Prognostic significance of blood markers of inflammation in patients with ST-segment elevation myocardial infarction undergoing primary angioplasty and effects of pexelizumab, a C5 inhibitor: a substudy of the COMMA trial

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Aims Pexelizumab, a monoclonal antibody inhibiting C5, reduced 90 day mortality and shock in the Complement inhibition in Myocardial infarction treated with Angioplasty (COMMA) trial without apparent reductions in infarct size. Inflammation is a critical component of ST-elevation myocardial infarction (STEMI); this substudy examines prognostic values of selected markers and treatment effects.

Methods and results C-reactive protein, interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α) serum levels were assessed in 337 patients enrolled in either the placebo or the pexelizumab 24 h infusion group. Higher C-reactive protein and IL-6 levels at baseline, 24 h, and 72 h were strongly associated with increased subsequent death \((P, 0.002 \text{ at baseline and 24 h, } P, 0.02 \text{ at 72 h})\); and all baseline marker levels with death or cardiogenic shock \((P, 0.03)\) within 90 days. C-reactive protein and IL-6 levels were similar at baseline, but significantly lower 24 h later with pexelizumab, when compared with placebo (17.1 vs. 25.5 mg/L, \(P = 0.03\) and 51.0 vs. 63.8 pg/mL, \(P = 0.04\), respectively). At 72 h, corresponding levels were similar, whereas TNF-α was slightly higher \((P = 0.04)\) in the treated group.

Conclusion Inflammation markers and their serial changes predict death and shock in patients with STEMI undergoing primary angioplasty. Pexelizumab reduced C-reactive protein and IL-6, suggesting treatment benefits mediated through anti-inflammatory effects.

KEYWORDS
Myocardial infarction; Primary angioplasty; Complement inhibition; Pexelizumab; C-reactive protein; Interleukin-6; Tumour necrosis factor

Introduction

Pexelizumab is a unique single-chain fragment of a humanized monoclonal antibody specifically directed against the complement protein C5.1 The administration of pexelizumab in patients with an acute ST-elevation myocardial infarction (STEMI), managed with primary percutaneous coronary intervention (PCI), was associated with a substantial reduction in mortality and cardiogenic shock compared with placebo in the Complement inhibition in Myocardial infarction treated with Angioplasty (COMMA) trial.2 The benefits were achieved with no detectable effect on infarct size as measured by the area under the curve of CK-MB release, the trial’s primary endpoint. This implied that non-conventional mechanisms could be operative and further exploration was warranted.

The complement system is an important innate humoral modulator; it mediates defence mechanisms against infection, initiates and amplifies inflammation, bridges innate and adaptive immunity, induces apoptosis, and clears autoimmune and apoptotic material. Complement is also recognized as being an important contributor to ischaemia-reperfusion injury.3–7 Markers of inflammation and activated complement proteins are elevated in acute coronary syndromes and are related to poor outcomes.5,6,8,9 Moreover, complement proteins deposit in the atherosclerotic plaque...
and in the infarct area, where they co-localize with C-reactive protein.\textsuperscript{7,10} Experimental and clinical studies have shown a protective effect in inhibiting the activation of complement.\textsuperscript{2,11,12}

The goals of this substudy are to assess the prognostic value of selected markers of inflammation on the 90 day incidence of death and cardiogenic shock, and the effects of pexelizumab on these markers, identifying possible mechanisms through which the drug reduced these events in the COMMA trial.

Methods

Study objectives and design

The primary objective of this substudy was to examine the prognostic value of the levels of markers on the occurrence of death (all-cause mortality) and of death or cardiogenic shock through the following 90 days, with the secondary objective to characterize the effects of pexelizumab on these markers. Cardiogenic shock was defined as systolic blood pressure $<90$ mm Hg for $\geq1$ h, associated with cool, clammy skin, oliguria, altered sensorium, and/or a cardiac index $\leq2.2$ L/min/m\textsuperscript{2}, unresponsive to fluid replacement alone, and considered secondary to cardiac dysfunction. Follow-up was assessed through in-hospital visits at 14, 30, and 90 days after randomization.

Study population

The COMMA study enrolled 960 patients with acute MI and ST-segment elevation or new left bundle branch block, with symptom duration $<6$ h for whom primary angioplasty (PCI) was planned.\textsuperscript{2} Patients were randomized to receive (i) a 2.0 mg/kg bolus of pexelizumab before PCI and a 24 h placebo infusion, (ii) a 2.0 mg/kg bolus and 24 h infusion (0.05 mg/kg/h) of pexelizumab, or (iii) a placebo bolus and placebo infusion. A study-wide cap of 20% of patients with isolated inferior wall MI was implemented during recruitment to ensure a substantial proportion of patients with large infarct size. As benefits in COMMA were observed most clearly in the pexelizumab bolus and infusion group, this substudy focuses on patients from this treatment group and the placebo group to gain insights on involved mechanisms. Among patients from the main trial, inclusion also applied to the availability of previously unthawed blood samples at specific time points thought to be especially germane in assessing treatment effects: at baseline, end of infusion at 24 h, and 48 h later. Patients who experienced an outcome of death or cardiogenic shock during this time period were also included as long as the baseline blood sample was available. In the main trial, blood had originally been sampled for the substudy.\textsuperscript{1965}

Blood sampling and analyses

Blood was obtained through a forearm venipuncture or, at baseline, from the arterial access site for coronary angiography using a red top vacutainer tube. The tubes were to be allowed to clot for 35–45 min and the blood centrifuged within 1 h of collection for 15 min at 3000 r.p.m. After centrifugation, 1 mL of serum was to be carefully transferred into a cryotube. All specimens were to be shipped after 6 days or at discharge in dry ice to a central laboratory located either in North America or in Europe, where it was stored at $-70^\circ$C. For the current substudy, the frozen serum samples were shipped to the Montreal Heart Institute, where all analyses on a certain patient were carried out on the same day. All kits came from the manufacturer from a single batch production. At baseline, 29 marker levels were missing from 1011 possible measurements, 19 were missing at 24 h from 984 possible measurements, and 29 missing at 72 h from 972 possible measurements.

C-reactive protein levels were measured by particle-enhanced immunonephelometry, using both a high sensitivity C-reactive protein reagent and the Dade Behring Nephelometer BN\textsuperscript{TM} ProSpec (Marburg, Germany). Interleukin-6 (IL-6) levels were assessed by the solid phase enzyme amplified sensitivity immunoassay (EASIA E\textsuperscript{TM} ELISA, BioSource International, Inc., Camarillo, CA USA), and tumour necrosis factor-\textalpha (TNF-\textalpha) levels by an ultra-sensitive solid phase sandwich enzyme linked-immunosorbent assay (BioSource International, Inc.) on an automated laboratory processing unit (Personal Lab\textsuperscript{TM} 05, Adaltis Inc., Montreal, Canada). The ranges of measurement reported by the manufacturers are 0.175–1100 mg/L for C-reactive protein, 2–2350 pg/mL for IL-6, and 0.09–64 pg/mL for TNF-\textalpha, with respective intra-assay and inter-assay coefficients of variation of 3.5 and 3.4%, 6 and 8%, and 6 and 9%, respectively. The respective upper limits of normal reported are 3 mg/L, 8.5 pg/mL, and 2.1 pg/mL.

Statistical analysis

For baseline data, categorical variables are summarized using per cent values, with continuous variables described as medians with interquartile range. Categorical variables were compared between treatment groups using Pearson’s $\chi^2$ test or Fisher’s exact test, whereas differences in continuous data were evaluated using the Mann–Whitney U test.

The prognostic values of marker levels at all three time points for subsequent clinical events of mortality, and baseline levels for events of cardiogenic shock and the composite endpoint of death or shock, occurring within the 90 day trial period, were evaluated through logistic regression analysis. The follow-up of 90 days represented the timing of the principal clinical endpoint of the COMMA trial. An epidemiological approach was implemented for outcome analyses, with age, Killip class, and MI location entered as covariates in logistic regression models. The Box-Tidwell transformation test was used to see whether the linearity assumption holds for continuous variables of age and marker level in the logistic regression models. When this procedure determined log-transformed values of marker levels to be most appropriate in the predictive models, the odds ratios (OR) were estimated according to a two-fold increase in marker level; otherwise, raw levels were entered into the model and OR were estimated according to an absolute change equivalent to a two-fold increase at the median. Treatment effects on marker levels were analysed by comparing patients in the placebo group with those in the bolus+infusion group. The effects were assessed using an analysis of covariance procedure, with baseline characteristics added as covariates; at 24 and 72 h, the baseline marker value was included as a covariate to account for initial levels. For this purpose, the 24 h levels were examined from patients who survived until that point, corresponding to the time of study drug discontinuation at the end of the infusion period. Levels at 72 h were also examined. Marker values were log-transformed to satisfy the assumptions of normality and equal
Multivariable regression analyses were used to assess the relationship of baseline variables with markers, and non-significant variables were excluded from the model using a stepwise elimination procedure. Baseline variables that were investigated in the secondary analysis included age, weight, gender, diabetes, Killip class >1, amount of ST-segment elevation, and MI location.

Statistical analyses were performed using SPSS 10.0 for Windows statistical software (Chicago, IL). A P-value <0.05 for a two-tailed test was considered statistically significant in all cases. However, given the lack of adjustment for multiple comparisons, even significant findings should be considered hypothesis generating rather than definitive.

Results

Patient characteristics

Table 1 displays selected baseline and post-randomization characteristics for all patients with an analysed blood sample after baseline. The groups were generally well balanced, except for a trend towards higher body weight and slightly more patients with an anterior MI in the placebo group. More than 95% of patients in both groups were of Killip class 1 or 2 at admission. These characteristics were similar to those in the entire cohort of patients from the main COMMA trial. When compared with other ST-segment elevation trials, there were more patients with an anterior MI by design.

The mortality rate during the entire 90 day period in the two study groups was 8.3% in the placebo and 3.2% in the bolus and infusion group in the current substudy; in the entire trial, rates were 5.9% in the placebo group and 1.8% in the treatment group. In the substudy, incidence rates of death or shock after randomization were 11.8% in the placebo group and 5.8% in the pex group (compared with 8.3 and 3.6%, respectively, in the COMMA trial). During the first 24 h of the study, nine patients died, with 23 either dying or experiencing shock.

Prognostic value

Elevated levels of C-reactive protein and IL-6 at all time points were highly predictive of the subsequent incidence of death and of death and cardiogenic shock within the 90

<table>
<thead>
<tr>
<th>Baseline and post-randomization characteristics</th>
<th>Placebo (n = 175)</th>
<th>Bolus + Infusion (n = 151)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>61 (53–71)</td>
<td>61 (50–74)</td>
<td>0.538</td>
</tr>
<tr>
<td>Female sex</td>
<td>23</td>
<td>25</td>
<td>0.715</td>
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<tr>
<td>Weight (kg)</td>
<td>84 (74–98)</td>
<td>82 (70–94)</td>
<td>0.089</td>
</tr>
<tr>
<td>Heart rate (per min)</td>
<td>77 (65–90)</td>
<td>77 (65–88)</td>
<td>0.828</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>130 (114–152)</td>
<td>132 (117–154)</td>
<td>0.481</td>
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<tr>
<td>Systolic</td>
<td>80 (68–92)</td>
<td>79 (70–92)</td>
<td>0.919</td>
</tr>
<tr>
<td>Anterior MI</td>
<td>81</td>
<td>71</td>
<td>0.029</td>
</tr>
<tr>
<td>Killip class</td>
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<td></td>
<td>0.510</td>
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<tr>
<td>I</td>
<td>82</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>17</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>0</td>
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<td>IV</td>
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<td>2</td>
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<tr>
<td>Hypertension</td>
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<td>19</td>
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<tr>
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<td>Smoking status</td>
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<tr>
<td>None</td>
<td>31</td>
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<td></td>
</tr>
<tr>
<td>Current</td>
<td>34</td>
<td>34</td>
<td></td>
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<tr>
<td>Sum ST-segment elevation (mm)</td>
<td>10.0 (7.0–16.0)</td>
<td>10.4 (5.5–16.9)</td>
<td>0.533</td>
</tr>
<tr>
<td>Symptom-onset-randomization (h)</td>
<td>2.45 (1.64–3.89)</td>
<td>2.58 (1.77–3.76)</td>
<td>0.486</td>
</tr>
</tbody>
</table>

Categorical variables are given as percentages and continuous variables as medians with interquartile range, for patients with a blood sample past baseline. MI, myocardial infarction; ACE, angiotensin converting enzyme; TIMI, thrombolysis in myocardial infarction; CK-MB, creatine kinase MB fraction.
day follow-up (Figure 1). Baseline levels of C-reactive protein [OR per two-fold increase 1.63 (95% CI, 1.24–2.16), \( P = 0.001 \)] and IL-6 [OR 2.19 (1.46–3.28), \( P < 0.001 \)] were highly predictive of death occurring during the 90 day trial, whereas TNF-\( \alpha \) was not [OR 1.26 (0.90–1.75), \( P = 0.173 \)]. In particular, patients in the highest quartile group of C-reactive protein (\( \geq 7.6 \) mg/L) and IL-6 (\( \geq 22.0 \) mg/L) had an increased risk in mortality (Table 2).

Levels of these markers obtained after baseline were also strongly associated with mortality. The predictive value of C-reactive protein (\( P < 0.001 \)), IL-6 (\( P < 0.001 \)), and TNF-\( \alpha \) (\( P = 0.020 \)) was most apparent at 24 h. At 72 h, levels of C-reactive protein (\( P = 0.003 \)) and IL-6 (\( P = 0.015 \)) were still significant but weaker predictors of mortality. Baseline values of IL-6 (\( P = 0.006 \)) and TNF-\( \alpha \) (\( P = 0.031 \)), but not of C-reactive protein, were associated with cardiogenic shock. Baseline values of C-reactive protein (\( P = 0.011 \)), IL-6 (\( P < 0.001 \)), and TNF-\( \alpha \) (\( P = 0.029 \)) were associated with the combined endpoint of death or shock.

### Changes in markers and pexelizumab treatment effects

The median values of all three markers increased significantly during the first 72 h; those of IL-6 peaked at 24 h.
(P < 0.001) to a five-fold increase above baseline in the placebo group, whereas those of C-reactive protein and TNF-α continued to increase throughout the 72 h observation period (P < 0.001) to reach 13-fold and two-fold increases, respectively (Figure 2 and Table 3). By the end of the infusion period at 24 h, the C-reactive protein (P = 0.03) and IL-6 (P = 0.04) levels were less elevated in the pexelizumab group when compared with those in the placebo. Thus, median C-reactive protein and IL-6 levels were 32 and 20% lower at 24 h in the pexelizumab vs. placebo groups. These reductions in IL-6 and in C-reactive protein were not, however, sustained 48 h following the discontinuation of study drugs (Table 2). There was no such treatment effect on TNF-α at 24 h (P = 0.12), although there was a slight increase at 72 h (P = 0.04) with pexelizumab.

Discussion

This substudy documents, a clear association between higher levels of inflammation markers at baseline with increased death or cardiogenic shock in patients with STEMI managed with primary angioplasty, and a link between higher levels during the ensuing 72 h with more frequent death through 90 days. It also displays a reduction in C-reactive protein and IL-6 levels during infusion with pexelizumab. The connection found between reductions in severe adverse clinical outcomes and levels of inflammation is an original description of acute myocardial infarction. It supports a novel mechanism in pexelizumab-associated treatment benefits, relating to its effect on inflammatory pathways as reflected by the markers.

Inflammation markers

Many studies have shown an elevation of inflammation markers in myocardial infarction.5–6,8,9 Observations from the SHOCK trial and registry have suggested that systemic inflammation plays a role in the pathophysiology of cardiogenic shock.14 In the current substudy, consisting predominantly of patients with acute anterior myocardial infarction, IL-6 and C-reactive protein levels were modestly elevated at first contact but increased markedly in the following days. Peak levels of IL-6 were seen at 24 h, whereas those of C-reactive protein and TNF-α increased until 72 h.

Prognostic implications

Levels of C-reactive protein and IL-6 throughout the study, especially at 24 h, were very strong predictors of subsequent death until 90 days after randomization. TNF-α had a weaker prognostic value that was most apparent after 24 h, suggesting different pathophysiological implications than C-reactive protein and IL-6. On the other hand, IL-6 and TNF-α at baseline, but not C-reactive protein, predicted cardiogenic shock events.

IL-6 is expressed by inflammatory cells located within the active culprit lesion and the infarct area15 and is one of the main endogenous mediators of the acute phase response.16 C-reactive protein, in addition to being a sensitive acute phase reactant, may play an active role in the pathophysiology of acute myocardial infarction and is a known activator of the classical pathway to complement activation.4,7,10 As a result, autopsy studies of infarcting myocardium in humans have shown the extensive depositions of C-reactive protein, activated complements, and C-reactive protein-complement complexes, and also significant correlations between amounts of each, in the hours and days following acute onset.10 Blood levels of C-reactive protein generally correlate with markers of infarct size, although this correlation is attenuated in patients with successful reperfusion.17 In contrast, TNF-α is expressed early and late in the infarct, peri-infarct, and normal zones of myocardium, suggesting it is part of an important intrinsic myocardial stress response system to injury.18 TNF-α also possesses multiple pleiotropic effects, most being proinflammatory but some cardio-protective and anti-apoptotic.19

Pexelizumab and inflammation markers

The administration of pexelizumab was associated with a 32% lower C-reactive protein and 20% lower IL-6 median levels relative to placebo after 24 h, at the end of the infusion. These reductions were not sustained, however, 48 h after the discontinuation of therapy, possibly in relation with the pharmacodynamic profile of pexelizumab. The bolus and 24 h infusion doses used in this study inhibited the hemolytic activity of complement for <12 h after drug discontinuation and the formation of the terminal complex.
of the complement for only a few hours.\textsuperscript{1,2,13} It can be speculated that the administration of the drug during the critical acute phase of ischaemic injury may prevent several consequences of the acute inflammatory response on self-amplification and on various aspects of the healing process. TNF-\(\alpha\) levels increased relatively late in this study and were slightly higher in the pexelizumab group, particularly at 72 h.

Although the role of complement in coronary artery disease and myocardial infarction has not been studied in detail, its role in inflammation and auto-immunity is fundamental and involves numerous proximal, as well as more distal, pathways to inflammation and immunity. Studies have shown that the neutralization of antibodies against the C5a receptor and IL-6\textsuperscript{20} protects against death in sepsis and that the inhibition of C5 protects against renal ischaemia-reperfusion injury.\textsuperscript{21}

Pexelizumab specifically blocks C5, whose pivotal function in complement activity consists of bridging the proximal activation pathways to the common terminal pathway. Thus, C5 is cleaved into C5a and C5b. C5a is a potent anaphylatoxin acting on a variety of cells that enhances vascular permeability, attracts and activates inflammatory cells resulting in an oxidative burst, releases granule-bound enzymes, and promotes secretion of a number of pro-inflammatory cytokines such as TNF-\(\alpha\) and IL-6. On the other hand, IL-6 promotes the expression of the C5 receptors while stimulating hepatic synthesis of C-reactive protein. The other cleavage product of C5 whose generation is prevented by pexelizumab is C5b, which leads to formation of the membrane attack complex. This complex is primarily a potent cytolytic that promotes apoptosis, the secretion of IL-6 and other cytokines, and facilitates cell–cell and cell–substrate adhesion.\textsuperscript{22}

In this study, levels of TNF-\(\alpha\) increased after the first 24 h and were slightly higher in the pexelizumab group at 72 h, and this late elevation carried a minor increased risk of mortality. As treatment was not present at that time, no major inferences can be made on effects of pexelizumab on this important cytokine.

**Study limitations and potential**

The results of this study should be considered hypothesis generating and preliminary. The substudy had a relatively small sample size, was not pre-planned, and was performed only once the results of the original trial were known. Because only \(\sim 60\%\) of patients were included owing to blood sample availability, it is possible that unrecognized selection bias had an impact on our findings, specifically those concerning treatment effects on marker levels, in spite of similar characteristics of the studied and overall populations, similar event rate proportions, and an adjustment for baseline characteristics. No analyses were performed on patients from the bolus alone group, although this analysis would have been of interest for data validation, as intermediate clinical benefits were observed in this group. Furthermore, the markers of inflammation described herein may not most adequately represent all components of the effects of pexelizumab; other potential mechanisms involved with the benefits warranting further investigation include inhibition of apoptosis, modulation of inducible nitric oxide synthase expression, attenuation of free
radical generation, autoimmunity, cardiac remodelling, and/or other modulators of cell damage.

Nonetheless, the observation of a prognostic ability of C-reactive protein and IL-6 levels combined with that of a reduction in inflammatory markers with pexelizumab, suggests a link exists between the presence and intensity of inflammation and susceptibility to death and shock, which may be favourably influenced by innovative therapeutic strategies such as complement inhibition or other approaches. These issues are now being addressed as part of a large ongoing phase 3 mortality trial with pexelizumab in patients with ST-segment elevation undergoing primary angioplasty.

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References


