innate immune response and forms a cascade of over 30 proteins present in plasma (fluid phase) or bound to the target surface (damaged/infected cells or pathogens). The molecular composition of the complement system has been studied and described in great detail.\textsuperscript{11-17} Summarized, the complement system can be activated through three pathways: the classical pathway, the lectin pathway and the alternative pathway (figure 2). Activation of the classical pathway is mediated by the C1 complex, consisting of C1q, C1s and C1r, while activation of the lectin pathway is mediated by MASP1/MASP2 together with a collectin (usually mannose-binding
Following coronary artery occlusion, cardiac ischemia causes exposure of phosphatidylserine (PS) on the cardiomyocyte membrane and upregulation of adhesion molecules, such as ICAM-1, on endothelial cells. When the infarcted myocardium is reperfused, IgM, C-reactive protein (CRP) and complement proteins (C) enter the myocardium from the systemic circulation. PS indirectly triggers exposure of lysophospholipids (Lyso-PC), which function as an attachment site for IgM, CRP and C to cardiomyocytes that are reversibly damaged by ischemia, after which the complement system is activated. Complement activation results in the death of the damaged cardiomyocytes and release of anaphylatoxins (C3a and C5a). The anaphylatoxins and the adhesion molecules both stimulate extravasation of pro-inflammatory macrophages (M) and neutrophils (PMN). As result of cardiomyocyte death, cardiac proteins such as troponins and creatine kinase (CK-MB) are released, which can be detected in the systemic circulation.

Upon binding of the recognition molecules of either pathway (C1q or MBL/ficolins, the cleavage of fluid phase C2 and C4 by proteases (C1s/MASP-2) is triggered, which bind to the target surface and form a C3-convertase. In the alternative pathway, C3 spontaneously hydrolyses in the fluid phase, which together with factor B results in the formation of an alternative C3-convertase (C3bBb) on the target surface. All three pathways converge at the point of C3, which is cleaved by C3-convertase. The C3 cleavage-product C3b formed in either the classical or lectin pathway, may serve as starting point in the alternative pathway as well. Thereby, the alternative pathway functions as an amplification loop for the other two activation pathways. In addition, C3b bind to the C3-convertase, forming a C5-convertase on the target surface, which in turn cleaves fluid phase C5. The complement cascades ends when C5b (cleavage product from C5) forms the membrane attack complex (MAC), together with C6, C7, C8 and C9. MAC forms a pore in the membrane, which triggers the lysis of the targeted host cells or pathogens. When C3 and C5 are cleaved, the respective anaphylatoxins C3a and C5a are released in the fluid phase. These have a wide variety of pro-inflammatory properties. Both stimulate recruitment of inflammatory cells and induce the oxidative burst of neutrophils and macrophages. C3a also stimulates pro-inflammatory cytokine
Figure 2. The complement system and its inhibitors
Schematic representation of the complement cascade (grey boxes and arrows). Complement inhibitors and their points of action within the complement cascade are listed in light orange boxes. Inhibitors in purple have been investigated as therapeutic agent in AMI animal models, and inhibitors in red have been investigated as therapeutic agent in both AMI animal models and clinical trials with AMI patients. Inhibitors marked with * are artificially created or isolated from non-human species. Unmarked inhibitors are endogenous to humans. + indicates a stimulatory function.


production by macrophages. Finally, besides serving as component for C3/C5-convertase and activator of the alternative pathway, C3b can also directly bind to pathogen surfaces, which stimulates opsonisation of these pathogens.
Complement activation in the human post-AMI heart

The classical pathway of complement activation is well known to play a role in complement activation in the post-AMI heart. Ischemia activates apoptosis pathways in cardiomyocytes, which results in phosphatidylserine (PS) exposure on the outside of the cardiomyocyte cell membrane. Exposed PS in turn indirectly triggers exposure of lysophospholipids (lyso-PC), which functions as an attachment site for C-reactive protein (CRP) and type M immunoglobulins (IgM), which are both known activators of the classical pathway. Indeed, both IgM and CRP are found to coincide in time and location with activated complement in human infarctions, and also in increased levels in the serum of AMI patients.

The involvement of the lectin pathway in post-AMI complement activation on the other hand is a matter of controversy. Several collectins have been described to activate the lectin pathway. Of these molecules, MBL has been studied most extensively by far. Several studies have found increased MBL plasma levels in AMI patients compared to control patients, indicating participation of the lectin pathway (reviewed by Pagowska-Klimek and Cedzynski). Additionally, in murine studies, removal/blocking of MBL protected the mice/rats against cardiac ischemia-reperfusion injury. However, other studies described that increased MBL plasma levels are associated with a decreased risk of AMI. In addition, MBL is an acute phase protein produced by the liver during inflammation. Therefore, its presence in human plasma may not necessarily be related to lectin pathway activation. To the best of our knowledge, MBL has never been demonstrated in the infarcted human myocardium itself. Finally, involvement of the alternative pathway in complement activity in the post-AMI myocardium has never been investigated.

Summarized, a contributing role of the classical pathway in the post-AMI heart is well-established, but to assess possible involvement of the lectin and alternative pathways, further investigation is required.

Post-AMI complement activation timeframe in humans and model animals

A timeframe of complement activation in the post-AMI heart in humans and the four most commonly used animal models for AMI studies is depicted in figure 3. This timeframe is based on complement factors measured in cardiac tissue directly.

*Human.* Knowledge of the activation of the complement system in the human post-AMI heart is primarily based on pathological examination of cardiac tissue obtained post-mortem of patients who died as result of AMI. Schäfer et al. stained a small number of human cardiac sections with infarctions aged between 6 and 24 hours old with an antibody against the complement membrane attack complex (MAC, C5b-9), and found MAC to be present specifically in the infarcted myocardium in all sections, while the healthy myocardium from the same patients was negative. Following this, observational post-mortem studies
Figure 3. Complement activation in the post-AMI myocardium

Time course in which complement activity is present in the myocardium following acute myocardial infarction (AMI) in humans and the four most commonly used model animals in AMI research. Red bars indicate confirmed presence of complement activity. Pink bars in the human timeline also indicate complement activity, though not all studies find complement activity during this period.

on larger patient groups were performed. Lagrand et al. found complement activation products (C3c/d, C4c/d) in the infarcted myocardium from 12 hours until as late as 14 days following AMI, although the extent of activated complement decreased after 3 days. Other studies also found complement components (C3b/d, C4b/c/d, MAC) primarily present in the infarcted myocardium between 12 hours and 5 days following AMI.

Mouse. Charlagorla et al. found C3 depositions in the mouse heart after cardiac ischemia-reperfusion and observed that complement activation is detectable 1 hour after reperfusion and increased up until 24 hours. Extensive C3 depositions in the heart 24 hours after reperfusion were also observed by Oyama et al. A different timeframe was observed however by Wysoczynski et al., who found that MAC levels (determined by ELISA on cardiac homogenates) were the highest 48 hours after permanent coronary artery ligation, while after 24 and 72 hours MAC levels differed only minimally from controls.

Rat. Vakeva et al. systemically mapped several components of the complement system (C1, C3, C8 and C9) over time in the rat myocardium following permanent coronary artery ligation, and found that complement activation is detectable 2 and 3 hours after coronary artery ligation and remained detectable until at least 72 hours. Sumitra et al. performed a similar study in a
cardiac ischemia-reperfusion model and observed complement activation, visualized by staining soluble MAC, starting between 4 and 8 hours following reperfusion and remaining elevated up to 32 hours.\textsuperscript{40} A study by Fu et al. demonstrated that in cardiac ischemia-reperfusion, complement (C3) mRNA and protein are both still present in the heart 72 hours following reperfusion.\textsuperscript{41}

\textit{Rabbit.} Mathey et al. demonstrated in a rabbit model of permanent coronary artery ligation that MAC depositions were present in the infarcted heart from 5 hours up until 30 hours. In a cardiac ischemia-reperfusion model however, MAC depositions appeared as early as 15-30 minutes after reperfusion up to 6 hours after reperfusion.\textsuperscript{42} Time points beyond 30 hours were not included for either permanent coronary artery ligation or cardiac ischemia-reperfusion models.

\textit{Swine.} In a swine cardiac ischemia-reperfusion model, complement depositions were found in the heart 2 hours after reperfusion.\textsuperscript{9, 43} The presence of complement activation in infarcted tissue at later time points has never been investigated.

In conclusion, complement activation post-AMI has been observed within 3 hours after reperfusion of the infarcted myocardium in all four model animals. With regard to the duration of complement activity in the heart after induction of AMI, additional research is needed. In humans, complement activity can be detected in the infarcted myocardium as late as 14 days post-AMI. In rat and rabbit, no decline in complement activity has been observed in the latest studied time points (72 and 30 hours respectively), suggesting that complement activity may continue after these time points. Available data on this appears contradictory for mice, although this could be explained by the different AMI models used,\textsuperscript{36-38} or by technical complications. For instance, analysis of heart homogenates (used by Wysoczynski et al.\textsuperscript{38}) may have been contaminated by blood present in the intramyocardial blood vessels. For swine, no studies on the post-AMI complement activation timeframe have thus far been published.

**Permanent coronary artery ligation versus ischemia-reperfusion injury**

An important question is how reperfusion therapy affects the time course of complement activation. Mathey \textit{et al.} found in rabbits that experimental reperfusion resulted in an earlier start of complement activation compared to permanent coronary artery ligation.\textsuperscript{42} This observation indeed suggests that both types of AMI models (permanent coronary artery ligation versus cardiac ischemia-reperfusion) follow different timeframes of complement activation in the post-AMI heart. This is in line with the observation that reperfusion causes an accelerated PS exposure on cardiomyocytes in the infarct area,\textsuperscript{18} which likely contributes to the accelerated complement activation. In addition, the accelerated restoration of the blood flow can also cause complement components produced by the liver to reach the infarcted myocardium faster. In patients, it is known that percutaneous coronary intervention results in reperfusion injury, facilitating
myocardial damage and complement activation.\textsuperscript{44} In the study of Lagrand, a minority of AMI patients (24\%) had received reperfusion therapy, although the effect of reperfusion therapy on complement depositions was not discussed herein.\textsuperscript{24} For the other patient studies, it is not mentioned whether or not included patients received reperfusion therapy.\textsuperscript{23, 25, 34, 35} How reperfusion affects the total duration of complement activity after AMI is unknown.

\textbf{Search criteria for complement inhibitors}

Studies analysing therapeutic effects of complement inhibition in AMI, including studies of post-AMI interventional procedures such as coronary angioplasty or coronary artery bypass grafting were obtained through a PubMed search, and listed in \textit{table 1} (animal studies) and \textit{table 2} (clinical trials). The terms “myocardial infarction” and “ischemia” were each coupled with “complement” or the name of an inhibitor. The following 19 inhibitors were included in this search: anti-C5, anti-C5a, C1-inhibitor (C1-inh), C1s-inhibitor (C1s-inh), C4b-binding protein (C4BP), C5 shRNA, carboxypeptidase-N (CPN), clusterin, cobra venom factor (CVF), CD55 (Decay-accelerating factor, DAF), Disodium disuccinate astaxanthin (DDA), Factor H, Factor I, FUT-175, MBL/ficolin-associated protein 1 (MAP-1), CD46 (membrane cofactor protein, MCP), CD59 (protectin), soluble complement receptor 1 (sCR1) and vitronectin. The points of action of each inhibitor within the complement cascade is depicted in \textit{figure 2}.

For “ischemia”, only studies on cardiac ischemia were included. Proof-of-principle studies where an inhibitor was delivered to model animals prior to induction of AMI were not included in the analysis, as such studies have poor extrapolative value towards clinical practice for AMI patients. In the disease process of AMI, many non-cardiac organs/systems play an essential role, including the central nervous system\textsuperscript{45}, bone marrow\textsuperscript{46}, liver\textsuperscript{47} and spleen\textsuperscript{48}. Because of this, we also excluded \textit{ex vivo} studies on isolated hearts from this review.

\textbf{Inhibitors of the complement system}

Therapeutic application of a complement inhibitor in the context of AMI has been reported for 7 inhibitors: C1-inh, C1s-inh, FUT-175, sCR1, CVF, antibodies targeting C5 and clusterin (more details on these inhibitors can be found below). C1-inh and clusterin are both endogenous complement inhibitors isolated from human plasma, which have both been described to be present in the infarcted myocardium, coinciding with complement activation.\textsuperscript{49, 50} The other inhibitors are either artificially created (C1s-inh, FUT-175, sCR1, anti-C5) or isolated from non-human species (CVF). Details on the study design and results of these studies are summarized in \textit{table 1} for animal studies and in \textit{table 2} for clinical trials.

A soluble form of CD55 (membrane protein that prevents formation of C3-convertase and C5-convertase\textsuperscript{51, 52}) has been therapeutically applied in ischemia-
reperfusion models of several organs other than the heart.\textsuperscript{53} DDA (target not known), anti-C5a and C5 shRNA are artificially created inhibitors which have been investigated in proof-of-concept studies in cardiac ischemia-reperfusion models,\textsuperscript{54-58} although they have not yet been applied as therapeutic drug.

Besides C1-inh and clusterin, two other endogenous complement inhibitors, namely C4BP (fluid phase protein that simulates decay of C4b and C3b\textsuperscript{57}) and vitronectin (fluid phase protein that prevents MAC assembly\textsuperscript{58}) have also been described to be present in the human infarcted myocardium,\textsuperscript{35, 59} which suggests their endogenous involvement in post-AMI complement inhibition as well. A soluble form of CD59 (membrane-bound protein that prevents MAC assembly\textsuperscript{60}) has been described to be increased in human plasma following AMI.\textsuperscript{61} In addition, one study reports expression of endogenous factor H (fluid phase protein that acts as a cofactor in the degradation of C3b into iC3b\textsuperscript{62, 63}) in some cardiomyocytes in the human post-AMI myocardium.\textsuperscript{64} Therapeutic application of these inhibitors has not been studied however.

The remaining inhibitors (all endogenous): factor I (fluid phase protein that degrades C3b and C4b\textsuperscript{65}), CD46 (membrane-bound cofactor for factor I\textsuperscript{66, 67}), MAP-1 (fluid phase protein that prevents MBL-MASP1/2 assembly\textsuperscript{68}) and CPN (fluid phase inactivator of C3a and C5a\textsuperscript{69}) have never been investigated in the context of AMI.

**C1-inhibitor**

C1-inh is a fluid phase protein which covalently binds to C1s and C1r, which counteracts C1 assembly and subsequent activation of the classical pathway.\textsuperscript{70} C1-inh has also been described to inhibit the lectin pathway of complement activation in a similar way by binding MASP-1 and MASP-2. Besides inhibition of the complement system, C1-inh also inhibits the contact system and has been reported to inhibit leukocyte adhesion to endothelial cells (reviewed by Davis et al.\textsuperscript{71}).

In 1995, Buerke et al. published the first study analysing C1-inh as a therapeutic agent in an AMI animal model. In this cardiac ischemia-reperfusion study, C1-inh was injected intravenously in cats during coronary artery ligation, and a decreased infarct size and increased cardiac contractility was observed after a 4.5 hour reperfusion period.\textsuperscript{72} Murohara et al. studied the effects of C1-inh in a rat model of cardiac ischemia-reperfusion.\textsuperscript{73} When C1-inh was administered 1 minute before reperfusion, a decrease in plasma creatine kinase (CK) and PMN infiltration was observed 24 hours afterwards. In a follow-up study with the same rat model, Buerke et al. also found that C1-inh inhibited activation of the intramyocardial vascular endothelium in the first 8 hours after reperfusion. Also, positive effects on cardiac CK and PMN infiltration were observed after an extended period of reperfusion (48 hours).\textsuperscript{74} Later studies using rabbit\textsuperscript{75, 76} and mouse\textsuperscript{77} models of cardiac ischemia-reperfusion also found a decrease in infarct
size\textsuperscript{75-77} and a decrease in PMN infiltration and plasma markers of cardiac damage\textsuperscript{76, 77} 3 to 4 hours after reperfusion.

Effects of C1-inh were also studied in porcine models of cardiac ischemia-reperfusion. C1-inh injected intracoronarily 5 minutes after reperfusion resulted 2 hours afterwards in improved ejection fraction, fractional shortening, reduced infarct size and plasma markers of cardiac damage (CK and troponin T) and complement activation (C3a and C5a).\textsuperscript{78} In a follow-up study, wherein C1-inh was administered intravenously just before reperfusion, similar effects were found. Additionally, this study reported that the effect of C1-inh on infarct size disappears at high doses of C1-inh (100/200 U/kg).\textsuperscript{79}

In a clinical setting, human plasma-derived C1-inh concentrates have been administered as therapeutic for several decades to patients with hereditary angioedema, which is a safe treatment without significant side-effects.\textsuperscript{80} Several clinical trials have been performed administering C1-inh to AMI patients. Bauernschmitt \textit{et al.} found improvement of cardiac function of three AMI patients supplied with a C1-inh bolus (2000 U) just after failed emergency coronary surgeries, followed by two additional boluses (1000 U each) 12 and 24 hours after surgery.\textsuperscript{81} No controls however were included in this study. De Zwaan \textit{et al.} administered an intravenous bolus of 50-100 U/kg to AMI patients 6 hours after thrombolytic therapy, followed by a 48 hour C1-inh infusion (1.25-2 U/kg/hour), and found reduction in plasma levels of complement activation markers (C4b/c), troponin I and CK-MB levels compared to control patients.\textsuperscript{82} Effects on cardiac function and clinical outcome however were not reported. Following this, two trials were performed on ST-elevation myocardial infarction (STEMI) patients undergoing coronary artery bypass grafting (CABG). Similar to de Zwaan \textit{et al.}, Thielmann \textit{et al.} reported that C1-inh boluses given intravenously during (40 U/kg) and 6 hours after surgery (20 U/kg) resulted in decreased plasma levels of complement components (C3c and C4) and troponin I, but found no effect on clinical outcome (complication rate, hospitalization period and mortality) of the patients.\textsuperscript{83} Fattouch \textit{et al.} administered C1-inh intravenously as a bolus during surgery (500 U), followed by a 3 hour C1-inh infusion after surgery (also 500 U) and found improvement of cardiac function (stroke volume, cardiac index, arterial pressure) two hours post-surgery, and a reduced time of intubation and hospitalization. C1-inh however did not significantly affect mortality and complications such as bleeding, renal failure and arrhythmias.\textsuperscript{84}

Summarized, the C1-inh animal studies consistently showed beneficial effects (decreased infarct size, improved cardiac function and decreased cardiac inflammation). The clinical trials also showed some beneficial effects of C1-inh therapy, although improvement of clinical parameters (heart function, post-AMI complication rate) was not consistently reported.
<table>
<thead>
<tr>
<th>Study</th>
<th>Model Animal</th>
<th>Experimental protocol</th>
<th>Effect on infarct size</th>
<th>Effect on cardiac function</th>
<th>Other observed effects</th>
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<tbody>
<tr>
<td><strong>C1-inhibitor</strong></td>
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<tr>
<td>Buerke, 1995.</td>
<td>Cat</td>
<td>90m CAL – 4.5h REP</td>
<td>↓(NC/AAR)</td>
<td>↑ Cardiac contractility (dP/dt\text{max}) = Pressure Rate Index</td>
<td>↓ PCK in plasma ↑ Endothelial function ↓ MPO activity in AAR ↓ PMN binding to endothelium (in vitro) = Leukocytes in plasma</td>
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<td>Ref. 72</td>
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<td>Murohara, 1995.</td>
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<td>20m CAL – 24h REP</td>
<td>n.d.</td>
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<td>↓ Cardiac CK loss ↓ MPO activity</td>
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<td><em>Berinert</em>; 1x IV 1 min before REP; 100 U/kg</td>
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<td>Horstick, 1997.</td>
<td>Swine</td>
<td>60m CAL – 2h REP</td>
<td>↓ NC/AAR</td>
<td>↑ Apical Area EF ↑ Fractional Shortening = Cardiac Output</td>
<td>↑ AV lactate ↓ C3a in plasma ↓ C5a in plasma ↓ CK in plasma ↓ TroponinT in plasma</td>
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<td>Buerke, 1998.</td>
<td>Rat</td>
<td>20m CAL – 20m/8h/24h/48h REP</td>
<td>n.d.</td>
<td></td>
<td>↓ Cardiac CK (LVFW/septum) ↓ MPO activity (LVFW/septum) ↓ P-selectin in AAR ↓ ICAM-1 in AAR</td>
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<td>Ref. 74</td>
<td></td>
<td><em>Berinert</em>; 1x IV 2 min before REP; 10/50/100 U/kg</td>
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<td>Buerke, 2001.</td>
<td>Rabbit</td>
<td>60m CAL – 3h REP</td>
<td>↓(NC/AAR)</td>
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<td>Ref. 75</td>
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<td><em>Berinert</em>; 1x IV 5 min before REP; 100/200 U/kg</td>
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<tr>
<td>Horstick, 2001.</td>
<td>Swine</td>
<td>60m CAL – 2h REP</td>
<td>40 U/kg; ↓(NC/AAR) 100/200 U/kg = (NC/AAR)</td>
<td>n.d.</td>
<td>↑ Myocardial pO2 ↓ CK in plasma ↓ TroponinT in plasma ↓ C3a in plasma ↓ C5a in plasma ↑ TAT in plasma ↑ Infarct perfusion</td>
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<td>Schreiber, 2006.</td>
<td>Swine</td>
<td>2h CAL – 30m REP</td>
<td>= (NC/AAR)</td>
<td>= Stroke Volume = LVEDP = Aortic Pressure</td>
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<td>60m CAL – 3h REP</td>
<td>↓(NC/AAR)</td>
<td>n.d.</td>
<td>↓ CK in plasma ↓ MPO activity in LV</td>
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<td><em>Berinert</em>; 1x IV 5 min before REP; 100 U/kg</td>
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<td>Study</td>
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<td>Lu, 2013. Ref. 77</td>
<td>Mouse</td>
<td>30m CAL – 4h REP</td>
<td>↓(NC/AAR)</td>
<td>n.d.</td>
<td>↓ PMN infiltration in AAR</td>
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<td>1x IV 5 min before REP; 400 U/kg</td>
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<td>↓ TroponinT in plasma</td>
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<td><strong>C1s-inhibitor</strong></td>
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<tr>
<td>Buerke, 2001. Ref. 75</td>
<td>Rabbit</td>
<td>60m CAL – 3h REP</td>
<td>↓(NC/AAR)</td>
<td>= Pressure Rate Index</td>
<td>↓ CK in plasma</td>
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<td>C1s-INH-248: 1x IV 5 min before REP; 0.1/0.5/1.0 mg/kg</td>
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<td>↓ PMN infiltration in AAR</td>
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<td>↑ Superoxide dismutase in AAR</td>
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<td>↑ Troponin T in AAR</td>
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<td></td>
<td>↑ CK in AAR</td>
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<td></td>
<td>↑ Mitochondrial Protein P1 precursor in AAR</td>
</tr>
<tr>
<td>Buerke, 2006. Ref. 86</td>
<td>Rabbit</td>
<td>60m CAL – 3h REP</td>
<td>↓ (necrotic cells in AAR)</td>
<td>= Pressure Rate Index</td>
<td>↓ CK in plasma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C1s-INH-248: 1x IV 5 min before REP; 1.0 mg/kg</td>
<td></td>
<td></td>
<td>↓ PMN infiltration in AAR</td>
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<td></td>
<td>↑ Superoxide dismutase in AAR</td>
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<td>↑ Troponin T in AAR</td>
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<td>↑ CK in AAR</td>
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<td></td>
<td></td>
<td></td>
<td>↑ Mitochondrial Protein P1 precursor in AAR</td>
</tr>
<tr>
<td><strong>FUT-175</strong></td>
<td></td>
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<tr>
<td>Schwertz, 2002. Ref. 90</td>
<td>Rabbit</td>
<td>60m CAL – 4h REP</td>
<td>↓ (necrotic cells in AAR)</td>
<td>= Pressure Rate Index</td>
<td>↓ CK in plasma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1x IC 5 min before REP; 1mg/kg</td>
<td></td>
<td></td>
<td>↓ PMN infiltration in AAR</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>↑ αB-crystallin in AAR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ Superoxide dismutase in AAR</td>
</tr>
<tr>
<td>La Bonte, 2008. Ref. 91</td>
<td>Rat (diabetic)</td>
<td>30m CAL – 2h REP</td>
<td>↓(NC/AAR)</td>
<td>n.d.</td>
<td>↓ C3 in LV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1xIV 5 min before REP; 1mg/kg</td>
<td></td>
<td></td>
<td>↓ MPO activity in NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓ MPO activity in AAR</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>↓ ICAM-1 in LV</td>
</tr>
<tr>
<td>Schwertz, 2008. Ref. 76</td>
<td>Rabbit</td>
<td>60m CAL – 3h REP</td>
<td>↓(NC/AAR)</td>
<td>n.d.</td>
<td>↓ CK in plasma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1xIV 5 min before REP; 1mg/kg</td>
<td></td>
<td></td>
<td>↓ MPO activity in NC</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓ MAC in AAR</td>
</tr>
<tr>
<td><strong>Soluble Complement Receptor 1</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Smith, 1993. Ref. 98</td>
<td>Rat</td>
<td>30m CAL – 24h REP</td>
<td>= (NC/LV)</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>BRL55730; 1x IV 5 min before REP; 5 mg/kg</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Murohara, 1995. Ref. 73</td>
<td>Rat</td>
<td>20m CAL – 24h REP</td>
<td>n.d.</td>
<td>n.d.</td>
<td>↓ MPO activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>des-LHR-A; 1x IV 1 min before REP; 15 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Model</td>
<td>Animal</td>
<td>Experimental protocol</td>
<td>Effect on infarct size</td>
<td>Effect on cardiac function</td>
</tr>
<tr>
<td>------------------------</td>
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<tr>
<td><strong>Soluble Complement Receptor 1</strong></td>
<td></td>
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</tr>
<tr>
<td>Lazar, 1999.</td>
<td>Swine</td>
<td>90m CAL – 3h REP</td>
<td>↓(NC/AAR)</td>
<td>↑ Wall motion score</td>
<td></td>
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<td></td>
<td></td>
<td>30 min IV infusion starting 5 min after start CAL; 10 mg/kg</td>
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<tr>
<td>Zacharowski, 1999.</td>
<td>Rat</td>
<td>30m CAL – 2h REP</td>
<td>↓(NC/AAR)</td>
<td>= Mean arterial pressure = Pressure rate index</td>
<td>sCR1:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sCR1 (TP-10) or sCR1sLe&lt;sup&gt;x&lt;/sup&gt; (TP-20); 1x IV 5 min before REP; 1/5/15 mg/kg</td>
<td></td>
<td></td>
<td>= C5b-9 in plasma</td>
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<td></td>
<td>Mirococept (APT070); 1x IC 10 min before REP; 0.5 mg/kg</td>
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<td></td>
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<td></td>
<td>APT154; 1x IC 10 min before REP; 1.37 mg/kg</td>
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</tr>
<tr>
<td>Banz, 2007.</td>
<td>Swine</td>
<td>60m CAL – 2h REP</td>
<td>Mirococept; ↓(NC/AAR)</td>
<td>= EF = FS = mean arterial pressure = LVEDP = LVESP</td>
<td>APT154;</td>
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<tr>
<td>Hartmann, 1977.</td>
<td>Dog</td>
<td>24h CAL - no REP</td>
<td>↓ (necrosis in ST elevation areas)</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1x IV 30 min after start CAL; 20 U/kg</td>
<td></td>
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<tr>
<td>Maroko, 1978.</td>
<td>Dog</td>
<td>24h CAL – no REP</td>
<td>↓ (necrosis in ST elevation areas)</td>
<td>= Cardiac Output = Cardiac Contractility = LVEDP = LVESP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1x IV 30 min after start CAL; 20 U/kg</td>
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<tr>
<td></td>
<td></td>
<td>1x IV 5 min after start CAL; 20 U/kg</td>
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<tr>
<td></td>
<td></td>
<td>30 min IV infusion 30 min after start CAL; 125 U/kg</td>
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</tr>
<tr>
<td>Study</td>
<td>Model Animal</td>
<td>Experimental protocol</td>
<td>Effect on infarct size</td>
<td>Effect on cardiac function</td>
<td>Other observed effects</td>
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<td>-------------------------------------------------</td>
</tr>
<tr>
<td>Anti-C5</td>
<td>Rat</td>
<td>30m CAL – 4h/7d REP</td>
<td>↓(NC/LV)</td>
<td></td>
<td>n.a.</td>
</tr>
<tr>
<td>Vakeva, 1998.</td>
<td>Ref. 113</td>
<td>18A: 1x IV 5 min before REP; 20 mg/kg</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Rat</td>
<td>40m CAL – 28d REP</td>
<td>↓(NC/LV)</td>
<td>= Cardiac output = FS</td>
<td>↓ Mortality</td>
</tr>
<tr>
<td>Clusterin</td>
<td>Rat</td>
<td>3x IV 10m/1d/2d after REP; 0.3 mg</td>
<td></td>
<td></td>
<td>↑ macrophage infiltration in NC</td>
</tr>
</tbody>
</table>

**Abbreviations/symbols:** AAR: area at risk. CAL: Coronary artery ligation. CK: Creatine kinase. EF: Ejection fraction. FS: Fractional shortening IC: Intracoronarily. ICAM-1: Intercellular adhesion molecule-1. IV: Intravenously. LV: left ventricle. LVEDP: Left ventricular end-diastolic pressure. MAC: Membrane-attack Complex. n.a.: not applicable. n.d.: not determined. NC: necrotic core. NS: not significant. PMN: Polymorphonuclear leukocyte (neutrophil). Ref: Reference. REP: Reperfusion. TAT: Thrombin-antithrombin. ↓: significantly lower compared to untreated group. ↑: significantly higher compared to untreated group. = no significant difference compared to untreated group.

**Note:** Infarct sizes were determined at end of study protocol

### C1s-inhibitor

C1s-inh is a synthetic inhibitor that specifically targets C1s, thereby interfering with C1 assembly and inhibiting the classical pathway of complement activation.\(^{85}\) Unlike C1-inh, C1s-inh does not affect other complement activation pathways, nor has it known effects outside of the complement system. In a rabbit model of cardiac ischemia-reperfusion, Buerke et al. demonstrated that intravenous C1s-inh administration 5 min before reperfusion significantly reduced infarct size, plasma CK levels and PMN infiltration 3 hours later.\(^{75}\) In a follow-up study using the same model and treatment protocol, it was also observed that C1s-inh resulted in restored expression levels of several proteins in the post-ischemic myocardium, including superoxide dismutase (a reactive oxygen species scavenger), CK and troponin T, indicating that C1s resulted in decreased oxidative stress and increased cardiomyocyte viability.\(^{86}\)

Thus far, no clinical trials have been reported for C1s-inh.

### FUT-175

FUT-175 (also known as 6-amidino-2-naphthyl p-guanidinobenzoate dimethanesulfonate or Nafamostat Mesilate) is a synthetic protease inhibitor that inhibits complement activation through the classical pathway by binding to C1r and C1s, which prevents C1-mediated cleavage of C2 and C4. The alternative pathway is affected as well by the binding of FUT-175 to factor B, which inhibits assembly of a functional alternative C3-convertase.\(^{87-89}\) Besides the complement
system, FUT-175 also inhibits components of the coagulation system.\textsuperscript{87, 88} In a rabbit model of cardiac ischemia-reperfusion, Schwertz \textit{et al.} administered FUT-175 intracoronary 5 min before reperfusion, and found that FUT-175 decreased infarct size, PMN infiltration and plasma CK levels, as well as increased levels of superoxide dismutase and αB crystallin (a heat-shock protein that has a protective role in cardiomyocytes exposed to inflammatory stress) 4 hours after reperfusion.\textsuperscript{90} In a follow-up study, FUT-175 was administered intravenously instead. Again, a reduction in infarct size, PMN infiltration and plasma CK levels was found (3 hours after reperfusion), as well as a decrease in MAC depositions in the heart.\textsuperscript{76} La Bonte \textit{et al.} studied the effects of FUT-175 administered intravenously 5 min before reperfusion in a rat cardiac ischemia-reperfusion model, but then in diabetic rats. Also in this study FUT-175 decreased infarct size, PMN infiltration and plasma CK levels (2 hours after reperfusion).\textsuperscript{91}

FUT-175 has been used successfully in several clinical trials in the prevention of post-cholangiopancreatography pancreatitis without any significant adverse effects.\textsuperscript{92, 93} Thus far, to the best of our knowledge no clinical studies on AMI patients have been performed however.

\textbf{Soluble Complement Receptor 1}

Complement receptor 1 (CR1, CD35) is a cell membrane-bound receptor that binds C3b and C4b and inhibits all three pathways of complement activation by destabilizing C3-convertase and C5-convertase.\textsuperscript{94} It can also inhibit complement activation by serving as a cofactor for factor I, and stimulates clearance of immune complexes and regulates B-cell proliferation (reviewed in detail by Khera \textit{et al.}\textsuperscript{95}). CR1 can be detected in soluble form in human plasma, although its levels are very low compared to other complement inhibitors, making it difficult to use human plasma as a source of therapeutic soluble CR1.\textsuperscript{96} For therapeutic application, Weisman and colleagues therefore developed a recombinant form of soluble CR1 (referred to as sCR1).\textsuperscript{97} Smith \textit{et al.} and Murohara \textit{et al.} analysed sCR1 in a rat cardiac ischemia-reperfusion model where sCR1 was applied just before reperfusion.\textsuperscript{73, 98} Murohara \textit{et al.} found a significant reduction in infarct size, as well as decreased PMN infiltration and plasma levels of cardiac CK 24 hours after reperfusion, while Smith \textit{et al.} only described a non-significant decrease in infarct size. Zacharowski \textit{et al.} compared sCR1 with an alternatively glycosylated form of sCR1 (sCR1sLe\textsuperscript{a}) which, besides inhibiting complement activation, also binds endothelial selectins to reduce leukocyte extravasation. Both forms were administered just before reperfusion in a rat model of cardiac ischemia-reperfusion. They found infarct size reduction and reduced plasma troponin T levels for both forms 2 hours after reperfusion, although only sCR1sLe\textsuperscript{a} significantly inhibited PMN infiltration.\textsuperscript{99}

In a swine model of cardiac ischemia-reperfusion injury, Lazar \textit{et al.} observed that intravenous sCR1 administration immediately after coronary artery ligation resulted in an improved wall motion score (semi-quantitative scale
for wall contraction during systole) 3 hours after reperfusion, as well as a reduced infarct size.100 Finally, Banz et al. investigated mirococept (APT070), a sCR1 variant with an added membrane anchor to increase retention of the inhibitor in inflammatory sites.101 After intracoronary administration 10 minutes before reperfusion in a swine model of cardiac ischemia-reperfusion, mirococept resulted in a decreased left ventricular end-diastolic pressure (LVEDP), increased ejection fraction (EF) and fractional shortening (FS) and decreased infarct size 2 hours after reperfusion.102 However, only for the LVEDP and infarct size the differences were significant.

In a phase I clinical trial, TP-10 (same brand of sCR1 also used by Zacharowski et al.99) was administered to a small group of patients with respiratory dysfunction.103 Herein, TP-10 was found to be safe and able to inhibit complement activation in patients. Later, a larger trial was performed in patients undergoing cardiopulmonary bypass surgery.104 Again, no detrimental effects were found for TP-10. Also, the incidence of AMI and death was significantly reduced as result of TP-10 administration, albeit for male patients only. Thus far, no clinical trials administrating sCR1 to AMI patients have been performed.

**Cobra Venom Factor**

CVF is isolated from the venom of several cobra species of the *Naja* genus.105 In plasma, CVF utilizes complement factor B to form the complex CVF-Bb, which completely turns over (depletion) all complement C3.106 Its potency to inhibit virtually all complement-mediated effects has made CVF a widely used tool for proof-of-concept studies on the role of complement in various diseases. For instance, Hill & Ward treated rats with CVF 36 hours before induction of permanent coronary artery ligation and observed decreased PMN infiltration of the infarcted myocardium.4 The use of CVF as therapeutic drug was first described by Hartmann et al. In a dog model, intravenously administered CVF 30 minutes after permanent coronary artery ligation resulted in a decreased amount of necrosis, decreased inflammation and increased creatine kinase (CK) activity in the myocardium 24 hours post-AMI.107 Maroko et al. and Crawford et al. performed similar experiments in respectively a dog and baboon model of permanent coronary artery ligation. In both models, a decrease in necrosis and PMN infiltration and increased CK activity was observed 24 hours post-AMI.108, 109 In addition, Maclean et al. observed in rats that the infarct-reducing effect of CVF was still measurable as late as 21 days after permanent coronary artery ligation.110 A humanized form of CVF (a hybrid between human complement C3 and CVF) has been developed just over 5 years ago.111 Gorsuch et al. demonstrated in a proof-of-concept study that humanized CVF can improve cardiac function and reduce infarct size in a mouse model of AMI when administered two hours before induction of AMI.112 Animal studies applying humanized CVF as post-AMI treatment however have thus far not been performed. Thus far, no clinical trials have been performed for CVF.
### Table 2 Effect of complement inhibitors in clinical trials on AMI

| Study                      | Trial name | Included patients                                                                                                                                                                                                 | Treatment protocol                                                                                     | Observed effects of treatment                                                                                     |
|----------------------------|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|
| **C1-inhibitor**           |            |                                                                                                                                                                                                                 |                                                                                                           |
| Bauernschmit, 1998. Ref. 81| n.a.       | emergency coronary surgery procedure after failed PTCA procedure: n=3 – treatment group no control group                                                                                                      | *Berinert*: 1x bolus® 2000U 50-170 min after surgery + 2x bolus 1000U 12h/24h after surgery           | Rapid improvement of cardiac function and survival of all patients *(time point unknown)*.  
↑ Systolic pressure *(8h post-I)*  
↓ Pulmonary wedge pressure *(8h post-I)* |
| De Zwaan, 2002. Ref. 82    | n.a.       | AMI patients receiving thrombolytic therapy <2h post-AMI: n=13 – treatment group n=18 – control group                                                                                                        | *Cetor*: 1x IV bolus 6h post-AMI 50-100 U/kg + IV infusion → 1x 50-100U/kg + 1.25-2.0 U/kg/h, 48h       | ↓ plasma C4b/c *(6-72h post-I)*  
↓ plasma troponin T *(6-72h post-I)*  
↓ plasma CK-MB *(10-48h post-I)* |
| Thielmann, 2006. Ref. 83   | n.a.       | STEMI-patients undergoing emergency CABG: n=28 – treatment group n=29 – control group                                                                                                                            | *Berinert*: 1x IV bolus 40 U/kg 5 min before end of CABG + 1x IV bolus 20U/kg 6h after CABG            | ↓ plasma C3c *(1-48h post-S)*  
= plasma C4 *(1-48h post-S)*  
↓ plasma troponin I *(36-48h post-S)*  
= mortality *(30d post-S)*  
= post-AMI complications *(30d post-S)* |
| Fattouch, 2007. Ref. 84    | n.a.       | STEMI-patients undergoing emergency CABG: n=38 – treatment group n=42 – control group                                                                                                                            | *C1-inhib*: 1x IV bolus 500 U 10 min before end of CABG + IV infusion of 500 U over 3h (after CABG)  | ↑ Mean Arterial Pressure *(2h post-I)*  
↑ Stroke Volume *(2h post-I)*  
↑ Cardiac Index *(2h post-I)*  
↓ plasma C3a *(10m-2h post-I)*  
↓ plasma C4a *(40m-2h post-I)*  
↓ plasma troponin I *(12-48h post-I)*  
↓ Wall Motion Score *(30m-3h post-I)*  
= in-hospital mortality  
= in-hospital post-AMI complications  
↓ duration of intubation and hospitalization |

**Anti-C5**

|                          |            | AMI patients receiving coronary angioplasty: n=262 – treatment group 1 n=281 – treatment group 2 n=271 – control group                                                                 | *Pexelizumab*: 1x bolus® 2 mg/kg right before first balloon inflation *(group 1)*  
*Pexelizumab*: 1x bolus® 2 mg/kg right before first balloon inflation + 20h infusion 0.05 mg/kg/h starting 4h after bolus *(group 2)* | = plasma CK-MB *(group 1+2: 0-72h post-I)*  
↓ mortality *(group 2 only: 90-180d post-I)*  
= post-AMI complications *(group 1+2: 6d-90d post-I)* |
<p>| Granger, 2003. Ref. 115   | COMMA (phase II) |                                                                                                                                                                                                                  |                                                                                                           |                                                                                                           |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>Trial name</th>
<th>Included patients</th>
<th>Treatment protocol</th>
<th>Observed effects of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mahaffey, 2003.</td>
<td>COMPLY (phase II)</td>
<td>STEMI patients receiving fibrinolytic therapy: n=304 – treatment group 1 n=309 – treatment group 2 n=307 – control group</td>
<td>Pexelizumab: 1x bolus® 2 mg/kg right before start fibrinolytic therapy (group 1) Pexelizumab: 1x bolus® 2 mg/kg before start fibrinolytic therapy + 20h infusion 0.05 mg/kg/h starting 4h after bolus (group 2)</td>
<td>= plasma CK-MB (group 1+2: 0-72h post-I) = mortality (group 1+2: 6d-180d post-I) = post-AMI complications (group 1+2: 6d-90d post-I)</td>
</tr>
<tr>
<td>Theroux, 2005.</td>
<td>COMMA (phase II)</td>
<td>STEMI patients receiving coronary angioplasty: n=151 – treatment group n=175 – control group</td>
<td>Pexelizumab: 1x bolus® 2 mg/kg right before first balloon inflation + 20h infusion 0.05 mg/kg/h starting 4h after bolus.</td>
<td>↓ plasma hsCRP (24h post-I) ↓ plasma IL6 (24h post-I) ↑ plasma TNF-α (24h post-I)</td>
</tr>
<tr>
<td>Armstrong, 2007.</td>
<td>APEX-AMI (phase III)</td>
<td>STEMI patients receiving coronary angioplasty: n=2860 – treatment group n=2885 – control group</td>
<td>Pexelizumab: 1x IV bolus 2 mg/kg before start of procedure + 24h infusion of 0.05 mg/kg/h.</td>
<td>= mortality (30-90d post-I) = post-AMI complications (30-90d post-I)</td>
</tr>
<tr>
<td>Martel, 2012.</td>
<td>APEX-AMI (phase III)</td>
<td>STEMI patients receiving coronary angioplasty: n=175 – treatment group n=181 – control group</td>
<td>Pexelizumab: 1x IV bolus 2 mg/kg before start of procedure + 24h infusion of 0.05 mg/kg/h.</td>
<td>↓ plasma C5a (24h post-I) ↓ plasma IL6(24h post-I) = plasma sCSb-9, CRP, IL4, IL10, IL12, IL1β, IL1ra, TNFα, IFNγ, CCL5, IP10, NT-proBNP (24h post-I)</td>
</tr>
</tbody>
</table>

**Abbreviations/symbols:** CAGB: Coronary artery bypass grafting. CCL5: Chemokine Ligand 5. CK-MB: Creatine kinase MB. (hs)CRP: (High-sensitivity) C-reactive protein. IFNy: Interferon gamma. IL: Interleukin. IP10: IFNy-induced protein 10. IV: Intravenously. NT-proBNP: N-terminal pro-Brain Natriuretic Peptide. Post-I: post-intervention. STEMI: ST-elevation myocardial infarction. TNF-α: Tumour necrosis factor-α. ↓: significantly lower compared to untreated group. ↑: significantly higher compared to untreated group. = no significant difference compared to untreated group.

**Trial names:** APEX-AMI: Assessment of Pexelizumab in acute myocardial infarction. COMMA: Complement inhibition in myocardial infarction treated with angioplasty. COMPLY: Complement inhibition in myocardial infarction treated with thrombolytics.

**Notes:** (A) C1-inhibitor brand not mentioned in article. (B) Administration route not mentioned in article. (C) Primarily for CABG. Clinical Outcome consists of mortality and the incidence of post-AMI complications.

**Anti-C5**

Vakeva and colleagues isolated antibodies (termed 18A) from C5-deficient mice immunized with rat C5, which were able to block formation of MAC and release of the anaphylatoxin C5a. In a rat model of cardiac ischemia-reperfusion injury
Vakeva et al. demonstrated that a bolus of 18A antibodies just before reperfusion significantly reduced the infarct size in rats, measured after 4 hours and 7 days. Next to antibodies targeting C5, cardioprotective effects have also been described for antibodies specifically targeting C5a and shRNAs against C5 in respectively a rat model and a swine model. However, in these studies the therapy was administered to animals before induction of ischemia, and whether these compounds also exhibit a beneficial effect when administered after induction of ischemia has not yet been determined.

In two phase II clinical trials, humanized monoclonal anti-C5 antibodies (Pexelizumab) were given to STEMI-patients in combination with either fibrinolytic therapy (The COMPLY trial) or coronary angioplasty (the COMMA trial). Both studies tested two Pexelizumab treatment protocols: a single bolus (2mg/kg) or a bolus (2mg/kg) followed 4 hours later by a 20 hour infusion (0.05 mg/kg/hour). Plasma CK-MB levels were measured, as well as mortality and incidence of complications such as heart failure and arrhythmia. In the COMPLY trial, no beneficial effects of Pexelizumab were reported. However, the COMMA trial found a significant reduction in mortality, especially when the Pexelizumab bolus was followed by a period of Pexelizumab infusion. Furthermore, the Pexelizumab treatment in the COMMA trial was also associated with a decrease in inflammation markers (C-reactive protein and interleukin-6) in plasma. The COMMA trial was therefore succeeded by a phase III clinical trial (the APEX-AMI trial), to determine if the positive effects were reproducible in a larger patient cohort. In this trial, a reducing effect of Pexelizumab was found in plasma levels of C5a and interleukin-6. However, in contrast to the COMMA trial, no effects on the incidence of clinical complications such as death, heart failure and shock were found.

Clusterin
Clusterin, also known as Apolipoprotein J, is an endogenous plasma inhibitor that binds to complement components C7, C8 and C9 and prevents functional assembly of MAC. Besides inhibiting complement, other described functions of clusterin include lipid transportation, control of cell adhesion and protection against oxidative stress. In a rat model of ischemia-reperfusion injury, van Dijk et al. administered three boluses of clusterin intravenously 10 min, 1 day and 2 days following reperfusion and found a decrease in infarct size and mortality 28 days after reperfusion. Also, increased numbers of macrophages in the infarcted heart were described. Some improvement in cardiac output and reduction in PMN infiltration was also reported, although this was not statistically significant.

To the best of our knowledge however, clusterin has never been therapeutically administered to patients.
Summary of current studies and issues of concern related to complement inhibitor treatment of AMI.
In this review, we have focused specifically on AMI rather than all forms of ischemia-reperfusion injury and restricted our analyses to in vivo treatment of AMI in animal models and clinical trials. Summarized, protective effects (decreased infarct size, improved cardiac function and decreased cardiac inflammation/damage) in animal models have been demonstrated for C1-inh, C1s-inh, FUT-175, sCR1, CVF, anti-C5 and clusterin, confirming the involvement of complement activation in AMI pathophysiology. Of two complement inhibitors, namely C1-inh and pexelizumab, therapeutic application was also studied in clinical trials. For neither of these inhibitors significant reproducible improvement of clinical end-points such as mortality or incidence of post-AMI complications was found. For C1-inh however, some improvement in cardiac function and plasma markers of cardiac damage and inflammation were reported. In our analysis, several important issues became evident that will require more attention in future research.

Inhibitory targets inside and outside the complement cascade
As mentioned above, C1-inh is the only complement inhibiting compound thus far for which reproducible protective effects have been described for AMI patients. A reduction in plasma levels of complement activation markers has been described in three of four trials,82-84 which indicates that complement inhibition has occurred in these patients. However, this does not exclude the possibility that the protective effects of C1-inh were, at least partially, the result of effects outside the complement system, such as inhibition of leukocyte extravasation or inhibition of the coagulation system.122 Indeed, Lu et al. found that C1-inh also resulted in a reduction in infarct size and PMN infiltration in mice deficient in complement C3.77 This is important to consider, as differences in efficacy between complement inhibitors may to certain extent be the result of activities not related to the complement system.

Another factor which may cause differences in efficacy between different complement inhibitors is the point of action within the complement cascade (see figure 2). For instance, C1-inh affects the complement cascade at the start of the classical and lectin pathways.122 However, the role of the alternative pathway in post-AMI complement activity remains unclear. Therefore, it is theoretically possible that complement activation occurs via this pathway, circumventing the effects of C1-inh. Alternatively, pexelizumab prevents cleavage of C5 into C5a and C5b, which affects the terminal pathway of all three complement activation routes. However, it does not prevent release of the pro-inflammatory anaphylatoxin C3a.

In summary, complement inhibitors differ in point of action within the complement system and also in actions outside of the complement system. Knowledge on this is vital for the selection of the most suitable complement
inhibitor for research. In line with this, the possible participation of the lectin and alternative pathways in the post-AMI heart requires more clarification.

*Duration of the complement-inhibiting therapies*

The second concern is the duration of the therapy. As pointed out in this review, it is not known how long complement stays activated in the animal heart following ischemia-reperfusion injury. This is important, as many complement inhibitors have a half-life of several hours in murine models. The half-life is especially short when inhibitors are administered intravenously, the most common administration route for complement inhibitors in pre-clinical animal studies (table 1). If an inhibitor (supplied as a bolus following AMI) is already degraded while complement activity remains present in the infarcted myocardium, the long-term treatment efficacy may be limited.

Human plasma-derived C1-inh has a plasma half-life of 4.5 hours in rats and around 33 hours in humans. De Zwaan *et al.* demonstrated that a C1-inh bolus followed by 48-hour infusion protocol results in increased plasma levels of C1-inh which likely stay increased for several days after the end of the infusion protocol. Based on the knowledge on the duration of complement activity in the human post-AMI myocardium, it is plausible that C1-inh plasma levels stayed increased for the entire period in which complement is highly active in the infarcted myocardium. Related to the studies from Thielmann and Fattouch, it is difficult to make a comparison based on C1-inh pharmacokinetics. However, in these two studies, the surgical procedure (emergency CABG caused) itself caused a decline in C1-inh plasma levels. The C1-inh administration compensated for this loss, but did not elevate the C1-inh plasma levels above the baseline values measured in plasma obtained pre-operatively.

Several clinical trials on AMI patients have been performed using pexelizumab, despite the limited amount of pre-clinical data on antibodies targeting C5 as therapeutic option for AMI. Prior to the COMMA and COMPLY trials, a pharmacokinetics study was performed for pexelizumab, revealing that a bolus of 2 mg/kg constituted a plasma half-life of 14 hours and a decreased plasma complement activity for up to 24 hours. In the COMMA, COMPLY and APEX-AMI trials a 2 mg/kg bolus was followed by an infusion of 0.05 mg/kg/h covering a 24 hour period following start of the therapy. With this treatment protocol, complement inhibition declined quickly after the infusion stopped and complement activity was back to baseline value 48 hours after start of therapy. Given the longer timeframe in which complement is activated in the infarcted heart, extension of the infusion period to cover the first five days after start of the AMI may have resulted in a more substantial effect. However, to test this hypothesis, additional clinical studies need to be performed.

Finally, for future studies on the timeframe of post-AMI complement activation, a difficulty should be noted. Even though immunohistochemical staining of complement components on cardiac tissue gives a more accurate
representation of complement activity compared to plasma measurements, complement components may linger in tissue after the complement activity has ceased. Therefore, this may result in an overestimation of the total duration of complement activation in the post-AMI heart.

In summary, the efficacy of complement inhibitor therapy may be increased when duration of therapeutic application is similar to the period of complement activity in the human/animal post-AMI heart. This hypothesis is thus far untested however.

The model system of choice for animal studies.
As mentioned above, permanent coronary artery ligation and ischemia-reperfusion injury differ in the timeframe of complement activation. This distinction is important to keep in mind when designing animal studies on the effects of complement inhibitors. All included animal studies using CVF as complement inhibitor employed permanent coronary artery ligation, while all included studies investigating other inhibitors used a ischemia-reperfusion model. Also, the CVF-studies deviate from the other animal studies in parameters used to measure the effects of the complement inhibitor, making it impossible to compare both model systems in the animal studies included here. However, reperfusion therapy is fundamental in modern clinical practice for AMI patients, also evident by the fact that all included clinical trials have administered C1-inh and pexelizumab in combination with a form of reperfusion therapy. Therefore, ischemia-reperfusion therapy is a more logical choice for future pre-clinical studies compared to permanent coronary artery ligation.

Time point of analysis.
The first concern is the time points at which the effects of complement inhibition were measured. For no animal study, therapeutic effects of C1-inh were measured at later time points than 48 hours after reperfusion. The positive effects described for C1-inh in clinical trials (improvement of cardiac function and reduction in plasma markers of cardiac damage and inflammation) were all measured only between 1 and 72 hours following AMI. Even though a reducing effect of C1-inh on cardiac necrosis and other markers of cardiac damage can be observed after a few hours of reperfusion, it cannot be excluded that C1-inh might delay the AMI-related damage rather than inhibit it. To study this further, therapeutic effects of C1-inh need to be measured also at time points later than 72 hours post-AMI.

For pexelizumab, it is difficult to make a comparison between clinical trials and animal studies as pexelizumab was never tested in an AMI animal model to the best of our knowledge. For CVF\textsuperscript{110} and clusterin\textsuperscript{121}, long term effects were studied in rats (21 and 28 days post-AMI respectively), and both studies reported a decrease in infarct size at these time points. However, for CVF cardiac function was not measured, and for clusterin only a small (non-significant) increase in
cardiac output was found. Long-term effects of therapeutic complement inhibition should be included in future animal studies, so that findings can be extrapolated better towards clinical practice.

In summary, previous studies have insufficiently addressed long-term effects of therapeutic administration of complement inhibitors. This is important, as a short-term benefit may not necessarily remain beneficial at later time points.

**Adverse drug effects**

Finally, we looked at reported adverse effects of complement inhibitor administration in patients, as complement deficiency is associated with an increased susceptibility for infectious diseases.\(^{128}\) The clinical trials that investigated C1-inh or pexelizumab in AMI patients all reported no drug-related adverse effects however. Additionally, sCR1 and FUT-175 have been administered to patients in clinical trials as well, albeit not directly related to AMI.\(^{92, 93, 103, 104}\) For FUT-175, no drug-related adverse effects were described.\(^{92, 93}\) For sCR1 however, a number of adverse effects were described in one study, including rash, hypotension, anuria, pneumonia and renal failure.\(^{103}\) These adverse effects were not considered to be related to infections however. The second clinical trial however, using the same sCR1 brand (TP-10), reported no adverse effects.\(^{104}\)

Summarized, adverse drug effects are not a major concern for compounds investigated thus far in the context of AMI, and patients were not reported to have an increased vulnerability for infections. The concern for infections is primarily linked to genetic complement defects, or to chronic therapeutic complement inhibition.\(^{129}\) A short (<48h) therapeutic complement inhibition likely provides a neglible window of opportunity for opportunistic infectious agents.

**Future perspectives**

Overall, we have found no solid reason why the concept of complement inhibition as therapeutic option for AMI patients should be abandoned as a whole. For C1-inh, we encourage the advancement towards larger clinical trials, as the previously published clinical trials and animal studies do not provide any basis why C1-inh would have insufficient efficacy for AMI patients. FUT-175 and sCR1 are also suitable candidates to consider for clinical trials, as animal studies show favourable results and administration to patients is considered to be safe. However, we would like to stress the importance of the issues of concern raised in this review (time point of analysis, therapy duration and the precise therapeutic targets), which should be taken into consideration in the design of future preclinical and clinical studies on complement inhibitory therapies for AMI.
References


