Endothelial cell dysfunction after coronary artery bypass grafting with extracorporeal circulation in patients with type 2 diabetes mellitus

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Abstract

Objective: Type 2 diabetes mellitus is a well-known risk factor in patients with severe coronary artery disease undergoing coronary artery bypass grafting (CABG). The aim of the study was to analyze the endothelial dysfunction in these patients by evaluating postoperative soluble inflammatory cytokines. Methods: Patients undergoing CABG without (n = 15, group A) and with (n = 14, group B) diabetes mellitus were analyzed for their release of E-selectin, interleukin-6 (IL-6), and tumor necrosis factor (TNF) up to 3 days postoperatively. A pharmacokinetic quantitative kinetic evaluation (Kinetica 2000) of maximum concentrations (c max), time to reach c max (t max), area under the curve (AUC 0-inf), and terminal elimination half time (t 1/2) was performed using a non-compartmental model. Results: There was no difference in preoperative plasma concentrations of the cytokines and in the postoperative kinetic analyses of TNF when comparing both groups. However, the release of IL-6 was restricted with c max of 1055 ± 543 pg/ml for group B versus 2112 ± 1532 pg/ml for group A (p ≤ 0.05), paralleled by a decrease in the absolute amount (AUC 0-inf) of IL-6. The t 1/2 remained unaffected (13.9 ± 6.6 h and 12.7 ± 4.6 h, respectively). The AUC 0-inf of E-selectin decreased by a factor of 1.7 (p ≤ 0.05) with unchanged c max but reduced t 1/2 (12.9 ± 10 h for group B vs 33.1 ± 20.4 h for group A; p ≤ 0.01) referring to an augmented endothelial uptake and degradation of E-selectin. Conclusions: CABG with extracorporeal circulation could be used to verify a specific endothelial dysfunction in diabetic patients characterized by an impaired release of IL-6 and an increased turnover of E-selectin.

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1. Introduction

Diabetes mellitus is a well-known risk factor in patients with severe coronary artery disease undergoing coronary artery bypass grafting (CABG) [1–3]. The reason for this heightened risk is unclear. It has been assumed that the greater surgical risk associated with diabetes mellitus might be a consequence of an endothelial dysfunction and an altered inflammatory response to CPB, characterized by alteration in the secretion of cytokines (interleukin-6, tumor necrosis factor) or of adhesion molecules [4–9].

The aim of the present study was to analyze endothelial dysfunction and inflammatory response after CABG employing cardiopulmonary bypass (CPB) in patients with coronary artery disease and type 2 diabetes mellitus during extracorporeal circulation (ECC).

2. Patients and methods

2.1. Patients

Two hundred and seventy-four consecutive patients (100%) undergoing elective CABG with CPB were screened. A subgroup of n = 75 (27%) with cold crystalloid cardioplegic solution (Janostil, Fresenius) was selected and, according to the presence of diabetes type 2 (n = 14; 19%), included in the study. A control group (n = 61; 81%) was selected and matched for age (n = 15; 20%). The restriction of patients to one particular cardioplegic solution during ECC introduced an unbalance with respect to gender (Table 1). Informed consent was obtained from all patients. Demographic data and surgical parameters are shown in Table 1. Patients with diabetic type 2 were on oral anti-diabetic therapy for at least ≥1.5 years. In one patient, 7 months before CABG, a change from oral anti-diabetics to insulin administration was necessary. None of the patients suffered from a complicated diabetes (i.e. the presence of polyneuropathy, renal insufficiency, reduced vision, and/or leg and foot ulcers). Patients with type 1 diabetes, as well as those with diffuse arteriosclerosis, preoperative signs of infection (white cell
All other data are mean ± standard deviation. HbA1c, glycosylated hemoglobin; FPG, fasting plasma glucose; po, postoperatively; preop, preoperatively; CK, creatine kinase; CK-MB, MB isoenzyme of CK; CRP, C-reactive protein; AUC0—3 days, area under the curve (time course from day 0 to 3 po); Cmax, peak plasma concentrations; tmax, time to reach Cmax.

Anesthesia was comparable for all patients and consisted of intravenous etomidate and fentanyl application. Pancuronium was used for neuromuscular blockage. None of the patients received aprotinin. Heparin (150 IU kg−1) was administered before cannulation of the aorta and right atrium. A Stöckert® heart-lung machine with a Maxima Plus CB3380® hollow fiber oxygenator (Medtronic, Anaheim, CA, USA) was used for non-pulsatile extracorporeal circulation. The latter was primed with 1.3 l electrolyte solution (Janosteril, Fresenius) and 250 ml mannit solution (20%). Cold crystalloid cardioplegia was applied for cardiac preservation. Residual blood remaining in the circuit after CPB was salvaged with a cell saver and retransfused. Transfusion of red blood cells was necessary in six patients in group A (450 ± 250 ml) and in three patients in group B (567 ± 57 ml).

2.2. Serial analyses

Serial blood samples were withdrawn from the radial artery catheter or peripheral line before induction of anesthesia, at the end of ECC, on administration to the intensive care unit, as well as 6, 12, 18, 24, 48, and 72 h after termination of CPB. Blood samples were collected into sterile vacuum tubes with ethylenediaminetetraacetic acid and in serum-monovettes. Samples of plasma and serum were frozen for analysis of TNF, IL-6, and E-selectin using enzyme-linked immunosorbent assay (Beckman Coulter, Hamburg; R&D, Wiesbaden). Plasma albumin was measured with a calorimetric assay (bromcresol green method, MP3R, Boehringer Mannheim, Mannheim), while hemoglobin content was analyzed with an automated blood gas analysis system (ABL510, Radiometer, Copenhagen). For all parameters, a volume correction was made to eliminate hemodilution effects according to Diago et al. [10]. The correction factor was calculated as the ratio of c/c(t1), with c being the concentration of hemoglobin (or albumin) preoperatively and c(t1) being the concentration at the time of sampling. In 13 healthy volunteers (age 24—35 years) TNF, IL-6, and E-selectin were measured as a reference.

After weaning from ECC, the hemoglobin level dropped to about 40% and remained low throughout the 3-day observation period (p ≤ 0.001) in both patient groups (Fig. 1).
Similar observations were made for plasma albumin concentrations. During CPB, plasma albumin decreased significantly (20.4 ± 3.6 mg/ml vs 43.3 ± 3.5 mg/ml; p < 0.001), and only began to normalize about 3 days after surgery (35.9 ± 1.4 mg/ml).

2.3. Kinetic analysis

For quantitative kinetic analysis of the release of IL-6, TNF, and soluble E-selectin (sE-selectin) maximum concentrations (c_{max}), time to reach c_{max} (t_{max}), area under the curve (AUC), and terminal elimination half time (t_{1/2}) were assessed using a non-compartmental model (trapezoidal rule) (Kinetica 2000, version 3.0) [11]. The parameter levels obtained before CPB were set at zero to be able to correlate the absolute amount of IL-6, TNF, and soluble E-selectin with the AUC. Fasting plasma glucose concentrations (FPG) were obtained before CPB were set at zero to be able to correlate the absolute amount of IL-6, TNF, and soluble E-selectin with the AUC. Fasting plasma glucose concentrations (FPG) were assessed to determine the extent of the diabetic disease [12] (Table 2). Blood samples were drawn immediately prior to the beginning of CPB. Therefore, in one patient of the control group an intramuscular (i.m.) application of insulin was administered (Table 3). Oral anti-diabetic therapy of the patients was restarted 2–3 days post-op after leaving intensive care unit (Table 3).

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients</th>
<th>Days on therapy</th>
<th>Dose per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without anti-diabetic</td>
<td>Control</td>
<td>14/15</td>
<td></td>
</tr>
<tr>
<td>therapy</td>
<td>Diabetic</td>
<td>6/14</td>
<td></td>
</tr>
<tr>
<td>Insulin therapy(^{a,b})</td>
<td>Control</td>
<td>1/15</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>6/14</td>
<td>1.7 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>90 ± 55 IU</td>
</tr>
<tr>
<td>Gilbenclamide(^b)</td>
<td>Diabetic</td>
<td>2/14</td>
<td>2.1 ± 2.5</td>
</tr>
<tr>
<td>Metformin(^b)</td>
<td>Diabetic</td>
<td>3/14</td>
<td>3.3 ± 3.1</td>
</tr>
<tr>
<td>Acarbose(^b)</td>
<td>Diabetic</td>
<td>2/14</td>
<td>1.1 ± 3.5</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD.

\(^{a}\) Continuous infusion, intravenous bolus, and intramuscular (i.m.) application of insulin were triggered by a plasma glucose level of ≥200 mg/dl (11.1 mmol/l).

\(^{b}\) Data included single therapy and a combination therapy of different oral anti-diabetics or of insulin and oral anti-diabetics.

3. Results

3.1. Clinical findings

Demographic data and operative details were similar between the groups as shown in Table 1. There was no significant difference in blood loss, blood product transfusion requirement, postoperative myocardial infarction or stroke, as well as in the new onset of atrial fibrillation. No re-operation for bleeding or early bypass occlusion and no prolonged postoperative intensive care were necessary. The diabetic patients of group A demonstrated a significantly increased fasting plasma glucose level of 7.0 mmol/l as compared to 4.4 mmol/l for the non-diabetic control patients. Mean HbA1c level in the diabetic patients was 8% (Table 2).

Initiation of postoperative anti-diabetic therapy with insulin was triggered by a plasma glucose level of ≥200 mg/dl (11.1 mmol/l). The actual plasma glucose level determined after insulin administration (2.0 ± 1.8 days post-op) amounted to 250 ± 21 mg/dl (13.9 ± 1.2 mmol/l). In all control patients plasma glucose levels increased with the beginning of CPB. Therefore, in one patient of the control group an intramuscular (i.m.) application of insulin was administered (Table 3). Oral anti-diabetic therapy of the patients was restarted 2–3 days post-op after leaving intensive care unit (Table 3).

3.2. Soluble mediators before CPB

There was no significant difference in the preoperative serum concentration of TNF, soluble E-selectin, and IL-6 between the two patient groups (Fig. 2). Healthy volunteers showed comparable concentrations for TNF and IL-6, whereas the concentrations for E-selectin of volunteers were significantly decreased (Fig. 2).

3.3. Soluble mediators peri- and postoperatively

Fig. 2. TNF, IL-6, and soluble E-selectin blood levels of healthy volunteers (gray bars), non-diabetic (black bars), and diabetic (open bars) patients before CPB. Results are expressed as mean with standard deviation.
and 8.8 ± 2.9 h (groups A and B; p = 0.982) following termination of CPB. The underlying pharmacological non-compartmental model showed no difference in the elimination half time (t1/2), but a 30% decrease of both the absolute amount (AUC) and the maximal release of IL-6 in the diabetic patient group (Figs. 3 and 4). The values remained increased up to 3 days after termination of CPB (p ≤ 0.001). Serum concentrations of soluble E-selectin peaked 10.9 ± 4.7 and 11.3 ± 3.9 h (group A and group B; p = 0.645) (Fig. 3). The absolute amount (AUC0–inf) of soluble E-selectin in the 3-day follow-up was significantly reduced in the diabetic group (p = 0.02) accompanied by a reduction of the terminal elimination half time of about 60% (p = 0.001) (Fig. 5).

4. Discussion

The inflammatory process, i.e. the release of cytokines and soluble adhesion molecules during and after CPB, is well documented in clinical studies [13,14]. However, diabetic patients were mostly excluded in these investigations for an assumed endothelial dysfunction. Here, we analyzed for the first time the altered endothelial inflammatory response in diabetic patients following CPB. Our data demonstrated not only an increased production of cytokines (IL-6, TNF) and of soluble adhesion molecules (E-selectin) during and after CPB but also a qualitatively different inflammatory reaction in patients with type 2 diabetes mellitus.

CPB induced an increase in plasma TNF in patients with and without diabetes that lasted for the duration of the study. Peak levels for TNF were noted at 2 h after surgery, i.e. prior to the maximum concentrations of IL-6 and E-selectin. These results are in line with other studies [15]. The effect of TNF to implicate in the pathogenesis of myocardial ischemia and reperfusion injury [16,17] was independent of the diabetic disease. Due to the wide interindividual variability, postoperative serum TNF levels alone do not allow us to draw exact conclusions with regard to endothelial function.

The IL-6 levels peaked 4 h postoperatively and decreased over the next 48 h in both study groups, which is evidence for a strong pro-inflammatory response. Even 3 days after the
termination of CPB, IL-6 concentrations remained elevated in both groups compared to baseline levels. Steinberg et al. had reported that IL-6 levels increased after protamine administration reached a maximum at 3 h after bypass and remained above the baseline levels [18]. Similar time—response curves have been reported by Franke et al. [19]. The kinetic analysis over a 3-day period in our patient cohort proved the impaired release of IL-6 in diabetic patients, and also demonstrated that the elimination of IL-6 was not affected. Apart from IL-6, the release of IL-8 and TNF is impaired in patients with diabetes undergoing elective CPB as well [20]. The pathophysiolo of this inflammatory response in diabetic patients is still unclear [20]. An increased oxidative stress and complement activation in diabetic patients was discussed by Matata and Galinanes [20].

In both patient groups, elevated overall glucose levels were observed during the early postoperative period. As the increased glucose levels were similar, the incremental plasma glucose excursions (AU(C0—3 days)), which would influence the kinetics of soluble mediators, could be excluded as an objectionable parameter. The presence of insulin and/or oral anti-diabetics had no influence on the kinetic profile of TNF, IL-6, and E-selectin (data not shown). Hemodilution effects issue from the need of priming solution for ECC. So far, only a few studies corrected the concentrations of soluble inflammatory markers with regard to hemodilution [10,15]. Our data demonstrate a significant reduction of parameter levels at the end of CPB after quantification of the dilution factor using hemoglobin or albumin concentration in the respective plasma sample. A future perspective might be the application of minimal extracorporeal circulation for diabetic patients to reduce the maximum stimulating effects after CPB.

In summary, CAD patients with diabetes mellitus demonstrate a specific endothelial dysfunction during CABG with extracorporeal circulation characterized by an impaired release of IL-6 and an increased turnover of E-selectin.

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References


