Changes of plasma asymmetric dimethylarginine levels after coronary artery bypass grafting

INGA KARU1,2,3, KERSTI ZILMER1, JOEL STARKOPF2 & MIHKEL ZILMER1

1Institute of Biochemistry, University of Tartu, Estonia, 2Clinic of Anaesthesiology and Intensive Care, University of Tartu, Estonia, and 3Clinic of Anaesthesiology, North Estonia Regional Hospital, Estonia

Abstract

Objectives. We investigated whether coronary artery bypass grafting affects plasma asymmetric dimethylarginine (ADMA) concentrations and whether precardiopulmonary bypass (CPB) cardioplegia influences ADMA release from the heart. Patients. Twenty-two patients were randomly assigned to control (n = 11) and hyperoxia (n = 11) groups. Arterial and coronary sinus blood was sampled before cardiopulmonary bypass and during early reperfusion. The arterial samples were drawn 60 min after declamping of the aorta, and an additional sample was collected on the 1st postoperative day. Results. Baseline arterial values of ADMA were not different between groups (0.59 ± 0.18 μmol/L in control, 0.63 ± 0.13 μmol/L in hyperoxia group). Negligible release of ADMA into coronary sinus was detected 20 min after cardioplegia. A significant decrease of arterial ADMA was observed by the 1st postoperative morning (0.42 ± 0.16 μmol/L in control, and 0.38 ± 0.01 μmol/L in hyperoxia group, p < 0.01 compared to baseline). Conclusions. Cardiac function and endothelial dysfunction are important determinants of ADMA release. A decrease of ADMA with hyperoxia was observed.

Keywords: Asymmetric dimethylarginine, ADMA, hyperoxia, precardiopulmonary bypass, coronary artery bypass grafting

Endothelium plays a crucial role in maintenance of vascular tone and structure. One of the major vasoactive mediators, derived from endothelium is nitric oxide (NO), formed in the endothelial cells by isoforms of nitric oxide synthase (NOS). In the heart, most of NO is produced by endothelial NOS, present in the endothelium of coronary vessels and myocardium (1,2). Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NOS and thereby modulates NO production (3). Elevated levels of circulating ADMA has been suggested as a marker of endothelial dysfunction (4), leading to increased resting vascular tone and decreased myocardiial vascular tone and decreased myocardiial vasodilatory capacity.

Cardiac surgery with cardiopulmonary bypass (CPB) is associated with myocardial ischemia during cardioplegia followed by reperfusion upon declamping of the aorta. As a result, ischemia-reperfusion (IR) injury, including damage of both myocardial and endothelial cells, develops. Whether increased ADMA levels interfere with postischemic endothelial dysfunction is not clear. Furthermore, to the best of our knowledge, it is not known whether coronary artery bypass grafting (CABG) with CPB has an influence upon myocardial release or circulating levels of ADMA.

In our previous experimental studies we have shown that preischemic exposure of experimental animals to low-grade oxidative stress, induced by hyperoxia, can reduce both myocardial and endothelial injury (5,6). In the model of IR injury a decrease in endothelial NOS expression following chronic hyperoxia has been reported (7). Whether the exposure to hyperoxia influences levels of circulating ADMA is not known.

In present preliminary report we describe the changes in arterial and coronary sinus concentrations of ADMA during CABG procedure. Additionally we investigated the effect of hyperoxic pretreatment on plasma levels of ADMA.

Material and methods

Patients

The study design was approved by the Ethics Review Committee on Human Research of the University of Tartu and written informed consent was obtained.
from all patients. Twenty-two adult patients scheduled for isolated primary elective aortic-coronary bypass grafting with at least three distal anastomoses were recruited and randomized into the control (n = 11) and hyperoxia (n = 11) groups. The exclusion criteria included preoperative ejection fraction of the heart below 40%; unstable angina or elevated markers for myocardial necrosis; treated diabetes mellitus; hepatic, renal or pulmonary disease. All medications, except salicylates, were allowed up to the day before surgery.

Study protocol
After induction of anesthesia and intubation of the trachea the patients were ventilated until the beginning of CPB with either 40% or >96% oxygen. After CPB mixture of oxygen and air was adjusted to obtain normal pO2 levels. Arterial blood gases were analyzed (Radiometer ABL 700 series, Ratiometer Medical A/S, Denmark) 15 min after randomization and verified again before CPB.

Anesthesia and operative procedure
Intravenous anesthesia with midazolam, fentanyl and pancuronium was used in all cases, no volatile anesthetics were added. CPB was performed with roller pump (Stöckert Instrumente GmbH, Munich, Germany) and membrane oxygenator (Diodeo, Mirandola, Italy) under mild hypothermia (naso-pharyngeal temperature 33–35°C). Manually inflatable 15 Fr coronary sinus cannula (Medtronic Inc., Minneapolis, USA) was inserted transfarially. For achieving cardioplegia cold crystalloid solution (St.Thomas' II) was infused antegrade into the aortic root and retrogradely into the coronary sinus. Infusion technique of cardioplegia was standardized in all cases. Coronary sinus pressure was carefully monitored and kept between 20 and 40 mmHg not to cause coronary venous injury. Infusion was repeated after completion of each anastomosis or at least every 20 min. Both distal and proximal anastomoses were performed under a single cross-clamping period. All operations were performed by the same surgical team.

ADMA measurement
Blood samples were collected from coronary sinus and radial artery cannulae before CPB and at 5 and 20 min after declamping. The balloon of the coronary sinus cannula was manually inflated at these time points to get blood exclusively from the coronary sinus. Additional arterial samples were drawn 60 min after declamping of the aorta, and on the morning of the first postoperative day. Blood was centrifuged immediately after collecting and serum stored at -80°C until analyses.

ADMA was determined using the competitive ADMA-ELISA (DLD Gesellschaft für Diagnostika und Medizinische Geräte mbH, Hamburg, Germany). The kit uses the microtiter plate format, where ADMA is bound to the solid phase of the plate. ADMA in the samples is acetylated and competes with the solid phase bound ADMA for a fixed number of rabbit anti-ADMA anti-serum binding sites. After the system has reached equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase is then detected by anti-rabbit-IgG-peroxidase. The substrate tetramethylbenzidine-peroxidase reaction is monitored at 450 nm.

Myocardial release was calculated as the difference between arterial and coronary sinus concentrations, thus negative values indicate a release from the heart during reperfusion.

Statistical analysis
Student’s t-test was used to distinguish differences in demographic data between groups. Friedman’s ANOVA followed by Dunnet’s test was applied to locate significant differences over time. Differences between groups were assessed by the analysis of variance for repeated measures. Correlation is expressed as Pearson correlation coefficient. Data are represented as mean ± SD, with a value of p < 0.05 considered as significant.

Results
Demographic data
Patients’ demographic data are presented in Table I. There were no inter-group differences regarding age of the patients, and ischemic time, but there were more male patients in the hyperoxia group. Three to five coronary arteries were bypassed in both groups. No inotropic support was needed during the perioperative period.

Arterial levels of ADMA
The baseline arterial levels of ADMA, measured before CPB, were similar in both groups (Figure 1). The minimum and maximum values were 0.34 and 0.80 μmol/L among controls, and 0.36 and 0.76 μmol/L in hyperoxia pretreated patients. During the first hour of reperfusion no alterations in arterial concentrations were seen, but a significant decrease (p < 0.01) was observed by the first postoperative morning in both groups (mean 0.42 ± 0.16, mini-
Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=11)</th>
<th>Hyperoxia-pretreated (n=11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.8 ± 9.6</td>
<td>69.7 ± 5.5</td>
<td>0.23</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>6/5</td>
<td>9/2</td>
<td>0.17</td>
</tr>
<tr>
<td>Preoperative ejection fraction (%)</td>
<td>58 ± 11</td>
<td>53 ± 7</td>
<td>0.23</td>
</tr>
<tr>
<td>pO₂ 15 min after induction (mmHg)</td>
<td>132.8 ± 39.0</td>
<td>356.5 ± 65.3</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>pO₂ before cardiopulmonary bypass (mmHg)</td>
<td>124.1 ± 35.0</td>
<td>300.8 ± 118.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Exposure time to oxygen (40% vs &gt;95%, min)</td>
<td>135 ± 20</td>
<td>148 ± 24</td>
<td>0.18</td>
</tr>
<tr>
<td>Aortic cross-clamping time (min)</td>
<td>85 ± 18</td>
<td>89 ± 13</td>
<td>0.56</td>
</tr>
<tr>
<td>Vessels bypassed</td>
<td>3.7 ± 0.8</td>
<td>4.1 ± 0.7</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Discussion

This study provides the first evidence that CAGB surgery is associated with a significant decrease of arterial ADMA concentrations by the first postoperative morning. We demonstrated that a cardiopulmonary arrest did not cause a release of ADMA from the human heart, and ventilation with hyperoxic gas mixture had no influence on either arterial or coronary sinus levels of ADMA.

Free ADMA — an endogenous competitive inhibitor of NOS — is released into the plasma after proteolytic breakdown, and is eliminated via renal excretion (8) and hydrolytic degradation by dimethylarginine dimethylaminohydrolase (DDAH) (9).

Whether ADMA is involved in reducing NO levels and causing endothelial dysfunction of reperfused heart is not fully clear. NO, although decreased during reperfusion (10), has been shown to afford cardioprotection, as described by reduced infarct size and neutrophil accumulation in the reperfused heart (11). In the present study, no significant changes in arterial and coronary sinus

![Figure 1](image1.png)

Figure 1. ADMA concentrations in arterial plasma of control (40%) and hyperoxia (>95%) pretreated patients before cardiopulmonary bypass (CPB) and during reperfusion and early the first postoperative morning (1 POP). Data are given as mean ± SD. *p < 0.01 compared to baseline values.

![Figure 2](image2.png)

Figure 2. Myocardial release (arterio-coronary sinus difference) of ADMA in control (40%) and hyperoxia (>95%) pretreated patients before cardiopulmonary bypass and during immediate reperfusion. Data are given as mean ± SD.
ADMA were detected during the immediate, 20 min reperfusion of cardioplegic heart. This allows us to speculate, that other mechanisms rather than altered metabolism of ADMA are responsible for endothelial dysfunction after cardioplegia. In hyperoxia pretreated patients we observed the correlation between coronary sinus ADMA and aortic cross-clamping time, which could be interpreted as a relation between the severity of endothelial reperfusion injury and duration of ischemic period. Why such correlation occurred only in the hyperoxia treated but not in control patients, remains unclear. It cannot be excluded that additional oxidative stress induced by hyperoxia aggravated endothelial dysfunction. Also, a small number of patients as important limiting factor of the study should be accounted while interpreting the results. Despite of careful selection of patients, possible influence of variations in retrograde infusion of cardioplegic solution and degree of coronary atherosclerosis cannot be ultimately excluded.

Two weeks of medical therapy after acute coronary syndrome has been previously shown to reduce ADMA levels (12). We observed attenuation of the plasma ADMA already at the first post-operative morning after CABG procedure. The measured levels were on the very low end and in some cases even lower than recently proposed reference limits for ADMA in healthy population (13). Decrease of ADMA concentration may reflect adaptive changes, aimed to enhance NO release through better functioning of endothelial NOS. Whether these are only temporary alterations of immediate postoperative period, or reflect true improvement of endothelial functioning after surgery remains to be elucidated. Number of different powerful stimuli interferes in the early postoperative period, and therefore, the changes during this short period of time are difficult to interpret. Obviously, only persisting changes in ADMA could reflect the modification of cardiovascular risk in particular patients.

Ischaemic heart disease is characterized by a mismatch of myocardial oxygen demand and supply. All our patients presented with diffuse coronary artery disease as they had 3–5 vessels to bypass and had concurrent stenoses in smaller arteries. By ventilation with hyperoxic gas mixture oxygen supply to the myocardium can be improved, but also formation of reactive oxygen intermediates may occur. Oxidative stress may decrease the activity of DDAH (14) with concomitant increase in ADMA levels. In our study, exposure to 96% of oxygen in average for 135 min before bypass did not cause increased production of ADMA in the heart (Figure 2). During reperfusion period, there was a tendency, albeit non-significant, for ADMA release form hyperoxia pretreated but not from control hearts (Figure 2).

Conclusion
We have provided the first preliminary evidence regarding arterial and coronary sinus ADMA concentrations after cardioplegia and coronary artery bypass grafting operations.

CABG surgery with cardioplegia resulted in a significant decrease in arterial ADMA concentrations by the first postoperative morning. Pretreatment with hyperoxia had no influence on either myocardial release or arterial levels of ADMA.

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References