Inflammatory Activation During Coronary Artery Surgery and Its Dose-Dependent Modulation by Statin/ACE-Inhibitor Combination

Alberto Radaelli, Claudia Loardi, Maria Cazzaniga, Giulia Balestri, Caterina DeCarlini, M. Grazia Cerrito, Elena Negro Cusa, Luca Guerra, Stefano Garducci, Danilo Santo, Lorenzo Menicanti, Giovanni Paolini, Arianna Azzellino, Maria Luisa Lavitrano, Giuseppe Mancia, Alberto U. Ferrari

Background—On-pump coronary artery bypass graft (CABG) surgery triggers an inflammatory response (IR) which may impair revascularization. The study aimed at (1) characterizing the temporal profile of the IR by assaying appropriate markers in both systemic and coronary blood, and (2) determining whether (and which doses of) cardiovascular drugs known to have antiinflammatory properties, namely statins and ACE-inhibitors (ACEI), inhibit the response.

Methods and Results—Patients scheduled for CABG (n=22) were randomized to statin/ACEI combination treatment at standard doses (STD, ramipril 2.5/simvastatin 20 mg, or atorvastatin 10 mg, or enalapril 20 mg/simvastatin 80 mg, or atorvastatin 40 mg). Plasma levels of interleukin 6, tumor necrosis factor alpha, E-selectin, von Willebrand factor (vWF), and sVCAM-1 were serially assayed (ELISA) before, during, and after CABG. Blood was drawn from an artery, a systemic vein, and the coronary sinus. Myocardial perfusion scans were obtained before and 2 months after surgery in 19 out of 22 subjects. In the STD group both IL-6 and TNF displayed striking increases which were similar at all sites and peaked 10 to 60 minutes after aortic declamping. Such increases were drastically attenuated in the HiDo group. Instead, only modest increases in venous E-selectin, vWF, and sVCAM-1 were observed. Scintigraphic ischemia scores were entirely normalized after versus before CABG in the HiDo but not in the STD treatment group.

Conclusions—On-pump CABG surgery is associated with an intense systemic inflammatory response, which can be almost completely prevented by early treatment with high (but not standard) doses of ACE-inhibitors and statins.

Key Words: coronary artery surgery • inflammation • statins • ACE-inhibitors

It is increasingly recognized that the inflammatory response triggered by on-pump coronary bypass graft surgery (CABG)1–3 is of clinical importance as it may contribute to the genesis of common postoperative complications and may even exert adverse vascular biological effects on native or grafted coronary vessels.4–6 However, several aspects and possible implications of this phenomenon are a subject of controversy or have so far not been addressed.

One, limited information exists on the magnitude and time course of the CABG-related release of circulating and vascular wall inflammatory factors.7,8 Two, it is not established whether the release of such factors has systemic extension or is confined to the cardiac tissues exposed to surgical trauma.9 Finally, conflicting results were reported as to whether and at which doses commonly used cardiovascular drugs can attenuate the inflammatory response and its consequences.10–16 This applies in particular to statins and ACE-inhibitors whose pleiotropic antiinflammatory properties are now well established.16–22

To address such open questions, we examined in patients undergoing on-pump CABG surgery: (1) the patterns of the surgery-related inflammatory response, by assaying a range of mediators throughout the perioperative period; (2) the trends of inflammatory mediator release assayed in parallel from the systemic and the coronary circulation, and (3) the possible differential effects of 2 doses of statins plus ACE-
Table 1. Baseline Characteristics of the Two Groups

<table>
<thead>
<tr>
<th></th>
<th>Standard (n=11)</th>
<th>High Dose (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>65±1.6</td>
<td>64±1.6</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>120±4</td>
<td>115±3</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>80±2</td>
<td>75±3</td>
</tr>
<tr>
<td>EF, % (scintigraphic measure)</td>
<td>57±1</td>
<td>56±2</td>
</tr>
<tr>
<td>Serum cholesterol, mg · dL⁻¹</td>
<td>178±11</td>
<td>164±15</td>
</tr>
<tr>
<td>No. of grafts</td>
<td>3±0.2</td>
<td>3±0.2</td>
</tr>
<tr>
<td>No. of grafted vessels</td>
<td>2.8±0.1</td>
<td>2.7±0.2</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Diabetes mellitus, type II</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

EF indicates ejection fraction.

Table 2. Treatment Characteristics of the Two Groups

<table>
<thead>
<tr>
<th></th>
<th>Standard (n=11)</th>
<th>High Dose (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presurgery treatment duration, days</td>
<td>28.7±4.9</td>
<td>21.9±2.7</td>
</tr>
<tr>
<td>Dose of simvastatin, mg</td>
<td>15±3 (3)</td>
<td>67±13* (3)</td>
</tr>
<tr>
<td>Dose of atorvastatin, mg</td>
<td>11±2.6 (6)</td>
<td>40±2* (8)</td>
</tr>
<tr>
<td>No statin, n° pts</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Dose of ramipril, mg</td>
<td>1.8±0.6</td>
<td>6.4±0.9* (10)</td>
</tr>
<tr>
<td>Dose of enalapril, mg</td>
<td>20 (1)</td>
<td></td>
</tr>
</tbody>
</table>

Figures in parenthesis refer to No. of patients.

Method

Inhibitors on the inflammatory process as well as on the success of myocardial revascularization. The latter was assessed by performing effort radionuclide scans before and within 2 months of surgery.

**Results**

**Study Population**

Twenty-two patients with angiographically documented multi-vessel coronary artery disease not amenable to coronary angioplasty were recruited 20 days before the scheduled coronary revascularization surgery (Table 1). Patients were randomly allocated to either a standard therapy group (n=11, 2 females), in which the ongoing drug treatment was continued, or to a high dose therapy group (n=11, 1 female), in which ongoing drugs were maintained but their dose were uptitrated to ramipril 10 mg or enalapril 20 mg per day and to simvastatin 80 or atorvastatin 40 mg per day; such doses were viewed as to represent the highest recommended/tolerated dose for a patient in the pre-CABG phase and were previously shown to be associated with expression of the antinfiammatory activity of either drug class.21,22

Actual drug doses in each group are reported in Table 2.

Exclusion criteria were unstable myocardial ischemia, sustained atrial or ventricular arrhythmias, chronic obstructive pulmonary disease, or any other major system disease. The protocol was approved by the San Gerardo Hospital Ethical Review Board. All patients were informed about its nature and purpose.

**Surgical Procedure**

Each patient received preanesthesia with morphine 0.1 to 0.15 mg/kg and diazepam 6 mg. Thirty minutes later, anesthesia was induced with fentanyl 0.005 to 0.01 mg/Kg, midazolam 0.08 to 0.2 mg/kg, and pancuronium 0.1 mg/kg and maintained with propofol 3 to 6 mg/kg/h, fentanyl, sevoflurane, and pancuronium as needed. Radial or femoral arterial pressure, heart rate (HR), and central venous pressure (CVP) were monitored throughout. A longitudinal sterno-tomic approach was used in all patients. After harvesting the left internal mammary artery, or both mammary arteries, and a segment of saphenous vein, and after opening the pericardium, 300 UI/Kg of heparin was administered to obtain an activated clotting time target more than 480 sec. A standard cardiopulmonary bypass through the cannulation of ascending aorta and right atrium was instituted, maintaining a blood flow of 2.2 to 2.5 l/min/m² and a mean systemic pressure around 50 to 70 mm Hg. Mild systemic hypothermia (34°C to 35°C) was obtained in each patient.

Cardiac arrest was induced by administration of intermittent cold ematic cardioplegic solution through antegrade and retrograde route according to the Buckberg protocol.

After weaning off cardiopulmonary bypass, heparin was neutralized with protamine sulfate. The average aortic clamping time was 89±5.5 and 93±5.5 minutes (means±SEM) in the standard and high-dose patient group, respectively (P=ns). The corresponding durations of extracorporeal circulation were 110±6.0 and 115±7.0 minutes (P=ns). Each patient was then transferred into the intensive care ward. No major complications, nor any fatalities, occurred in either patient group.

**Biochemical and Scintigraphic Assessments**

**IL-6, TNF-α, E-selectin sVCAM-1, and vWF**

Plasma concentrations of these substances were determined by ELISA using venous, arterial, and coronary sinus blood samples at different time points. Blood from the coronary sinus was obtained 5 minutes before aortic clamping and 10 minutes after aortic declamping (with extracorporeal circulation still on). Arterial blood was obtained at the same times as coronary sinus blood and additionally at the 1, 6, and 24 hours after aortic declamping. Venous blood was obtained at the same times as arterial blood and additionally at the 15 and 1 days before as well as at the 7 and 60 days after surgery. Blood was directly drawn into heparinized tubes, immediately mixed by several gentle inversions, placed on ice, and then spun down for 10 minutes in a centrifuge (3000 g, 4°C) after withdrawal. The serum was stored at −80°C until it was assayed. To measure serum concentration of IL-6, TNF-α and E-Selectin, a quantitative sandwich enzyme immunoassay technique (ELISA; Bender Medsystems) was used on 100 µL plasma aliquots in triplicate. According to manufacturer’s protocol, plates were coated overnight and blocked with 0.5% Bovine Serum Albumin (BSA; Sigma-Aldrich). Plasma was diluted in PBS (Euroclone) and then incubated overnight in a 96-well antibody-coated plate at 4°C. All washing steps were carried out with PBS using an automated plate washer (DAS). Incubation with biotin-conjugate antibody was performed at room temperature for 1 hour, followed by washing and Tetramethylbenzidine (Sigma-Aldrich) substrate solution addition. Reaction was stopped using H3PO4 1 mol/L (Sigma-Aldrich), and plates were immediately read at 450 nm (DAS).

Serum concentration of sVCAM-1 and vWF were measured by quantitative sandwich enzyme immunoassay technique (ELISA) using different commercial kits (American Diagnosticfor sVCAM1 and R&D Systems for vWF). Standard manufacturer recommendations were followed and all tests were run in triplicate.

Other measurements included: (1) total serum creatinphosphokinase and its MB fraction, which were serially assayed in the perioperative period to assess enzyme baseline values and peak elevations, for the severity of surgery-related injury to skeletal muscle or to the myocardium to be evaluated; (2) complete blood cell counts (aiming at monitoring leukocytes and platelets), which were obtained preoperatively, immediately after aortic declamping, every 12 hours for at least 3 days as well as 14 days after surgery; (3) myocardial radionuclide scans, which were obtained 5 to 10 days before and 60 days after surgery to assess resting as well as effort myocardial perfusion and left ventricular systolic function. The single photon emission computed tomographic (SPECT) imaging with 99mTc-Tetrofosmin or 99mTc-MIBI were used according to a 2-day protocol as specified in the procedure guidelines for radionuclide myocardial perfusion imaging.21–23 The extent of ischemia was expressed as the summed difference score (SDS), which was...
pattern was observed for IL-6 (Figure 1, dotted bars, lower panel). A similar increase was evident in the samples drawn from an artery (Figure 1, dotted bars, upper panel) or a systemic vein (data not shown), as well as from the coronary sinus (Figure 2, dotted bars, upper panel). A similar increase was also observed for IL-6 (lower panels of Figures 1 and 2), although in this case there was a later rise so that in the time window between 6 and 24 hours the pattern of IL-6 changes was similar in the 2 groups.

**Statistical Comparisons**

Because IL-6, TNF, and E-selectin were found to be not normally distributed (Shapiro-Wilk normality test: \( P<0.05 \)), significance of the procedure-related changes within groups at the different times was assessed by the Wilcoxon rank test whereas the between group differences were assessed by the Kruskal-Wallis rank test (\( P<0.05 \)).

Pre- versus postoperative blood cell counts, CK enzyme assays, and SDS scintigraphic indexes were tested by the Mann–Whitney U test. For each test, the level of statistical significance was set at \( P<0.05 \).

**Results**

The baseline patient characteristics were largely similar in the 2 groups and are summarized in Table 1 (means±SEM), whereas the respective treatment characteristics and the marked between group differences in the actually administered drug doses are shown in Table 2; in the latter it is apparent that most patients received distinctly low doses of both ACEIs and statins. The types and doses of drugs administered to the high dose group patients before randomization were largely similar to those of the standard group patients (data not shown).

**Cytokines**

In the standard therapy group, plasma concentrations of TNF-\( \alpha \) were low (<2 pg.ml\(^{-1} \)) at baseline but showed a striking CABG surgery-related increase. The increase was in the order of 15- to 20-fold and was evident in the samples drawn from an artery (Figure 1, dotted bars, upper panel) or a systemic vein (data not shown), as well as from the coronary sinus (Figure 2, dotted bars, upper panel). A similar pattern was observed for IL-6 (Figure 1, dotted bars, lower panel; Figure 2, dotted bars, lower panel). The increase in plasma concentrations of cytokines was sustained in time and did not vanish until at least 6 hours post-surgery for TNF-\( \alpha \) and even much longer than that (up to at least 24 hours) for IL-6 (Figures 1 and 2).

At variance with the former group, the high dose therapy group was characterized by virtually entirely suppressed surgery-related changes in the plasma concentrations of TNF-\( \alpha \) in both arterial (Figure 1, hatched bars, upper panel) and coronary sinus blood (Figure 2, hatched bars, upper panel). A marked early suppression of the surgery-related increase was also observed for IL-6 (lower panels of Figures 1 and 2), although in this case there was a later rise so that in the time window between 6 and 24 hours the pattern of IL-6 changes was similar in the 2 groups.

**E-Selectin**

The plasma concentrations of E-selectin underwent a modest but significant surgery-related increase, with venous values about twice as large as before surgery since the time of anesthesia induction through the following 24 hours; arterial as well as coronary sinus samples revealed E-selectin values largely similar to the time-matched venous ones. At variance with the patterns observed for cytokines, the surgery-related increase in E-selectin was modestly and inconsistently affected by high-dose statin/ACE-inhibitor treatment (data not shown).

**vWF and sVCAM-1**

Similarly to E-selectin, these factors also failed to undergo sizeable surgery- or treatment-related modifications (data not shown).

**Leukocytes**

In both groups the preoperative leukocyte count was similar and within normal limits (6028±368 and 5875±414 wbc.ml\(^{-1} \), in the standard and high dose treatment group, respectively; means±SEM, \( P=\text{ns} \)). The leukocyte count increased significantly and markedly within 12 hours post-declamping, its peak being however significantly attenuated.
in the high dose (9400±614 wbc.ml⁻¹) compared with the standard dose treatment group (11714±864 wbc.ml⁻¹, \( P<0.03 \)).

Platelets

In both groups the preoperative platelet count was similar and within normal limits (181000±15000 and 150000±12000 plt.ml⁻¹ in the standard and high dose treatment group, respectively; means±SEM, \( P=ns \)). The platelet count was found to be significantly increased at the sampling performed 10 days after surgery; the increase was, however, significantly smaller in the high dose (325000±35000 plt.ml⁻¹) compared with the standard dose treatment group (452000±24000 plt.ml⁻¹, \( P<0.005 \)).

CK Enzymes

The patterns of total CK enzymes at baseline and after surgery were similar in the 2 groups, amounting to 97.9±18.5 and 75.3±10.9 U.l⁻¹ and peaking at 749±101 and 694±170 U.l⁻¹ in the standard and high dose therapy group, respectively (between group comparisons, both \( P=ns \)). No significant changes of CK enzyme MB fractions were observed in either group, the peak values being 27.3±2.79 versus 41.5±8.8 U.l⁻¹ in the standard and high dose therapy group, respectively (\( P=ns \)).

Myocardial Scintigraphy

Before surgery, the ischemic index SDS was not significantly different in the 2 groups (3.60±0.92 and 3.12±0.75 points in the standard and high dose treatment group, respectively, \( P=ns \)). After surgery, the score was significantly improved (reduced) in the standard dose treatment group (1.90±0.62 points, \( P<0.02 \) versus presurgery), but it was entirely normalized in the high dose treatment group (0.0±0.0 points, \( P<0.02 \) versus standard dose treatment group).

Discussion

Our study provides several original findings. The first point of novelty relates to a much more complete characterization of the inflammatory response to CABG than previously available. To begin with, the inflammatory response is huge in magnitude and far from short in duration, because there was an up to 20-fold increase in the assayed inflammatory markers and the elevation of some of them was detectable for as long as 24 hours; it was alongside shown that early scheduling of the blood samplings is crucial to avoid overlooking or incompletely appreciating the response.\(^{10–12,15}\) In addition, the response reflects preferential activation of circulating (leukocytes, platelets, macrophages) rather than vascular wall (endothelium) cellular elements, because there was a weak or null surgery-related increase in E-selectin, sVCAM-1, and vWF compared with the striking increases in TNF-\(\alpha\) and IL-6. Finally, the response was not limited to the heart and rather had a systemic nature because there were no gradients between coronary sinus and systemic cytokine concentrations.

The second point of novelty relates to the effects of cardiovascular drugs. First of all, statins and ACE-inhibitors administered in combination and well in advance of the time of surgery displayed an antiinflammatory activity that is much more extensive and potent than previously reported (10 to 12, 14 to 16): this is supported by their documented efficacy in presence of an exceedingly strong inflammatory stimulus such as on-pump CABG surgery, as well as by the observation that the suppression of cytokine release extended to TNF-\(\alpha\), an effect that was not seen with eg, treatment by statins alone.\(^{12,13}\) In addition, our results provide the first indication that drug dosage is crucial in the present setting because only the high but not the standard dose regimen was associated with the antiinflammatory action, a notion that to our best knowledge has not been previously documented nor explicitly addressed and has obvious practical implications. Finally, further noteworthy observations were that the high dose combination did not induce any undesirable skeletal muscle or myocardial cell damage, as shown by the lack of excess CK elevations, and extended its effects to blunt the surgery-related early increase in leukocyte count as well as the later increase in platelet count; the latter observation may be in relation with the attenuated release of IL-6,\(^{25}\) whereas we are so far unable to identify the mechanism(s) underlying this phenomenon and to clarify whether it may affect the risk of postoperative thrombotic events.

A third point of novelty relates to the differential results of the effort myocardial perfusion scans obtained in the high versus standard-dose groups, in which evidence of complete versus only partial revascularization was obtained. This suggests that the inflammatory phenomena associated with on-pump CABG surgery contribute to hinder the midterm (2 months) success of revascularization; such a possibility may not be unexpected considering that even short-lasting delivery of a noxious stimulus may trigger a much longer-lasting impairment of endothelial function and of vascular biological homeostasis;\(^{26,27}\) on the other hand, we obviously cannot exclude that other differential actions of the higher compared with the lower drug dose, not necessarily related to inflammatory phenomena, may have contributed to the scintigraphic results. Whatever the exact underlying mechanism(s), our results demonstrate the superiority of the higher—compared with the lower-dose treatment option—if any more are needed, a further argument in favor of the use of sizeably higher doses of cardiovascular drugs than is commonly done in clinical practice.

We believe that taken together, our findings have at least 1 more important clinical implication, ie, that the antiinflammatory effects of drug combinations such as that used in the present study represents one of the mechanisms contributing to their protective actions, this being suggested in light of the growing evidence that the development of common postoperative complications of CABG surgery such as atrial fibrillation, acute infection, defective wound repair, etc, is determined or facilitated by inflammatory phenomena.\(^{4,5,7}\) In particular, one may speculate that such phenomena concur to the augmented risk of complications associated with the on-versus off-pump surgical approach;\(^{28,29}\) should this be the case, then routine implementation of the drug regimen described in the present study would represent an easy strategy to minimize the excess risk associated with the former compared with the latter surgical technique.
Some limitations of our study are also to be commented. One, our demanding protocol prevented us from expanding the number of enrolled patients without unduly prolonging the recruitment period: even with the current population size, however, we could demonstrate highly significant intergroup differences and thus did not incur in the risk of type 2 statistical error that often impairs the reliability of small-sized studies. Two, although most patients received a ramipril/atorvastatin combination, in few cases different agents (enalapril in one and simvastatin in three cases) were administered: this was done to keep to a minimum the interference of the research protocol with the ongoing therapy, i.e., to modify at most the doses but not the molecules administered; furthermore, the antiinflammatory properties of both statins and ACE-inhibitors are believed to represent a class rather than a single agent effect. Three, we were unable to include a “placebo”, untreated group, which would have clearly been interesting but would have unquestionably been unethical. Finally, we elected to assess the effects of a drug combination rather than of single agents: this was done to maximize our chances to document the antiinflammatory potential of treatment and in light of the practical consideration that the overwhelming majority of coronary artery disease patients receives both statins and ACE-inhibitors. Even more importantly, there is both basic and clinical pharmacology evidence to support the notion that the antiinflammatory effects of the 2 drug classes may be additive rather than redundant, which would not be unexpected considering that ACEIs largely work by antagonizing the effects of a potent proinflammatory agent such as AII whereas statins are known to interfere with multifold molecular and cellular inflammatory pathways. This strategy proved to be successful, but it cannot be denied that it leaves the issue open as to whether the antiinflammatory effects are to be ascribed to the drug combination rather than to just the statin or the ACE-inhibitor. Although it is conceivable that the strongest effects were determined by the drug combination, the issue will have to be directly addressed in future randomized studies to fully characterize in larger numbers of patients and for longer follow-up periods the effects of each of the two drug classes—as well as of their combination—on the perioperative inflammatory processes and on the outcome of myocardial revascularization.

In conclusion, we characterized the intense systemic inflammatory activation associated with the on-pump CABG surgical procedure and the ability of prolonged, high-dose statin/ACE-inhibitor pretreatment to powerfully inhibit this phenomenon in absence of any detectable untoward effects and with a documented benefit on the success of the revascularization procedure.

Disclosures

None.

References


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