Administration of the Mitochondrial Permeability Transition Pore Inhibitor, TRO40303, prior to Primary Percutaneous Coronary Intervention, Does Not Affect the Levels of Pro-Inflammatory Cytokines or Acute-Phase Proteins

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Keywords

STEMI · Primary percutaneous coronary intervention · Reperfusion injury · Cytokines · Mitochondrial permeability transition pore

Abstract

Objectives: In the MITOCARE study, reperfusion injury was not prevented after administration of the mitochondrial permeability transition pore (mPTP) opening inhibitor, TRO40303, in patients with ST-segment elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (pPCI). The effects of TRO40303 on pro-inflammatory cytokines and acute-phase proteins were assessed. **Methods:** STEMI patients ($n = 163$, mean age 62 years) with chest pain within 6 h before admission for pPCI were randomized to intravenous bolus of TRO40303 ($n = 83$) or placebo ($n = 80$) prior to reperfusion. We tested whether

the groups differed in levels of IL-1 β , IL-6, IL-10, TNF, and high-sensitive C-reactive protein at various time points (0, 12, and 72 h) after PCI. Further, potential differences between groups in the change of biomarker levels between 0 and 72 h, 0 and 12 h, and 12 and 72 h were tested. **Results:** There were no statistically significant differences between the two groups, neither in levels of pro-inflammatory cytokines nor in levels of acute-phase proteins, and there were no statistically significant differences in the change of biomarker levels between the groups considering the time intervals from 0 to 72 h, from 0 to 12 h, and from 12 to 72 h. **Conclusion:** The administration of the mPTP, TRO40303, prior to reperfusion does not alter the pharmacokinetics of pro-inflammatory cytokines or acute-phase proteins during the first 72 h after PCI.

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Clinicaltrialsregister.eu; EudraCT number 2010-024616-33.

Introduction

Despite the proven success of rapid restoration of epicardial coronary blood flow with thrombolytics or primary percutaneous coronary intervention (pPCI) in patients admitted with ST-segment elevation myocardial infarction (STEMI) [1, 2], additional myocardial damage may occur after reperfusion. The process known as *reperfusion injury* affects myocyte viability, contractile performance, arrhythmia threshold, and endothelial function [3–5]. Reperfusion injury is partly related to the effect of toxic substances released from the anoxic mitochondria after reperfusion due to opening of the mitochondrial permeability transition pore (mPTP), and partly related to the activation of a general myocardial inflammation [6]. Numerous adhesion molecules and inflammation mediators are expressed and/or upregulated and lead to the fixation of leukocytes to the endothelium and the cardiomyocytes [7]. Cytokines also take part in the cardio-protection cascades that act on the mPTP [8, 9].

The tissue injury in acute myocardial infarction (AMI) triggers inflammation. The inflammatory response is complex and may be unpredictable, partly explaining why clinical interventions against reperfusion injury have failed.

Pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor (TNF), induce tissue destruction, while anti-inflammatory cytokines suppress inflammation and promote healing. Some cytokines, such as interleukin-10 (IL-10), which is found within the atheromatous plaque, are both anti-inflammatory and pro-inflammatory.

Inflammatory cytokines like TNF and IL-6 initiate dysregulation of intracellular Ca^{2+} . TNF reduces Ca^{2+} sensitivity of myofibrils and inhibits expression of SERCA2a [10]. These effects seem to be inhibited by radical scavengers indicating that the negative effects of the inflammatory cytokines are partially mediated with reactive oxygen species (ROS) leaking from the hypoxic mitochondria [11].

In the MITOCARE study, administration of the mPTP opening inhibitor, TRO40303, prior to pPCI in patients with STEMI did not reduce infarct size [12, 13]. Whether the blockage of the mitochondrial transition pore with TRO40303 has a modulating effect on levels of pro-inflammatory cytokines as well as acute-phase proteins has not previously been assessed in this population.

Methods

The MITOCARE study was a multicenter randomized, double-blind, placebo-controlled trial carried out in 4 European countries between October 2011 and September 2013. STEMI patients ($n = 167$) undergoing pPCI with acute chest pain within 6 h before admission were randomized to intravenous bolus of TRO40303 or placebo prior to reperfusion. Patients with prodromal symptoms and patients with multivessel disease were not eligible. Two patients did not meet the inclusion criteria and 2 patients in the TRO40303 group received treatment but did not undergo PCI. A total number of 163 patients were included, of whom 83 received TRO40303 and 80 received placebo (Fig. 1). Two patients in the TRO40303 group did not undergo PCI but were included in the main trial for safety analysis. The kinetics of cytokines and high-sensitive C-reactive protein (hsCRP) were analyzed in 161 patients of whom 79 were in the placebo group and 82 were in the treatment

Fig. 1. Patient flow. PCI, percutaneous coronary intervention.

Table 1. MITOCARE patient characteristics

Variable	Overall (<i>n</i> = 161)	Placebo (<i>n</i> = 79)	TRO40303 (<i>n</i> = 82)	<i>p</i> value ^a
Median age, years (IQR)	62 (53–70)	60 (53–69)	63 (53–71)	0.536
Median BMI (IQR)	27.3 (25–30)	26.7 (24–30)	27.5 (25–31)	0.198
Sex, <i>n</i> (%)				0.165
Male	135 (83.9%)	63 (79.7%)	72 (87.8%)	
Female	26 (16.1%)	16 (20.3%)	10 (12.2%)	
Diabetes, <i>n</i> (%)				0.519
Yes	12 (7.5%)	7 (8.9%)	5 (6.1%)	
No	149 (92.5%)	72 (91.1%)	77 (93.9%)	
Hypertension, <i>n</i> (%)				0.654
Yes	47 (29.2%)	25 (31.6%)	22 (26.8%)	
No	114 (70.8%)	54 (68.4%)	60 (73.2%)	
Smoking, <i>n</i> (%)				0.851
Yes	67 (46.2%)	33 (46.5%)	34 (45.9%)	
No	78 (53.8%)	38 (53.5%)	40 (54.1%)	
Stratum, <i>n</i> (%)				0.607
Anterior	64 (39.8%)	33 (41.8%)	31 (37.8%)	
Posterior	97 (60.2%)	46 (58.2%)	51 (62.2%)	
Killip class, <i>n</i> (%)				0.984
1	112 (70.4%)	55 (70.5%)	57 (70.4%)	
2–5	47 (29.6%)	23 (29.5%)	24 (29.6%)	

IQR, interquartile range; BMI, body mass index. ^a*p* value results from χ^2 tests if the variable was categorical and *t* tests if the variable was continuous. BMI was non-normally distributed in the TRO40303 group, and therefore, the Mann-Whitney test was used instead.

group (Fig. 1). The study was in accordance with the Declaration of Helsinki, and approved by the regional ethics committees. Informed consent was obtained from all subjects.

The patient characteristics have previously been reported (Table 1) [12]. The core lab FIRALIS (Firalis SAS Huningue, Paris, France) was responsible for the blood analysis of 161 patients to determine the levels of the pro-inflammatory cytokines (interleukin-1 β [IL-1 β], IL-6, IL-10, TNF) and hsCRP before reperfusion, at 12 and 72 h after pPCI. IL-1 β , IL-6, IL-10, and TNF levels were quantified by the use of an MSD Proinflammatory Panel I kit. Levels of hsCRP were quantified by an automated analyzer (Dimension EXL 200) from Siemens. To reduce measurement error, each cytokine was measured in duplicate at the respective time points. The repeated measures had high internal consistency for IL-6, IL-10, and TNF (Cronbach's α >0.9, intraclass correlation coefficient >0.9, Appendix 1), but IL-1 β had below the acceptable internal consistency (α = 0.458, intraclass correlation coefficient = 0.297, Appendix 1). The mean of the repeated measures for the cytokines and hsCRP was used in all analyses.

Not all patients had measurements for each biomarker at each specified time point (Appendix 2). There were in total 4 missing values for each cytokine analyzed. The remaining missing values were nondetectable due to a result below the detection method used. Twenty patients had extreme values far beyond the expected range of the biomarker on at least one repeated measure. Extreme outliers potentially caused by blood sampling or analysis error were removed from the dataset.

Statistical Analysis

All biomarkers had right-skewed distributions according to Q-Q plots and Shapiro-Wilk tests (p < 0.05). Thus, to test whether the groups differed in levels of IL-1 β , IL-6, IL-10, TNF, and hsCRP at the time points 0, 12, and 72 h, Mann-Whitney U tests were performed. Further, to find out whether groups differed in the progression of the biomarker and adjust for baseline biomarker levels, the Mann-Whitney U test was repeated on the change of biomarker for the time intervals 0–72 h, 0–12 h, and 12–72 h. A significance level of α = 0.05 was chosen for all tests. To visualize the differences between treatment groups, boxplots were created for each biomarker. In addition, line graphs were made to show the temporal changes in the levels of each biomarker during the first 72 h after STEMI. The Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) v.23 was used for all tests, while R version 3.2.3 was used to create graphs.

Results

The study population consisted of 161 patients, with a mean age of 62 years (IQR: 53–70), and of 83.9% men. There were no statistically significant differences in baseline characteristics between the TRO40303 and the placebo group (Table 1). Levels of IL-1 β increased during the

Table 2. Median (interquartile range) and *p* values (Mann-Whitney U test) for each biomarker at 0, 12, and 72 h following PCI for placebo (*n* = 79) and TRO40303 (*n* = 82)

Biomarker	Time	Median placebo	Median TRO40303	<i>p</i> value
IL-1 β , pg/mL	0 h	0.022 (0.008–0.022)	0.025 (0.012–0.045)	0.521
	12 h	0.028 (0.005–0.067)	0.024 (0.005–0.050)	0.524
	72 h	0.026 (0.006–0.051)	0.039 (0.015–0.093)	0.183
IL-6, pg/mL	0 h	1.21 (0.8–1.8)	1.16 (0.8–2.2)	0.604
	12 h	3.60 (1.2–5.5)	3.11 (1.9–5.0)	0.500
	72 h	1.89 (1.3–3.6)	1.93 (1.2–4.2)	0.736
IL-10, pg/mL	0 h	1.19 (0.6–2.7)	0.79 (0.3–2.5)	0.132
	12 h	0.17 (0.1–0.3)	0.17 (0.1–0.3)	0.749
	72 h	0.15 (0.1–0.2)	0.16 (0.1–0.3)	0.450
TNF, pg/mL	0 h	6.34 (4.9–7.7)	6.24 (5.1–7.5)	0.898
	12 h	6.99 (5.7–8.4)	6.96 (5.7–8.3)	0.940
	72 h	7.59 (6.3–9.9)	8.16 (6.7–9.9)	0.735
CRP, mg/L	0 h	2.46 (1.2–5.6)	2.92 (1.5–8.7)	0.511
	12 h	6.75 (3.4–9.0)	6.49 (3.5–10.5)	0.623
	72 h	16.65 (6.0–44.0)	24.42 (8.9–52.9)	0.108

Table 3. Median (interquartile range) and *p* values (Mann-Whitney U test) of the change over time between 0 and 12 h, between 0 and 72 h, and between 12 and 72 h following PCI for each biomarker in placebo (*n* = 79) and TRO40303 (*n* = 82)

Biomarker	Time interval	Median placebo	Median TRO40303	<i>p</i> value
IL-1 β , pg/mL	0–12 h	0.0036 (–0.010 to 0.021)	0.0000 (–0.016 to 0.025)	0.608
	0–72 h	0.0054 (–0.025 to 0.028)	0.0097 (–0.006 to 0.065)	0.088
	12–72 h	0.0025 (–0.039 to 0.019)	0.0060 (–0.014 to 0.056)	0.151
IL-6, pg/mL	0–12 h	2.25 (0.20–4.24)	2.13 (0.52–3.56)	0.416
	0–72 h	0.91 (0.15–2.19)	0.94 (–0.13 to 2.40)	0.698
	12–72 h	–0.88 (–2.72 to 0.24)	–0.81 (–1.78 to 0.42)	0.722
IL-10, pg/mL	0–12 h	–1.053 (–2.47 to –0.17)	–0.485 (–2.23 to –0.035)	0.096
	0–72 h	–1.025 (–5.23 to –0.18)	–0.665 (–4.49 to –0.040)	0.256
	12–72 h	0.0075 (–0.05 to 0.07)	0.0050 (–0.07 to 0.07)	0.939
TNF, pg/mL	0–12 h	0.617 (–0.32 to 1.44)	0.785 (0.13–1.55)	0.375
	0–72 h	2.018 (0.43–3.41)	2.133 (0.64–3.43)	0.496
	12–72 h	0.988 (0.15–2.30)	1.470 (0.29–2.09)	0.388
CRP, mg/L	0–12 h	2.54 (0.7–5.3)	2.69 (1.4–5.1)	0.401
	0–72 h	12.65 (3.9–68.0)	20.13 (6.7–80.3)	0.235
	12–72 h	8.26 (2.3–35.0)	15.57 (5.2–37.9)	0.095

first 12 h of STEMI, but returned to baseline levels after 72 h (Table 2; Fig. 2a, b). Levels of IL-6 appeared to increase at 12 h, and gradually decreased during the next 60 h, but at this point, thus still had not returned to baseline levels (Table 2; Fig. 2c, d). From baseline to 12 h, levels of IL-10 decreased, and between 12 and 72 h, they re-

mained stable at approximately the same value (Table 2; Fig. 2e, f). During the first 12 h, levels of TNF increased, and from 12 to 72 h, they increased further, but at a slower rate (Table 2; Fig. 2g, h). Levels of hsCRP increased gradually and at approximately the same rate from baseline to 72 h (Table 2; Fig. 2i, j).

Fig. 2. The kinetic profile of cytokines and CRP during the first 72 h following PCI for TRO40303 and placebo. Shown for levels of IL-1 β (a, b), IL-6 (c, d), IL-10 (e, f), TNF (g, h), and CRP (i, j). Round points denote the median level of the corresponding biomarker at the time measured (0, 12, and 72 h). Whiskers represent the interquartile range.

(For legend see next page.)

There were no statistically significant differences between the two groups, neither in levels of pro-inflammatory cytokines nor in levels of hsCRP at any time point ($p > 0.05$, Table 2). Moreover, there were no statistically significant differences in the change of the biomarker levels for the time intervals from 0 to 72 h, from 0 to 12 h, and from 12 to 72 h between the two groups ($p > 0.05$, Table 3). The similarity between groups is illustrated in the boxplots (Fig. 3).

Discussion

The main finding from the current substudy of the MITOCARE randomized controlled trial was that blockade of the mitochondrial transition pore had no significant effect on the temporal changes in circulating levels of the inflammatory cytokines IL-1 β , IL-6, IL-10, and TNF or the acute-phase protein, hsCRP, in STEMI patients treated with pPCI during the first 72 h after PCI. The inability of TRO40303 to modulate inflammation seems consistent with the lack of cardioprotective effects during the reperfusion period in the main trial [12].

The *reperfusion injury* which occurs after the restoration of the epicardial blood flow plays a major role in the development of infarct size. The reperfusion injury cascade is thus a complex interplay between several mechanisms ending up with inflammation and cell death.

Pharmacological interventions targeting various mechanisms like calcium channel blockers, anti-inflammatory agents such as pexelizumab and modulators of the mPTP have all failed in reducing reperfusion injury [14–17].

The sudden death of myocardial cells results in activation of the immune system that plays an important role in the acute phase and in the postinfarction reparatory phase. Different pathways in the immune system are involved in the cardiac repair and remodeling process. The activated complement cascade results in the activation of TNF and cytokine induction. IL-1 β and IL-6 play an important role in the damaged myocardium. IL-6 stimulates the release of CRP from the liver, which also promotes

inflammation, and high levels are associated with negative remodeling and adverse outcome after AMI [18, 19]. In addition, reparative processes are activated following AMI to stimulate healing of the infarcted myocardium [20]. Pharmacological intervention targeted to modify inflammation has so far failed to prevent left ventricular remodeling [21].

The Role of Specific Interleukins in STEMI

Our choice of inflammatory markers in this study has been extensively evaluated in experimental studies of myocardial infarction. It has been documented that levels of the pro-inflammatory cytokines IL-1 β , IL-6, and TNF are upregulated and are of prognostic significance [21, 22]. Moreover, several studies have shown involvement of inflammatory mediators in progressive myocyte loss caused by necrosis and/or apoptosis, suggesting that these cytokines are involved in the progression of cardiac remodeling [23]. This is further reinforced by the findings of a significant correlation between IL-6 and baseline NT-pro-BNP as documented in the APEX AMI study [24]. In the current study, there was an increase in levels of IL-6 and TNF during the first hours after PCI consistent with earlier findings. The hsCRP levels also increased up to a maximum and followed the increase in levels of IL-6 and TNF.

IL-6 and IL-1 β are pro-inflammatory while other cytokines are important to promote myocardial salvage. IL-10 downregulates the inflammatory response in AMI. So far, experimental data are ambiguous, showing both protective and negative effects of IL-10. One experimental model has shown an important suppressive role of IL-10 that improves left ventricular function and remodeling by suppressing matrix metalloproteinase-9 and thus inhibiting fibrosis [25]. IL-10 and pro-inflammatory-resolving lipid mediators have been identified as inhibitors of the postinfarction inflammatory reaction [26]. So far, no clinical data exist on the role of IL-10 in STEMI patients treated with pPCI. Inhibition of mPTP in our study did not show any significant difference in the temporal levels of IL-10.

Levels of IL-1 β after STEMI revascularized by pPCI have previously been shown to have a strong association with impaired myocardial function and infarct size assessed by cardiac magnetic resonance imaging after 1 year [27]. The mechanisms are unclear, but it has been shown that IL-1 β is involved in the increased proliferation of bone marrow hematopoietic stem cells after a myocardial infarction and by inhibiting the IL-1 β there is a possibility to reduce infarct size [28]. In our study popula-

Fig. 3. Boxplots illustrating the differences between TRO40303 and placebo in levels of pro-inflammatory cytokines and CRP during the first 72 h following PCI. The difference is shown for IL-1 β (a), IL-6 (b), IL-10 (c), TNF (d), and CRP (e). Notches represent the confidence interval around the median. Overlapping notches indicate nonsignificant differences between groups.

tion, treatment with TRO40303 did not have any statistically significant effect on the circulating levels of IL-1 β , indicating that the substance did not modulate the inflammation.

TNF is upregulated in AMI [29]. This cytokine is pro-inflammatory, but it can also promote healing by regulating the postinfarction remodeling process [30]. The action of TNF is decided by binding to two different receptors that cause cardiac injury or protect cardiomyocytes from apoptosis [31]. Binding to TNF receptor 1 leads to myocardial cell death, but the role of TNF receptor 2 is unclear. There is only one small clinical trial to our knowledge on 26 STEMI patients that investigated the effect of blocking TNF. The authors concluded that blockage of TNF did not affect the myocardial salvage [32]. Our sub-study showing no effect of TRO40303 on circulating levels of circulating TNF is, therefore, not unexpected, and in accordance with results of the main study.

Mitochondrial Dysfunction

The major sources of ROS in the ischemic-reperfused myocardium are the mitochondria, xanthine oxidase, and phagocyte-derived nicotinamide adenine dinucleotide phosphate oxidase [33–35]. ROS stimulate the production of inflammatory cytokines and inversely, inflammatory cytokines stimulate ROS formation [36]. However, circulating levels of biomarkers may not necessarily reflect the accurate changes in myocardial levels. The phenomenon of mitochondrial permeability transition was detected in studies by Hunter and Haworth [37–40] in the late 1970s and was confirmed in the late 1980s by Crompton and colleagues [41–43]. The calcium overload as a result of ROS generation led to necrosis of the cardiac myocytes mediated by increased permeability of the mitochondrial membrane. The mitochondrial metabolism is depressed in STEMI resulting in a reduced level of adenosine triphosphate and increased oxidative stress with formation of ROS. The mPTP opens in response to the calcium overload that occurs in the ischemic cardiomyocytes *after* reperfusion. The opening of mPTP can be modulated directly by different pharmacologic agents, for example, cyclosporine or indirectly for instance by preconditioning [44]. In vitro studies have shown that the blockage of the mPTP in ischemic cardiomyocytes resulted in reduced levels of oxidative stress [45]. So far, 5 randomized trials have investigated the effect of cyclosporine on reperfusion injury. A small study carried out by Piot et al. [46] showed a reduction in infarct size in patients that were treated with cyclosporine before PCI. On the other hand, Cung et al. [47] failed to show any benefit of

administering intravenous cyclosporine to inhibit the opening of mPTP in STEMI patients undergoing primary PCI [47, 48]. Additionally, cyclosporine treatment before thrombolysis in another study population did not show any beneficial effects [49]. There may be several reasons why inhibition of mPTP by TRO40303 was not achieved in our study.

TRO40303, a novel substance, modulates the mPTP by binding to the mitochondrial translocator protein at the cholesterol site. Thereby, it reduces mitochondrial-mediated cell death by blocking the mPTP, which leads to reduced levels of ROS in an experimental model of myocardial infarction [50]. TRO40303 has been shown to reduce infarct size when it is administered before reperfusion. The reduction in infarct size is related to the reduction in factors released by the mitochondria into the cytoplasm contributing to apoptosis and cell death. Effective inhibition of the mitochondrial transition pore should thus theoretically reduce the levels of pro-inflammatory cytokines. However, this did not occur in our study. Several other explanations are possible.

Drug Dose

The evaluation of expected active dose of TRO40303 in humans was carried out in a small selected population of 72 healthy males and postmenopausal and hysterectomized females [51]. A sublethal dose of TRO40303 has also been tested in an experimental hepatitis model where the authors showed a significant reduction in mortality [45]. The dose chosen in the MITOCARE trial might have been inadequate to achieve the optimal delaying of the mPTP opening and higher dosage could be essential in order to reduce oxidative stress in the ischemic reperfused cardiomyocytes more efficiently.

Clinical versus Experimental AMI

TRO40303 administration alone did not show promising results in our population, and this lack of clinical effect was accompanied by unchanged levels of cytokines. We could explain our neutral findings by the difference in the pathophysiology of an acute STEMI in an experimental model compared to STEMI due to a plaque rupture in a clinical setting. The rupture of a coronary plaque results in a systemic inflammatory response due to myocardial ischemia. In addition, the reperfusion itself results in marked immediate accumulation of leukocytes, mainly neutrophils into the ischemic myocardial area during the first 6 h [52]. In addition, endothelial cells and platelets are involved in the inflammatory reaction in the clinical setting. Since different mechanisms are involved in

reperfusion injury, it could be more appropriate to intervene on several levels in the reperfusion cascade [53]. Simultaneous inhibition of different inflammatory pathways could affect the outcome. Targeting both the mPTP and improving plaque stability could be a strategy. Blocking IL-6, known to destabilize plaque in acute coronary syndromes, with the IL-6 receptor monoclonal antibody, tocilizumab, that also targets the mitochondria, has been shown to reduce infarct size in patients with non-ST-segment elevation myocardial infarction [54].

Time
The mean time between pain and PCI was reported to be median, minimum and maximum at 180, 60, and 2,880 min, respectively, indicating very fast reperfusion limiting the infarct size. Whether TRO40303 would have been more efficient in reducing infarct size in patients that do not achieve rapid reperfusion is still a question. Larger infarcts show more negative remodeling and would perhaps gain benefit from mPTP inhibition by TRO40303. A clinical trial with TRO40303 performed in patients whom, due to geographical location, arrive late for mechanical reperfusion by percutaneous techniques might enlighten this issue. Thrombolysis is still an option for patients living in rural areas with long transport distances to PCI centers. One might speculate if administration of TRO40303 in addition to thrombolysis in these patients might show positive results in reducing reperfusion injury.

Conclusion
Administration of the mitochondrial transition pore inhibitor, TRO40303, had no statistically significant effect on the temporal changes in the levels of the circulating cytokines IL-1 β , IL-6, IL-10, and TNF or the acute-phase protein, hsCRP, in STEMI patients treated with pPCI during the first 72 h following PCI.

Acknowledgments
We are grateful for the participation of study personnel in the MITOCARE study, the core lab FIRALIS for the biomarker analysis, and the patients taking part in the project. We also appreciate the help of study nurse, Jorunn Nilsen, for her participation in the local organization of the trial. The authors thank F. Hoffmann-La Roche Ltd. for full access to the database after their acquisition of Trophos. The MITOCARE project is supported by the European Union under the 7th Framework Programme for RTD – Project MITOCARE – Grant Agreement HEALTH – 2010-26-261034.

Conflict of Interest
N.B., L.K.B.-M., J.E.N., V.T., P.S.M., V.B., H.F, T.S.H., S.E.J., S.H., and A.I.L. do not have any conflicts of interest. D.A. received honoraria as trial leader from the EU-FP7 grant No. H – 2010-26-261034. No other conflicts of interest are declared.

Appendix 1
Internal Consistency between Repeated Measures of Each Cytokine

Biomarker	Cronbach α	Intraclass correlation coefficient
IL-1 β , pg/mL	0.458	0.297
IL-6, pg/mL	0.995	0.991
IL-10, pg/mL	0.991	0.982
TNF, pg/mL	0.973	0.947

Appendix 2
Number of Missing Values for Each Biomarker at 0, 12, and 72 h following PCI for 161 Patients

Biomarker	Time, h	Missing
IL-1 β , pg/mL	0	37 (23%)
	12	30 (19%)
	72	50 (31%)
IL-6, pg/mL	0	8 (5%)
	12	14 (9%)
	72	21 (13%)
IL-10, pg/mL	0	14 (9%)
	12	10 (6%)
	72	18 (11%)
TNF, pg/mL	0	8 (5%)
	12	8 (5%)
	72	20 (12%)
CRP, mg/L	0	5 (3%)
	12	10 (6%)
	72	25 (15%)

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