Coronary artery bypass grafting surgery is associated with a marked reduction in serum homocysteine and folate levels in the early postoperative period

Simona Storti*, Alfredo Giuseppe Cerillo, Antonio Rizza, Isabella Giannelli, Giuliana Fontani, Mattia Glauber, Aldo Clerico

Clinical Chemistry Laboratory Unit and Operative Unit of Adult Cardiac Surgery, Ospedale 'G. Pasquinucci', Institute of Clinical Physiology, The National Research Council, Via Aurelia Sud 54100 Massa, Italy

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Abstract

Objective: It has been suggested that cardiac surgery-related ischemia-reperfusion injury to the myocardium is caused by the oxidative stress, derived from the formation of reactive oxygen species in the early phases of the reperfusion. The availability of cysteine, mostly deriving from homocysteine (Hcy) via the trans-sulfuration pathway, is determinant for the synthesis of glutathione (GSH), which in turn acts as a first line of defence against oxidative stress. Indeed, an increased consumption of Hcy in response to oxidative stress has been described. Previous research has shown that Hcy levels increase during the first weeks or months after coronary artery bypass grafting (CABG). This study was designed to evaluate the Hcy metabolism in CABG patients during the early postoperative period.

Methods: Serum Hcy and folate were measured by an automatic immunometric system on blood samples obtained from 48 (mean age 67±10 years) consecutive patients undergoing CABG, preoperatively and at 0, 12, 48, and 120 h after surgery. A subgroup of 22 of these patients was also studied 6 months postoperatively.

Results: A significant decrement of Hcy and folate was observed throughout the study (P, 0.0001) from 17.3±9.3 to 8.5±5.6 μmol/l at 12 h postoperatively for Hcy, and from 5.1±2.8 to 2.5±1.5 ng/ml at 48 h for folate. The reduction of Hcy and folate levels remained significant after correction for haemodilution (as assessed by measurement of plasma proteins). Furthermore, the use of cardiopulmonary bypass significantly interacted with the time of sampling in affecting the Hcy levels. Hcy levels returned to near-baseline values 48 h postoperatively, and were similar to baseline at follow-up.

Conclusion: Our study indicates that serum Hcy and folate levels are markedly reduced during the early postoperative period after CABG. This reduction is at least in part independent of haemodilution, and may be caused by an altered Hcy turnover, due to an increased consumption of GSH during and soon after CABG.

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1. Introduction

Myocardial damage due to ischemia-reperfusion injury has been described during procedures that involve temporary interruption of coronary blood flow, such as coronary artery bypass grafting (CABG) surgery [1].

The two landmarks of ischemia-reperfusion injury are the Ca++ overload and the oxidative stress, which are strictly related to each other. Oxidative stress is usually associated with an increased formation of reactive oxygen species (ROS) [1]. The glutathione (GSH), which is synthesized from L-cysteine, is considered to play an essential defensive role in the response to oxidative stress [2]. The trans-sulfuration pathway, whose initial entering substrate is homocysteine (Hcy), converts methionine to cysteine [2], which is in turn converted to GSH via the GSH biosynthetic pathway (Fig. 1). Indeed, an increase of the Hcy flux through the trans-sulfuration pathway, resulting in increased consumption of Hcy, has been described in the human hepatoma cell line HepG2 in response to oxidative stress [3].

The results of previous research have shown that Hcy levels tend to increase during the first weeks or months after cardiac surgery procedures [4,5], while they may be reduced...
in general surgical patients early postoperatively [6]. To our knowledge, however, the Hcy variation during the early postoperative period after CABG has not been deeply investigated.

In the present study, we analyzed the behavior of Hcy and folate levels during the first five postoperative days after CABG surgery, in order to evaluate whether and how the Hcy metabolism is affected during the early phases that follow surgical myocardial revascularisation. Since a sustained increase of the Hcy levels, possibly resulting in increased susceptibility to graft failure, has been previously reported to occur after CABG [4], we also checked serum Hcy and folate at 6 months follow-up in a subgroup of patients.

2. Materials and methods

2.1. Patients

Forty-eight consecutive patients (mean age 67 ± 10 years, 40 males and eight females) undergoing isolated CABG at our institution from 1st January, 2003 to 28th February, 2003 were included (Table 1). Significant renal dysfunction, severe psoriasis, and therapy with corticosteroid or oral contraceptive drugs were considered exclusion criteria [7]. Patients undergoing combined CABG and valvular procedures were excluded, as well as patients undergoing emergency procedures. Patients in stable haemodynamic conditions undergoing non-elective CABG (patients with significant lesions affecting the left main coronary artery, patients with unstable angina) were included. Finally, postoperative acute renal failure (creatinine ≥ 2.0 mg/dl at any time-point after the operation) was considered an exclusion criterion, since altered renal function is known to affect the Hcy concentration [7]. This study was approved by the Ethical Committee of the ‘G. Pasquinucci Hospital’ and of the ‘Institute of Clinical Physiology’ of the Italian National Research Council. Informed consent was obtained from all patients.

2.2. Blood samples collection and analysis

Blood samples for the measurements of Hcy and folate concentration were obtained from all patients at hospital admission, at the end of surgery, and then at 12, 48, and 120 h postoperatively. A subgroup of 22 patients was also studied at 6 months follow-up. All the samples were collected in serum-separated tubes, immediately chilled in ice, centrifuged and frozen at −20 °C. All samples from the same patient were analyzed in the same session, to minimize the intra assay error. The measurement of serum protein and creatinine concentrations was performed with the Synchron Chemical System CX7 Beckman (Beckman Coulter, Milan, Italy). Blood samples for the measurements of Hcy and folate concentration were obtained from all patients at hospital admission, at the end of surgery, and then at 12, 48, and 120 h postoperatively. A subgroup of 22 patients was also studied at 6 months follow-up. All the samples were collected in serum-separated tubes, immediately chilled in ice, centrifuged and frozen at −20 °C. All samples from the same patient were analyzed in the same session, to minimize the intra assay error. The measurement of serum protein and creatinine concentrations was performed with the Synchron Chemical System CX7 Beckman (Beckman Coulter, Milan, Italy). The measurements of Hcy and folate were performed by the AxSYM® platform (Abbott Laboratories, Diagnostic Division, Abbott Park, USA); in particular, a Fluorescence Polarized Immuno-Assay (FPIA) method was used for Hcy evaluation, while a ionic capture system was used for folate.
measurement. The reference intervals for our laboratory are as follows: Hcy 1–15 μmol/l, folate 5.3–14.4 ng/ml, and serum protein 6.00–8.20 g/dl.

2.3. Anesthetic technique and surgical management

Total intravenous anesthesia with diazepam, fentanyl, pancuronium and propofol was used in all cases. The decision to use or not cardiopulmonary bypass (CPB) was left to the operating surgeon, and was mostly based on the severity and extension of disease of the target vessels. CPB was conducted on moderate hypothermia (34 °C), and myocardial protection was achieved by using intermittent antegrade hyperkalemic warm blood cardioplegia. The surgical technique employed in this series has been previously described in detail [8].

2.4. Statistical analysis

Patient demographic, operative and postoperative characteristics were prospectively recorded.

All the analyses were performed using Statview 5.0 (SAS Institute Inc, Cary, NC). The statistical difference among mean concentrations at different time points of Hcy, folate, and plasma proteins were assessed by Sheffe post-hoc test after repeated measures ANOVA. The effect of CABG surgery on the postoperative course of the Hcy, folate, or serum protein concentrations was assessed by a repeated measures ANOVA model, in which the serum levels of Hcy (folate or serum proteins) were the dependent variable. Furthermore, a step-wise multiple regression analysis was used to test the influence of serum folate and proteins (as two independent variables) on serum Hcy (as dependent variable).

The continuous variables are expressed as mean ± standard deviation in the text and tables and as percentiles in the figures. Nominal variables are expressed as percentages. P ≤ 0.05 was considered significant.

3. Results

Baseline and operative patient characteristics are reported in Table 1. CPB was used in 31/48 patients (64.6%). The mean number of coronary anastomosis was 3.0 ± 0.6 in patients operated with CPB, and 1.3 ± 0.4 in patients undergoing off-pump CABG (P < 0.0001). All patients received a complete myocardial revascularisation. There were no in-hospital deaths. Thirty-one complications were observed in 22 patients (Table 2). In two patients, serum creatinine rose slightly during the early postoperative period (maximum value 1.4 on postoperative day 3 and 1.6 on postoperative day 2, respectively). No significant changes in serum creatinine concentration was observed throughout the study in all other patients.

Table 2
Postoperative complications

|blesing, n (%) | 3 (6.25) |
|Surgical re-exploration for bleeding, n (%) | 2 (4.17) |
|Acute respiratory failure, n (%) | 5 (10.4) |
|Atrial fibrillation, n (%) | 17 (35.4) |
|Ventricular fibrillation, n (%) | 1 (2.08) |
|Temporary creatinine increase, n (%) | 2 (4.17) |
|Pneumothorax, n (%) | 1 (2.08) |
|Temporary pace-maker, n (%) | 2 (4.17) |

As expected, the baseline Hcy concentration of the studied population (mean, 17.3 ± 9.3 μmol/l, minimum value 6.9 μmol/l, maximum value 55.2 μmol/l) ranged from the low- to high-risk factor levels for coronary artery disease [9].

A significant decrement in both Hcy and folate was observed during and several hours after CABG (P < 0.0001) (Fig. 2, Table 3). The serum Hcy concentration reached the minimum level 12 h postoperatively (8.5 ± 5.6 μmol/l), and then returned near to the baseline value (15.9 ± 7.9 μmol/l) at 48 h. On postoperative day 5, the Hcy concentration (17.0 ± 8.3 μmol/l) was similar to the baseline value, as it was at 6 months follow-up (22 patients) (17.0 ± 7.3 μmol/l).

The mean folate concentration reached the minimum value 48 h postoperatively (2.5 ± 1.5 ng/ml), and was still significantly reduced on postoperative day 5 (3.2 ± 1.9 ng/ml, P = 0.0001) (Fig. 2, Table 3). At 6 months follow-up, the serum folate concentration was similar to the baseline value (6.3 ± 1.3 ng/ml; P = 0.06 vs. admission) (22 patients).

Total plasma protein concentration decreased from 7.3 ± 0.7 to 4.8 ± 0.6 g/dl at the end of the surgical procedure, and returned to normal values only at follow-up (7.4 ± 0.6 g/dl) (22 patients). These data suggest that haemodilution occurred throughout the postoperative period.

The variations of the Hcy concentration (dependent variable) throughout the study were independently related to both folate and serum protein concentrations (as independent variables), as assessed by stepwise multiple regression analysis (total DF = 197, F-value = 14.52, P < 0.0001). The following multiple regression equation was found:

\[
\text{Hcy} = 2811 - 0.984 \text{Folate} + 2.751 \text{Proteins}
\]

The effect of CPB on variations of Hcy levels up to postoperative day 5 was also analyzed as an “effect factor” in repeated measures ANOVA analysis by comparing the time-course of Hcy levels of patients undergoing CPB surgery (n = 31) to those undergoing off-pump CABG (n = 17). Indeed, this analysis showed that the use of CPB significantly interacts with time of sampling in affecting Hcy levels (DF = 5, F-value = 5486, P = 0.0002) (Fig. 3).
4. Discussion

Oxidative stress is known to occur in association with ischemia-reperfusion in CABG patients, and this may lead to an increased consumption of GSH and of Hcy during and after surgery. Oxidative stress increases the activity of γ-glutamylcysteine synthetase, which catalyzes the transformation of L-cysteine to L-glutamate (i.e. the first limiting step of GSH biosynthesis) [2], leading to increased GSH synthesis in cells. One of the major determinants of the rate of GSH synthesis is the availability of cysteine [2], which derives from Hcy through the trans-sulfuration pathway (Fig. 1). Ischemia-reperfusion related oxidative stress might, therefore be expected to induce a decrement in plasmatic levels of cysteine and Hcy, its direct precursor. Our study indicates that CABG surgery is associated with a significant perioperative reduction of the blood levels of Hcy and folate, and that this reduction is at least in part independent of haemodilution.

Previous studies on the time-course of Hcy and folate levels after cardiac surgery mostly focused on the follow-up period (weeks or months after surgery) [4,5]. Interestingly, Jeremy and co-workers reported increased levels of Hcy 6 days to 6 weeks after CABG, and concluded that since Hcy is a known risk factor for atherosclerosis, these changes may be of importance to the pathophysiology of vein graft disease [4]. Lazzerini and co-workers found a similar increase of the Hcy levels 3–6 months after heart transplantation, and speculated that surgery-related

![Fig. 2. Box plot of Homocysteine, folate and serum protein concentrations in 48 patients who have undergone CABG: A, admission in the hospital; T0, soon after the end of surgery; T12, T48 and T120: 12, 48 and 120 h postoperatively, respectively; FU, follow-up visit 6 months postoperatively. The results are expressed as boxes with five horizontal lines, displaying the 10th, 25th, 50th (median), 75th, and 90th percentiles of the variable. All values above the 90th percentile and below the 10th percentile (outliers) are plotted separately (as circles). *P < 0.001 vs. admission; **P < 0.001 vs. previous time point.](https://academic.oup.com/ejcts/article-abstract/26/4/682/455260/26/4/682/455260)

![Fig. 3. Mean ± SE Hcy concentration in conventional CABG (CPB) and off-pump CABG (OPCAB) patients. The initial reduction, as well as the subsequent increase of the Hcy concentration are more marked in the CPB group, and this difference was statistically significant (P = 0.0002). Time abbreviations are the same as in Fig. 2.](https://academic.oup.com/ejcts/article-abstract/26/4/682/455260/26/4/682/455260)

<table>
<thead>
<tr>
<th></th>
<th>Hcy (μmol/l)</th>
<th>Folate (ng/ml)</th>
<th>Proteins (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17.3 ± 9.3</td>
<td>5.1 ± 2.8</td>
<td>7.3 ± 0.7</td>
</tr>
<tr>
<td>T0</td>
<td>11.6 ± 7.3*</td>
<td>4.9 ± 2.6</td>
<td>4.8 ± 0.6*</td>
</tr>
<tr>
<td>T12</td>
<td>8.5 ± 5.6*</td>
<td>3.8 ± 2.2*</td>
<td>5.1 ± 0.4*</td>
</tr>
<tr>
<td>T48</td>
<td>15.9 ± 7.9**</td>
<td>2.5 ± 1.3***</td>
<td>5.3 ± 0.5*</td>
</tr>
<tr>
<td>T120</td>
<td>17.0 ± 8.3</td>
<td>3.2 ± 1.9*</td>
<td>5.7 ± 0.4*</td>
</tr>
<tr>
<td>FU</td>
<td>17.0 ± 7.3</td>
<td>6.3 ± 1.3**</td>
<td>7.4 ± 0.6***</td>
</tr>
</tbody>
</table>

Hcy, homocysteine; A, admission time; T0, T12, T48 and T120, 0, 12, 48 and 120 h postoperatively; FU, follow-up visit 6 months postoperatively; *P < 0.001 vs. admission; **P < 0.001 vs. previous time point.
hyperhomocysteinemia could enhance the development of cardiac allograft vasculopathy [5]. On the other hand, a reduction in the Hcy levels has been reported in the early postoperative period after general surgical procedures by Foschi and associates, that explained this finding as a response to surgical stress [6]. Indeed, serum Hcy is considered a negative acute phase reactant, and its levels are decreased during the acute phase of vascular accidents, such as stroke or myocardial infarction [6].

In our series, a significant postoperative reduction of the Hcy levels was observed. Moreover, we found that Hcy levels were not different from base line at 6 months follow-up. These apparent discrepancies are probably due to differences in the time of sampling and protocol between our and others’ studies. In the Jeremy series [4], blood samples were taken at 1 and 6 days and 6 weeks postoperatively. In this study, a decrement in Hcy levels was observed on postoperative day 1, and this finding is in accordance with our results. The Authors ascribed the reduction in Hcy levels to the direct impact of haemodilution [4]. However, we found that the initial decrement in both Hcy and folate remained significant after correction for haemodilution, as assessed by changes in plasma protein concentration. It is, therefore, possible that serum Hcy levels decrease immediately after surgery, as a result of the combined effect of haemodilution and increased consumption in response to ischemia-reperfusion and oxidative stress; increase to supra-normal levels one to 6 weeks after surgery as a consequence of inflammation and reduced folate availability, as observed by Jeremy and co-workers [4] and confirmed by our data (Fig. 2, Table 3); and then return to near baseline levels 6 months postoperatively.

It is known that ischemia-reperfusion injury generates ROS during the early reperfusion [10], and several studies have shown the beneficial effects of endogenous and exogenous antioxidants (superoxide dismutase, catalase, vitamin E, GSH peroxidase) in protecting the myocardium [1]. Furthermore, a marked depletion of endogenous antioxidants has been observed in the ischemic heart upon reperfusion [11]. Our data confirm the observation that the reduced form of GSH acts as a first line of defense against oxidative stress: this finding is in accordance with previous studies demonstrating a protective effect on the myocardium of thiol-containing antioxidants, in particular N-acetylcysteine [12,13], and should renew the interest for this field of research.

A side finding of our study was the observation that CPB alters the immediate postoperative time-course of the Hcy concentration. In particular, we found that the use of CPB is associated with a faster decrease of the Hcy concentration during the first 12 h, and with a faster and higher increase of the Hcy concentration 12–48 h postoperatively (Fig. 3). However, this finding probably suffers by at least two important sources of bias: the early decrease is probably in relation to haemodilution, since CPB patients were likely to receive more fluids during and soon after the operation. Moreover, CPB patients in our series had a significantly more severe disease, and were therefore more exposed to ischemia-reperfusion.

In conclusion, our data indicate that Hcy and folate concentrations are markedly reduced after CABG surgery. In our opinion, these findings have two relevant clinical implications: first, they should encourage the development of strategies involving the use of antioxidants for myocardial protection; and second, they should lead to reconsider the possible role of reduced folate concentrations in the genesis of postoperative hyperhomocysteinemia, and the opportunity to employ perioperative folate supplementation in selected patients undergoing CABG surgery.

References


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