Pravastatin immunomodulates IL-6 and C-reactive protein, but not IL-1 and TNF-α, in cardio-pulmonary bypass

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ABSTRACT. ***Background.*** **While statins are increasingly used in cardiopulmonary bypass (CPB), the anti-inflammatory effects of individual statins, within the context of various treatment regimes, need further examination. The present study evaluates the anti-inflammatory effectiveness of the short-term, preoperative and intensive postoperative use of pravastatin in CPB.** ***Method*****. Forty three patients undergoing CPB were enrolled in a randomized, prospective clinical study. One group (n = 21), received pravastatin, the other (n = 22) did not. Patients in the pravastatin group received one dose of 40 mg per day for nine days, starting 48 hours before CPB, with an additional dose of 40 mg one hour after surgery. Plasma levels of selected inflammatory mediators were measured at baseline and tracked systematically.** ***Results.*** **Pravastatin reduced postoperative interleukin-6 (IL-6) levels significantly at 24 and 48 hours, and at seven days. Mean ± SD values, for treated versus untreated patients were: at 24 hours, 159.5 ± 58.5** ***versus*** **251.2 ± 53.0 pg/mL (p < 0.001); at 48 hours, 81.9 ± 31.5** ***versus*** **194.2 ± 56.3 pg/mL (p < 0.001); and at seven days, 16.4 ± 7.2 versus 30.8 ± 12.6 (p < 0.001). C-reactive protein (CRP) decreased significantly on the seventh postoperative day, when plasma levels were 3.6 ± 1.1 in the treated patients versus 8.2 ± 2.1 mg/dL in the controls (p < 0.001). No changes in plasma IL-1 and TNF-α were found during entire study.** ***Conclusions.*** **Pravastatin induced a precocious modulation of IL-6 expression and a later reduction of plasma CRP levels. Pravastatin′s effects on the expression of these pivotal inflammatory mediators strongly support its well-timed use in CPB.**

Keywords: CPB, inflammatory mediators, inflammation, revascularization, IL-6, CRP

It is well established that cardiopulmonary bypass (CPB) induces a systemic inflammatory response syndrome (SIRS) [1] through the release of inflammatory and anti-inflammatory cytokines [2, 3]. SIRS is the result of a complex mediator interrelationship of inflammation and its effects on cellular function, involving a generalized endothelial inflammation. This response appears a few hours after surgery through the immediate release of interleukin-6 (IL-6) and other inflammatory mediators [4]. Thus, IL-6 is a sensitive marker of the acute inflammatory response, stimulating endothelial activation, vascular smooth muscle cell proliferation, and leukocyte recruitment [5], all of which are events that may lead to plaque growth or instability [6]. This prothrombotic cytokine is the main stimulus for the production by the liver of most acute phase proteins including C-reactive protein (CRP) and serum amyloid A [7]. CRP, a prototypic marker of inflammation, activates endothelial cells to express adhesion molecules [8], and also induces the secretion of IL-6 and endothelin-1, thus decreasing the expression and bioavailability of endothelial nitric oxide synthase in human endothelial cells [9]. Because of the pivotal role of IL-6 and CRP after CPB, their immunomodulation is extremely important for the control of inflammatory expression and endothelial activation [10].

Statins have previously been shown to modulate inflammatory reaction in acute coronary syndromes (ACS) [11, 12] and after CPB [13, 14]. They rapidly improve endothelial function, increase nitric oxide production, and reduce CRP serum level, pro-inflammatory cytokines (*e.g.* IL-6), adhesion molecules, and other acute phase proteins [15].

Most previous studies analyzing the effects of statins on inflammatory reactions after CPB have been restricted, however, to single measurements of plasma mediators at arbitrary timing, and have included a number of different statins, with varied equipotentials and/or in unreported doses [13, 16]. Furthermore, there is no consensus on the optimal timing of statin therapy. Although some authors claim that short-term pleiotropic effects take up to two weeks to occur, some evidence suggests that these effects can be achieved in a shorter period [16].

In order to shed additional light on the efficacy and timing of statin therapy in CPB, we systematically tracked the sequential changes in levels of the key inflammatory

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mediators IL-6 and CRP in response to therapy with a single, very safe statin, pravastatin. The treatment regime we examined consisted of standard daily doses of pravastatin beginning 48 hours before surgery and continuing through the seventh post-operative day, but also included an early post-operative dose. We believed that this extra dose would help maintain the circulatory statin level during the period of maximal plasma expression of inflammatory markers.

PATIENTS AND METHODS

Patients

Forty-three patients, 36 men and seven women, scheduled to undergo non-emergent CPB to treat 2-, or 3-vessel coronary artery disease were enrolled in a randomized clinical study. Inclusion criteria were: age between 50 and 80 years old, stable angina, and ejection fraction over 35%. Exclusion criteria were: acute myocardial infarction, active or prior history of autoimmune disorders, medication with immunomodulating agents such as steroids or anti-inflammatory drugs, elevated white blood cell or C-reactive protein levels, any history or signs of infection before surgery, and renal or hepatic dysfunction. This sample adequately represented the population of patients submitted to non-emergent CPB. Informed consent was obtained from all patients and the investigation was approved by the local ethics committee.

Patients were randomly assigned to one of two groups, pravastatin (n = 21) or control (n = 22), equated approximately in terms of the ratio of men to women. The groups thus formed were similar in terms of clinical characteristics and risk factors *(table 1)*.

Table 1
Demographic clinical characteristics, risk factors and perioperative data

	Pravastatin group	Control group
Age, yrs		
Mean ± SD	68.2 ± 7.2	67.9 ± 7.3
Sex, n (%)		
Female	4	3
Male	17	19
Diagnosis, n (%)		
2-Vessel disease	2	2
3-Vessel disease	19	20
NYHA, n (%)		
Class I	8	9
Class II	13	13
Risk factors, n (%)		
HLP	16 (76.1)	17 (77.2)
Hypertension	17 (80.9)	16 (72.7)
Nicotine abuse	14 (66.6)	13 (59.0)
Diabetes mellitus	8 (38.0)	9 (40.9)
No. of grafts		
Venous grafts	55	58
LIMA	21	22
Radial graft	19	20
CEC time, min		
Mean ± SD	93.8 ± 9.1	94.1 ± 7.7
Range	76-110	81-105
Cross-clamping, min		
Mean ± SD	62.7 ± 7.0	63.2 ± 7.7
Range	50-70	49-77

NYHA: New York Heart Association classification; EF, ejection fraction; HLP: hyperlipoproteinemia; LIMA indicates left internal mammary artery.

Procedure

Patients in the pravastatin group received 40 mg of pravastatin per day starting 48 hours before surgery and continuing through the seventh post-operative day. In addition, they received an extra 40 mg dose one hour after CPB. Patients in the control group were not treated with statins. Both groups received aspirin six hours after CPB and diltiazem when the hemodynamics warranted. No patient was given an angiotensin-converting enzyme inhibitor (ACE-Is) during the study period. In agreement with international standards, none of the patients were transfused, since they used intraoperative cell saver and the hematocrit was not lower than 23% during the post-operative stage.

Intraoperative patient management

Standard anesthetic (fentanyl citrate, ethomidate, pancuronium bromide, isofluorane) and monitoring techniques were used in all patients. Hemodynamic monitorization was performed through a pulmonary artery catheter (IntelliCath, Baxter Edwards Critical-Care, Irvine, CA, USA) and continuous cardiac output (CCO) monitoring.

Cefazolin was used for antibiotic prophylaxis, and the first dose was administered before induction of anesthesia. None of the patients received tranexamic acid or aprotinin. The CPB circuit consisted of roller pumps (SARN), a membrane oxygenator (Affinity, Medtronic), an open hard shell reservoir (Medtronic), and a 40-µm arterial filter (Medtronic). Full-dose heparin (3 mg/kg) was applied before cannulation of the ascending aorta and the right atrium for installation of CPB. Hypothermic (32 to 34°C) continuous perfusion was maintained throughout the period of aorta cross-clamping. Standard blood cardioplegia was performed. After coming off the bypass, patients were administered protamine to completely neutralize the heparin.

Blood sampling

Blood samples were obtained during induction of anesthesia (baseline). Based on our previous finding of a high correlation between plasma levels of inflammatory markers and hemodynamic changes [17], samples were taken in the intensive care unit (ICU) between eight and 10 hours after surgery whenever considerable changes in the hemodynamia were observed (*i.e.* when systemic resistance fell significantly) and whenever cardiac output enhancement was more than 20% of that observed at ICU intake. Samples at fixed times were taken at 12, 24 and 48 hours, and on the seventh day. All samples were collected in evacuated blood collection tubes (10 mL; Monoject; Sherwood Medical; Ballymoney, N Ireland) containing ethylenediamine-tetra-acetic acid and aprotinin. Immediately after sampling, the blood was centrifuged at 3 500 rpm for 20 min, and plasma samples were stored at -70°C until measurements were made. Determinations of cytokines (TNF-α, IL-6, IL-1) and soluble IL-6 receptor

(sIL-6R) were made by a specific, sensitive, no cross-reactivity, commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantikine; R&D Systems, Minneapolis, MN, USA).
Measurements were carried out in duplicate and performed the same day to avoid inter-assay variation. The detection limit of the ELISA technique was 10 pg/mL. Serum concentrations of high-sensitive C-reactive protein (hs-CPR) were assessed using a latex-enhanced turbidimetric assay (Roche), and brain natriuretic peptide (BNP) was measured on site using the point-of-care Biosite Triage Assay (Biosite Diagnostic Corp).

Statistical analysis

Data are presented as mean ± SD. IL-6 and CRP plasma changes over time within each group were analyzed by a repeated measures ANOVA, and *post hoc* comparison were performed using the Bonferroni tests, while comparisons between groups were made using the non-parametric Wilcoxon rank sum test. A significance level of $p < 0.05$ was used in all statistical tests.

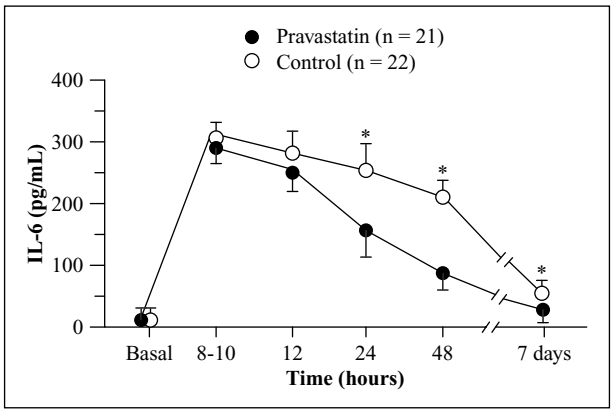

Figure 1
Effect of pravastatin on the temporal kinetics of plasma IL-6 in CPB patients.
The IL-6 kinetic pattern showed similar high levels of the cytokine during hemodynamic changes (8-10 h.) in both groups. A clear tendency of the reduction of plasma IL-6 started at 12 hrs. After surgery in the pravastatin group, reaching a significant value at 24 hours (non-parametric Wilcoxon matched pairs signed-rank test, * $p < 0.001$).

RESULTS

There were no substantial differences between the pravastatin group and the control group in terms of key perioperative data *(table 1)*. In both groups, a marginal rise in hepatic enzymes and an increase in total creatine-kinase were observed, but did not induce significant clinical alterations. In the pravastatin group, five cases of transitory arrhythmias of less than 48 hours duration were registered. In the control group, eight patients presented transitory atrial fibrillation. In all cases, frequencies were controlled by diltiazem and maintained under 120 beats/min it should be noted that the present study did not address the issue of statin's anti-arrhythmogenic effects). No severe adverse effects were observed in either group.

Plasma IL-6

Figure 1 shows the effect of pravastatin on the level of plasma IL-6 in CPB patients over time. Basal IL-6 levels were under the detection limit of the technique (< 10 pg/mL) in the treated and control groups. The maximum levels were observed when blood samples were taken during considerable changes in haemodynamia. In both groups, these changes occurred between nine and 10 hours after surgery, when IL-6 plasma levels were 287.1 ± 40.9 pg/mL for the statin group and 290.7 ± 52.1 pg/mL for non treated group. At 12 hours, the level of IL-6 measured was lower in the treated patients than in the control subjects (255.2 ± 42.0 *versus* 282.0 ± 48.7 pg/mL, $p < 0.06$), although not significantly so. This difference reached statistical significance at 24 and 48 hours: 159.5 ± 58.5 *versus* 251.2 ± 53.0 pg/mL ($p < 0.001$) and 81.9 ± 31.5 *versus* 194.2 ± 56.3 pg/mL ($p < 0.001$), respectively and on the seventh day (16.4 ± 7.2 *versus* 30.8 ± 12.6, $p < 0.001$).

Plasma CRP

Figure 2 shows the effect of pravastatin on the expression of plasma CRP over time. Since we expected only a slight increase and not an early peak expression during hemodynamic changes, we measured plasma CRP levels at fixed intervals. Basal levels were 0.51 ± 0.33 mg/dL in treated patients and 0.55 ± 0.35 mg/dL in the control group. Following surgery, increasing levels of plasma CRP were seen in both groups, reaching maximal concentrations at 48 hours, 21.5 ± 4.0 mg/dL in the pravastatin group and 24.4 ± 2.8 mg/dL in the control subjects. A significant reduction was achieved on the seventh day, when plasma CRP was 3.6 ± 1.1mg/dL in the treated group and 8.2 ± 2.1 mg/dL in the non-treated group ($p < 0.001$).

Other measurements

Throughout the entire study, both groups showed low plasma levels of IL-1 and TNF-α (below the detection limit of the technique < 10 pg/mL). In contrast, the concentrations of sIL-6R were consistently over 3,000 pg/mL in

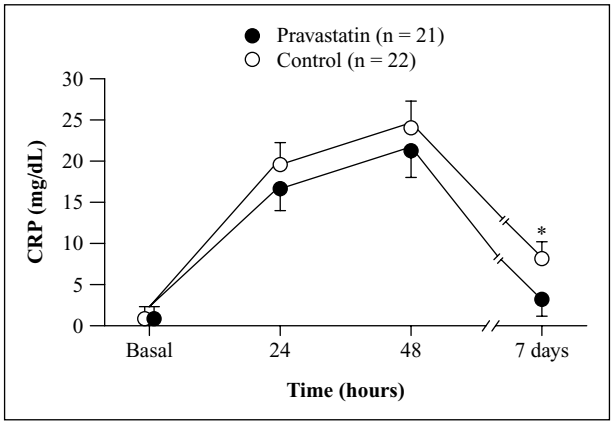

Figure 2
Effect of pravastatin on plasma CRP in CPB patients. Increasing post-operative plasma levels were seen in both groups, reaching a maximal level at 48 hours. A significant reduction was achieved on the seventh day in the pravastatin group (non-parametric Wilcoxon matched pairs signed-rank test, * $p < 0.001$).

both groups, with no statistically significant differences. No between-group differences in BNP and creatin-kinase levels were detected.

DISCUSSION

With other inflammatory models, such as ACS, evaluation of statin therapy is complicated by low plasma levels of key serum markers, and those studies that have demonstrated effects of statins have found greater effects on CRP than on IL-6 [18-20].

Here, using the CPB inflammation model [21] with higher levels of serum markers, we were able to demonstrate the therapeutic effectiveness of a single statin, pravastatin, identifying its effects on the expression of both IL-6 and CRP. Although we have shown that pravastatin has an effect on both of these acute-phase proteins, they do, in fact, behave quite differently.

The immunomodulatory effect of pravastatin on post-operative plasma levels of IL-6 was observed quite early. A substantial, but statistically non-significant, decrease was first seen in the pravastin-treated patients at 12 hours. After 12 hours, the levels of IL-6 were consistently lower in the pravastatin-treated patients than in the controls, with these differences being statistically significant at 24 and 48 hours, and at seven days. In contrast, pravastatin's effect on CRP levels was not observed until much later in the post-operative period. There was essentially no visible difference until 48 hours and no significant difference until the seventh post-operative day, when the treated patients showed CRP levels 56% lower. The radically different behavior of these two inflammatory markers would seem to make sense: pravastatin initially modulates the IL-6 mediator (the main stimulus for CRP production) and CRP modulation lags behind.

Consistent with other studies, a circulating peak of IL-6 was reached at approximately nine to 10 hours post-operatively [14, 22]. Since this peak level was essentially identical in both groups, our treatment regime does not appear able to control the endogenous stimulus at its point of maximal expression. Whether statin therapy could have a greater impact during this period remains an open question however. Since a significant reduction in plasma IL-6 level was not observed in the pravastatin-treated group until 24 hours postoperatively, the failure to detect an earlier effect could have been due to an insufficient serum level of statin. The testing of other treatment regimes using higher doses or different timings could help to answer this question.

Liakopoulus *et al*. [23] showed that different statins at different doses, attenuated the inflammatory response after cardiac surgery through diminished IL-6 and up-regulated release of IL-10. Recently, a combination of statins and ACE-inhibitors was also used, showing in untreated patients a significant, lesser inflammatory mediator expression [24]. We decided to evaluate only one statin, at a fixed dose, and excluding ACEIs and other drugs with well recognized anti-inflammatory properties.

Finally, considerable uncertainty still exists regarding the optimal timing of statin therapy in CPB [25]. Brull *et al*. [16] were the first to demonstrate a decrease in plasma IL-6 levels 24 hours post-operatively using statins administered the night before surgery (211 ± 156 *versus* 269 ± 141 pg/mL, $p < 0.01$). Other investigators, such as Florens *et al.* [13], who administered atorvastatin in 40 mg doses the evening before and the day of surgery, have not found the "night before" pre-operative regime to be effective. Some now advocate much longer pre-operative statin regimes. A recent European study, for instance, used atorvastatin three weeks before surgery, suggesting that this time would be necessary to achieve pleiotropic and anti-inflammatory effects [26].

While the present study was not designed to evaluate the merits of alternative timing regimes, our results do add to the body of evidence bearing on this issue. At least in the case of therapy using pravastatin in standard doses, it appears that the pre-operative treatment phase can be relatively brief when the post-operative regime is intensified by an extra standard dose, shortly after surgery.

In conclusion, immunomodulation of IL-6 and CRP is important in patients undergoing CPB [27] in relation to a host of factors bearing on their prognosis: hemodynamic stability, endothelial recovery, postoperative inflammatory phenomena, thrombocytosis, permeability of grafts, prevention of thrombogenesis and atherogenesis. Pravastatin safely and effectively immunomodulates these acute-phase proteins and should be considered a viable treatment option. While it has been suggested that effective immunomodulation with statins requires long-term, pre-operative therapy, we have demonstrated the utility of a regime employing a short pre-operative period and an intensified post-operative phase.

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