Ischemia/reperfusion injury : a metabolic meltdown

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IX

Short vs. prolonged statin therapy differentially affects the myocardial response to injury after cardiac surgery:

The PreOperative STatin InterVEntion trial

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ABSTRACT

Background: Preclinical studies suggest that acute administration of statins is superior to continued statin therapy in repressing the inflammatory response upon tissue injury. Human studies are scarce and results are controversial. Aim of this study is to determine whether acute preoperative treatment with simvastatin is more effective than prolonged preoperative treatment in mitigating the inflammatory response following on-pump cardiac surgery.
Methods: Patients scheduled for isolated valve surgery, not being on a statin regimen, were included (n=12) and randomized into acute (40 mg simvastatin 12 hours and 2 hours before surgery) or prolonged treatment regimen (40 mg simvastatin for at least two weeks preoperatively). Sequential arteriovenous sampling over the heart was performed to selectively assess the myocardial response. Plasma cyto/chemokines were assessed in a multiplex platform, and CRP, troponin and CPK levels in a certified clinical chemistry lab. The study was terminated prematurely after interim analysis indicated futility.
Results: The different treatment strategies did not affect the myocardial inflammatory and systemic post-operative response as no differences were found for any of the 30 cyto/chemokines in the multiplex platform, nor in the postoperative CRP response. However, prolonged statin treatment was associated with reduced levels of myocardial damage markers (troponin T ($P<0.027$) and CPK ($P<0.037$)).
Conclusion: This study does not confirm experimental evidence that acute statin therapy is superior to prolonged therapy in mitigating the inflammatory response following on-pump cardiac surgery. Unexpectedly, both myocardial damage markers troponin T and CPK were reduced in the prolonged treatment group.

INTRODUCTION

Cardiac surgery using a cardiopulmonary bypass (CPB) machine elicits an inflammatory response, leading to endothelial damage, free radical production, complement and thrombocyte activation, and cytokine release.[1-3] These effects are thought to negatively influence surgical outcome. Besides their cholesterol-lowering function, statins are known to exert potent anti-inflammatory effects.[4] As a consequence it is hypothesized that via these pleiotropic, anti-inflammatory actions, statins mitigate the inflammatory response caused by CPB.
Indeed, preclinical studies show promising results of peri-operative statin therapy in repressing the inflammatory response and limiting the infarct size.[4-6] However, data from clinical studies are less outspoken. Although this may reflect differences between experimental models and clinical reality, it is known that the anti-inflammatory potential of statins is most outspoken during acute treatment [7,8], and that the effect (partially) wanes during continued treatment.[9] Clinical studies so far, all report the effect of prolonged statin therapy on surgery outcomes[10,11] or do not differentiate between acute and continued treatment.[12]
Considering the preclinical observations that the anti-inflammatory potential of acute statin therapy is superior to maintenance therapy we hypothesized that short-term preoperative statin treatment is superior to continued treatment in mitigating the inflammatory response after on-pump cardiac surgery. The latter was tested in this exploratory randomized trial.

MATERIALS & METHODS

PATIENT POPULATION

The local medical ethics committee of the Leiden University Medical Center approved the study protocol. Patients received written and oral information before giving their informed consent. In order to achieve a homogenous study population, this study only included patients scheduled for isolated mitral valve surgery (or in combination with tricuspid valve surgery). Patients undergoing minimal invasive procedures or ablation for arrhythmia, patients with manifest atherosclerosis and those receiving treatment with statins were excluded. Three weeks before surgery, patients were randomized into the short or prolonged treatment group. This trial was registered at the 'Nederlands trial register', NTR2673.
The trial was terminated prematurely as interim analysis performed after inclusion of 12 patients indicated futility.

INTERVENTION

In the prolonged group 40 mg simvastatin (oral intake daily) was administrated at least for 14 days prior to surgery. The short-term treatment group received one dose the evening before and a second dose the morning before surgery. We have previously shown the anti-inflammatory potential of 40 mg simvastatin.[13] The dose of statins used in this study corresponds to doses used in everyday practice.

DATA COLLECTION

The details of anesthesia and surgical procedures, as well as plasma measurements and handling, are similar to that described in detail in our previous article.[3] In summary, systemic blood samples were obtained at the outpatient clinic prior to start of the statin intervention and the day before surgery from a vein, mostly antecubital. To selectively measure the myocardial response to injury we used the technique of arterial-venous concentration differences. To be more specific, sequential paired arterial (radial artery) and myocardial venous blood samples (coronary sinus) were collected simultaneously over the reperfused heart at 0, 30, 60 min, and 2, 6 and 24 hours after reperfusion (i.e. removing the aortic cross-clamp). Plasma preparation was performed as described earlier.[3]
A multiplex assay platform (X-plex, Biorad, Veenendaal, the Netherlands) was used to simultaneously measure selected cytokines and chemokines (TNF-a, IL-1B, IL-1ra, IL-2, IL-4, IL-5,, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-16, IL-17 and IL-18; IFN-γ, IP-10, MCP-1 (CCL-1), MIP-1α (CCL-3), MIP-1β (CCL-4) , RANTES (CCL-5), Eotaxin (CCL-11), G-CSF, GM-CSF, bFGF, PDGF-bb, and VEGF-A). We have previously shown that in the context of cardiac surgery plasma levels of these

factors exceed the sensitivity of the assay.
Peripheral Troponin T and CPK levels (reference values ‹0.050 µg/L and ‹145 U/L, respectively) were measured systemically before surgery (baseline), directly after surgery, and 6 and 12 hours after surgery in our certified clinical chemistry lab. Peripheral CRP levels (reference value ‹10 mg/L) were measured before surgery (baseline), directly after surgery, and 24 and 48 hours after surgery in our certified clinical chemistry lab.

STATISTICAL ANALYSIS

SPSS 22.0 (SPSS Inc, Chicago, III) was used for statistical analysis. Patient characteristics were analyzed by a t-test and expressed as mean ± SEM. For the arteriovenous plasma measurements, the area under the curve (AUC) was estimated, and compared between the treatment groups through a linear mixed model analysis for the total timeslot of measurements. The model contained as independent variables time, as categorical the group, and the interaction between group and time. The covariance model was specified as unstructured. The delta AUC between groups was calculated and the null hypothesis (AUC = 0) was tested by a Wald-test based on the estimated parameters of the linear mixed model. P-values ‹0.05 were considered significant.

RESULTS

STUDY POPULATION

Between January 2011 and October 2014, twelve patients were included in this single center study. In total, twenty-eight patients were approached. Ten patients refused to participate (refused study medication). Two patients were withdrawn from the study because of noncompliance since they did not take their medication, and for one patient statin therapy was started by the attending physician. Additionally, three patients were excluded because arteriovenous sampling failed due to dysfunction of the coronary sinus catheter (see Patient flow chart in Sup. Fig.1). Baseline characteristics of the included patients were similar (Table 1). None of the patients died during the study period.

Table 1. Patient characteristics

	Short treatment (n=6)	Prolonged treatment (n=6)	P-value
Age (years)	62.7 ± 2.8	68.0 ± 3.8	0.29
Gender (no. of males)	4	4	1.00
Body mass index (kg/m2)	25.5 ± 1.0	27.1 ± 1.3	0.35
Medical history (no.) ·Hypertension ·Diabetes mellitus ·Chronic kidney disease	 2 0 0	 2 0 0	
Current medication (no.) ·Beta-blocker ·Insulin ·Antiplatelet or anticoagulant ·Calcium-channel blocker ·ACE inhibitor	 4 0 1 0 3	 3 0 2 1 3	
Scheduled surgery (no.) ·Mitral valve surgery ·Mitral and tricuspid valve surgery	 4 2	 3 3	
Days of preoperative statin treatmen	2 ± 0.0	23 ± 2.7	‹0.005

Table 1. Patient characteristics showing no significant differences between the groups except for days of preoperative statin treatment, as analyzed by a t-test expressed as mean ± SEM.

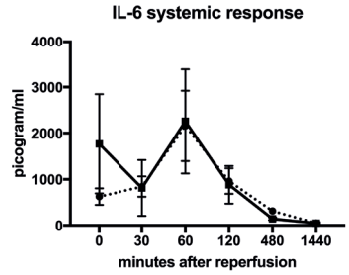
INFLAMMATORY RESPONSE

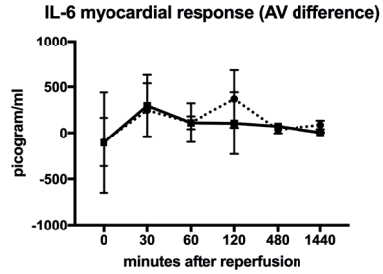
Systemic (arterial) and myocardial (arteriovenous differences) inflammatory responses in the short and prolonged treatment groups were similar. In fact, no differences were found for any of the cyto/chemokines tested. The dynamics of systemic and myocardial responses for the pro-inflammatory cytokines IL-6 and MCP-1 are illustrated in Figure 1A. The dynamics of all other cyto/chemokines are shown in Sup. Fig. 2. Similarly, no differences were found for plasma CRP levels at 24 and 48 hours after cardiac surgery (resp. P=0.29 and P=0.56; Figure 1A).

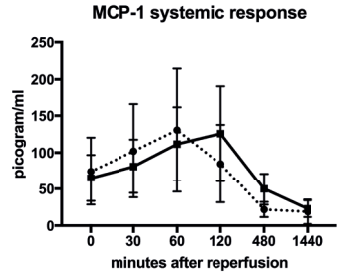
MYOCARDIAL DAMAGE

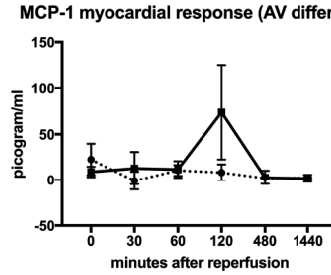
Differences were found for the biochemical damage markers troponin T and CPK. Figure 1B shows similar baseline values for the two patient groups but significantly higher Troponin T and CPK levels in the 12 hours following surgery in the short-term treatment group (differences in AUC for troponin T: P=0.004 and CPK: P=0.0003).

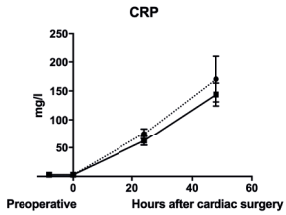
A. Inflammatory response



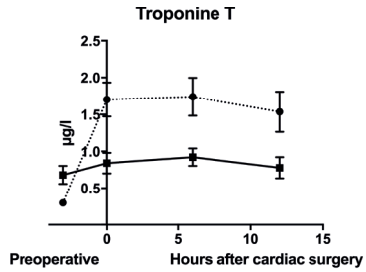








B. Myocardial damage



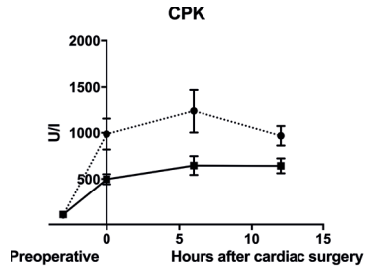


Fig. 1A. Pro-inflammatory cytokines (IL-6 MCP-1) and CRP levels. Levels of pro-inflammatory cytokines did not differ between treatment groups (IL-6: P=0.79, MCP-1: P=0.83) by comparing area under the curve.

Nor did the myocardial release, measured by arteriovenous differences over the reperfused heart (IL-6: P=0.53, MCP-1: P=0.50; Sup. Fig.1 provides myocardial release of the other measured cytokines). Systemic levels of CRP did not differ between the two treatment groups (P=0.11).
Legend: acute treatment group: dashed line, prolonged treatment group: solid line.

Fig. 1B. Levels of myocardial damage markers perioperatively.
Lower troponin T and CPK levels were observed in patients that received simvastatin 40 mg daily at least two weeks prior to surgery at 0, 6 and 12 hours postoperatively as compared to acute administration (red. day before and day of cardiac surgery). Troponin T: P=0.004; CPK: P=0.0003 (area under the curve was compared between the two treatment groups).
Legend: acute treatment group: dashed line, prolonged treatment group: solid line.

DISCUSSION

Results from this exploratory trial performed during planned myocardial ischemia/reperfusion do not confirm preclinical conclusions that acute preoperative statin treatment is superior to continued treatment in suppressing the post-reperfusion inflammatory response.

Preclinical studies convincingly show beneficial effects of statin therapy in limiting tissue damage following myocardial ischemia/reperfusion.[14-16] Positive effects are thought to be mediated by limiting the post-reperfusion response; an effect that is thought to involve increased endothelial NO production via activation of the PI3K-Akt-eNOS pathway.[4,17,18] Yet, clinical studies all fail to confirm these promising preclinical observations. This discrepancy between preclinical models and clinical reality may reflect interspecies differences and/or differences between the model context and real life ischemia/reperfusion. However, it may also relate to the fact that clinical studies and clinical evaluations are performed during continued statin therapy. Experimental data show that aspects of the statin anti-inflammatory potential fade during prolonged therapy, an effect attributed to weaning (desensitization) of the responses as result of metabolic adaptations and compensatory mechanisms.[5]
On this basis it was hypothesized that the negative conclusions from clinical trials may reflect the weaning phenomenon of continued statin therapy. It was therefore decided to perform an exploratory trial in patients with planned ischemia/reperfusion (on-pump valve surgery with cross-clamping of the aorta). As of unanticipated slow inclusion rate (mainly due to patients' unwillingness to participate because of concerns on side effects of statins or polypharmacy), it was decided on an interim analysis to test for futility. Conclusions obtained met all requirements for futility: results show parallel responses for *all* inflammatory mediators tested, and indicated suppressed post-operative troponin and CPK levels responses in the prolonged statin therapy group. Consequently the data fully refute the study hypothesis, and the decision was made to terminate the trial for reasons of futility.

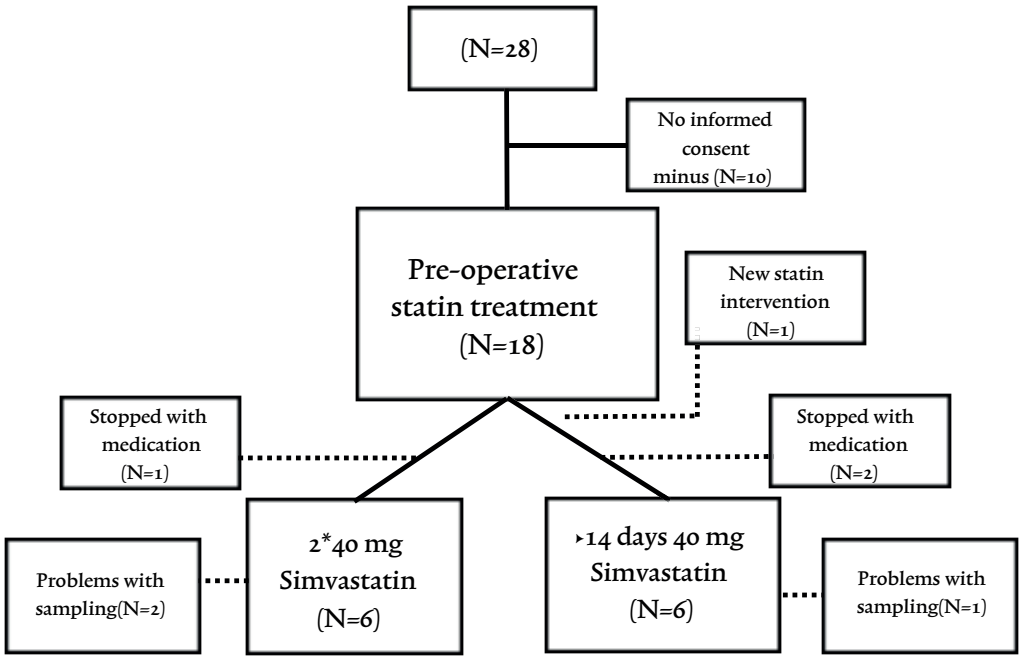
Although the findings from small studies are sensitive to a type II statistical error, we consider the observation of superior post-operative suppression of myocardial damage markers in the prolonged statin therapy realistic. Not only was the effect isolated, and observed for both, unrelated damage markers tested; the observations also fit in the reported beneficial effects of statin treatment on outcome (early mortality and duration of ICU/hospital admission) after cardiac surgery.[19,20]
A notable observation from this trial is that this beneficial effect appears

independent of the myocardial inflammatory response.

Moreover, the purpose of our trial was to test the hypothesis that acute statin therapy is superior to prolonged therapy, hence a reference group not receiving statin therapy is missing. As such we cannot quantify the impact of statin therapy per se on the post-reperfusion inflammatory response. Yet, the anti-inflammatory potential of statins has been firmly established in other contexts.[13] Moreover, on basis of this study it cannot be excluded that the apparent superiority of long-term statin therapy over acute statin therapy reflects a negative impact of the acute statin exposure. Finally, this trial has been performed with a hydrophilic statin (simvastatin). Since differences in cardio-protective effects have been reported between hydrophilic and lipophilic statins[21] results from this trial may not, or to a different extent, apply to lipophilic class of statins.

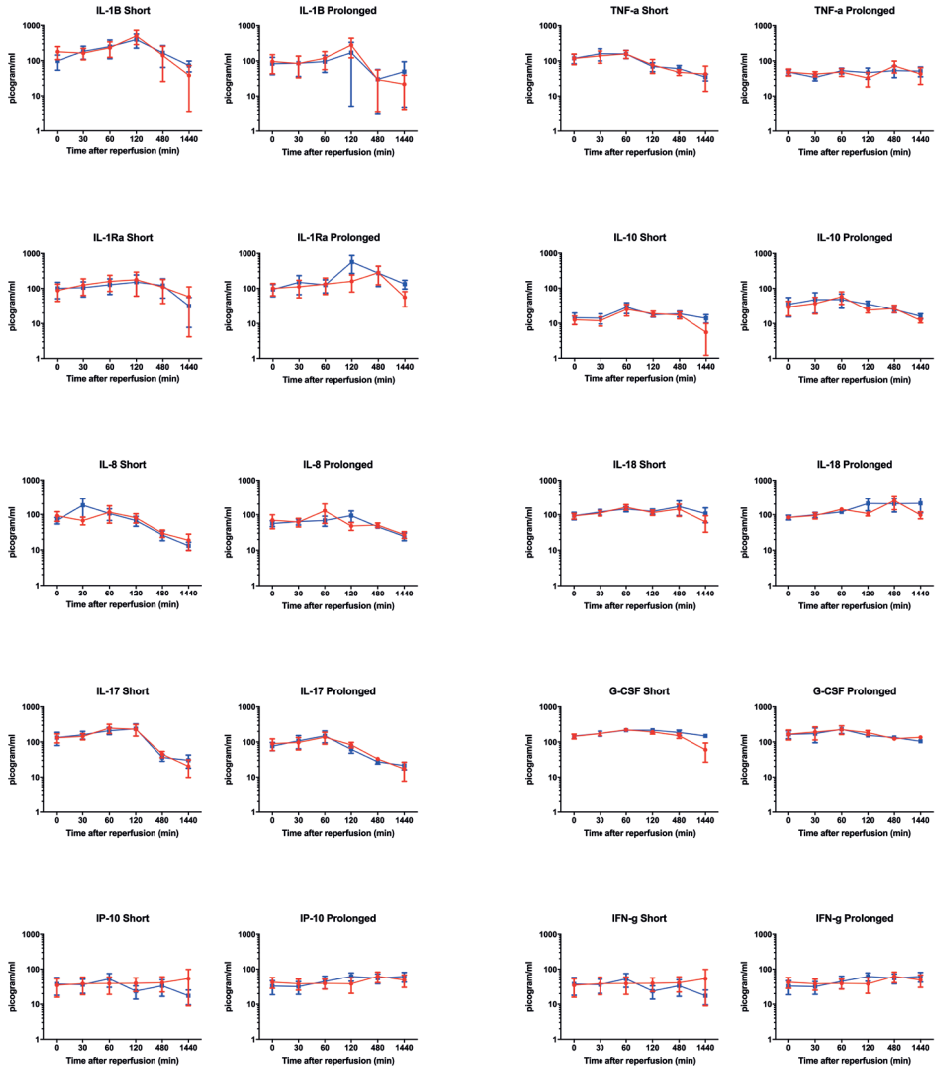
In summary, this trial does not support the hypothesis that acute statin therapy, by virtue of (partial) weaning of the anti-inflammatory potential during prolonged therapy, is superior to continued treatment in alleviating post-reperfusion inflammatory responses. On the contrary, indications were found for superior myocardial protection for prolonged statin therapy.

SUPPLEMENTARY FIGURES



Supplemental Figure 1. Flow-chart of our patient inclusion

Myocardial cytokine release



Supplemental Figure 2. Myocardial release of cytokines
Release (arteriovenous difference) of pro-inflammatory cyto/chemokines by the reperfused myocardium was absent. No differences were found between acute and prolonged treatment groups.
Legend: Red lines resemble arterial levels, blue lines venous levels (mean (SEM)). Left columns: acute treatment group; Right columns: prolonged treatment group.

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