Organ Perfusion During Cardiopulmonary Bypass: Therapeutic Effect of Dexamethasone.

**Dexamethasone: Benefit and Prejudice for Patients Undergoing On–pump Coronary Artery Bypass Grafting**

*A study on myocardial, pulmonary, renal, intestinal, and hepatic injury.*

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Abstract

Study Objectives

Cardiac surgery with cardiopulmonary bypass (CPB) results in perioperative organ damage caused by the systemic inflammatory response syndrome (SIRS) and ischemia/reperfusion injury. Administration of corticosteroids before CPB has been demonstrated to inhibit the activation of the systemic inflammatory response. However, the clinical benefits of corticosteroid therapy are controversial. This study was designed to document the effects of dexamethasone on cytokine release and perioperative myocardial, pulmonary, renal, intestinal and hepatic damage, as assessed by specific and sensitive (bio)markers.

Design and Patients

A prospective, double-blind, placebo-controlled, randomized trial for dexamethasone was conducted in 20 patients, receiving either dexamethasone (1 mg/kg before anesthesia induction and 0.5 mg/kg after 8 hours) (n=10) or placebo (n=10). Different markers were used to assess the SIRS: Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), C-reactive protein (CRP), tryptase, and organ injury: heart (plasma heart-type fatty acid binding protein H-FABP, Troponin I, Creatine kinase MB, CK-MB), kidneys (N-acetyl-glucosaminidase, NAG, microalbuminuria), intestine (intestine/liver-type FABP, I/L-FABP), liver (α-Glutathione S-transferase, αGST).

Results

Dexamethasone modulated the SIRS with lower pro-inflammatory (IL-6,8) and higher anti-inflammatory (IL-10) interleukin levels. CRP and tryptase were lower in the dexamethasone group. Cardiac Troponin I values were lower in the dexamethasone group at 6 h ICU (p=0.009). Patients in dexamethasone group had longer time to tracheal extubation (18.86±1.13 h versus 15.01±0.99 h, p=0.02) with lower oxygenation index at that time (PaO_2/FiO_2 ratios: 37.17±1.8 kPa versus 29.95±2.1 kPa, p=0.009). Postoperative glucose (10.7±0.6 mmol/L versus 7.4±0.5 mmol/L, p=0.005) was higher in the dexamethasone group. Serum glucose was independently associated with intestinal injury (urine I-FABP peak: R^2=42.5%, B=114.4±31.4, Sig.=0.002, urine L-FABP peak: R^2=47.3%, B=7714.1±1920.9, Sig.=0.001) and renal injury (urine NAG: R^2=32.1%, B=0.21±0.07, Sig.=0.009). Tryptase peaks correlated negatively with peaks of intestinal and renal injury (bio)markers.

Conclusions
Even while inhibiting SIRS, dexamethasone treatment offered no protection against transient, subclinical, perioperative abdominal organ damage. Tryptase release could have a preconditioning effect, offering protection against perioperative intestinal and renal damage. Dexamethasone treatment resulted in more pronounced postoperative pulmonary dysfunction, prolonged time to tracheal extubation and initiated postoperative hyperglycaemia in patients undergoing elective on–pump CABG.
2.1 Introduction

Organ damage after cardiac surgery with cardiopulmonary bypass (CPB) is caused by two related pathophysiological mechanisms: the systemic inflammatory response syndrome (SIRS) and ischemia/reperfusion injury. SIRS is triggered by the exposure of blood to large areas of synthetic materials of the extracorporeal circuit. It causes a complex inflammatory reaction involving activation of complement, platelets, neutrophils, monocytes and macrophages with increased blood concentrations of cytokines and leukotriens. Additionally, SIRS initiates activation of the coagulation, fibrinolytic and kallikrein cascades. A subsequent increase in endothelial cell permeability allows transvascular migration of activated leukocytes into the tissues with additional vascular and parenchymal damage. The ischemia/reperfusion injury is triggered mainly in heart and lungs secondary to aortic cross-clamping and cardioplegic arrest. During aorta cross clamping the heart is excluded from the circulation, being protected by cardioplegia and hypothermia. The lungs are deprived as well of pulmonary blood flow. Ischemia/reperfusion injury has been documented also in other organs such as kidneys and intestine, probably due to alterations in blood flow at the microcirculatory level. Preoperative administration of corticosteroids to patients undergoing cardiac surgery with CPB has been demonstrated to inhibit the activation of the plasmatic and cellular inflammatory response, to decrease the pro- to anti-inflammatory interleukins ratio, and to minimize tissue edema. Based on these findings corticosteroids are routinely used in a considerable number of institutions. The studies on the clinical benefits, however, show conflicting results when referring to changes in hemodynamic, pulmonary function and glucose metabolism. Recent clinical investigations by Chaney et al. indicated that methylprednisolone offers no clinical benefit, and may in fact be detrimental by initiating postoperative hyperglycemia and delaying postoperative tracheal extubation for undetermined reasons.

As an original contribution to the issue of CPB related SIRS and organ injury, we document the effect of dexamethasone on perioperative myocardial, pulmonary, renal, intestinal and hepatic damage, as assessed by newly available specific and sensitive (bio)markers. Furthermore, to describe the effects of corticosteroids on the systemic inflammatory response, we measured cytokine response and systemic tryptase release as a marker of mast cells activation. Finally, a new hypothesis relating tryptase to the attenuation of perioperative organ injury will be discussed.

2.2 Patients, Materials and Methods

Patients

The study was designed as a prospective, double blind, placebo-controlled, randomized trial for dexamethasone. After approval by the hospital ethics committee and
written informed consent, patients scheduled for first time coronary artery revascularization were studied. All patients included in the study had coronary artery disease with normal renal function (as assessed by a serum creatinine of less than 120 \( \mu \text{mol.liter}^{-1} \)), normal hepatic, cerebral and cardiac function (ejection fraction > 45%). Patients with diabetes, recent myocardial infarction, unstable angina, or recent use of radiocontrast agents and corticosteroids were excluded, as these conditions might be associated with increased baseline levels of the markers used in this study.

**Anesthetic management**

Patients (n=20) were randomized in a double–blinded fashion to receive either dexamethasone or placebo. A baseline serum glucose sample was obtained after overnight fasting. In the treatment group patients received dexamethasone 1 mg.kg\(^{-1}\) at induction of anesthesia and 0.5 mg.kg\(^{-1}\) 8 hours later. Patients in the control group received a placebo at the same time points. Anesthesia was provided according to a fixed protocol\(^{15}\). Premedication consisted of oral diazepam 10–15 mg 2 hours preoperatively. After insertion of peripheral venous and radial cannulae under local analgesia, general anesthesia was induced with sufentanil (2.5 \( \mu \text{g.kg}^{-1} \)) and midazolam (0.1 mg.kg\(^{-1}\)). Tracheal intubation was achieved with pancuronium (0.1 mg.kg\(^{-1}\)) and the lungs were ventilated with air and oxygen (FiO\(_2\)=0.4). A flow–directed pulmonary artery catheter was inserted into the right internal jugular vein, and an indwelling bladder catheter was used for urine collection. Anesthesia was maintained with sufentanil, midazolam, and pancuronium. Cefuroxim (1500 mg) was administered after induction. Hydroxyethyl starch 200/0.5 6% solution and lactated Ringers solution were used to obtain a mean arterial pressure (MAP) > 60 mmHg, to maintain filling pressures and cardiac output. Transfusion of packed cells were given at a hemoglobin < 4.5 mmol.L\(^{-1}\). According to standard care in our clinic, intravenous insulin was started at a serum glucose > 10 mmol.L\(^{-1}\). Inotropic support with dopamine was started at a cardiac index < 2.2 L.min.m\(^{-2}\). Diuretics, mannitol or aprotinin were not administered during the entire study period. Patient characteristics and perioperative variables were recorded prospectively.

**Cardiopulmonary Bypass**

Non–pulsatile CPB was performed using a roller pump (CAPS HLM, Stökert Instruments, Germany) and a membrane oxygenator (Cobe Optima; Cobe Laboratories; Lakewood, CO). The extracorporeal circuit was primed with 500 ml HES 200/0.5 6% and 1000 ml lactated Ringers solution. During CPB, the flow was maintained at 2.4 L.min.m\(^{-2}\) with moderate hypothermia (32° C) and \( \alpha \)-stat regulation of blood pH. Cold St. Thomas solution was infused into the aortic root to maintain cardioplegia during aortic cross–clamping. During CPB, the mean arterial pressure was allowed to vary between 60 and 90 mmHg. Deviations were corrected with phenylephrine or
Therapeutic Effect of Dexamethasone

nitroglycerine. The urine collection was divided in six intervals: (1) preoperative (baseline): during 12 hours prior to surgery, (2) preheparinization: from skin incision to systemic heparinization, (3) sternum closure: from heparinization to sternum closure, (4) 2 h ICU: during 2 hours postoperative, (5) 6 h ICU: 2 to 6 hours postoperative, (6) 24 h ICU: 6 h to 24 h postoperative. Urinary excretion of the measured biomarkers was calculated as ratio to urine creatinine concentration and adjusted to time interval in order to correct for changes in urinary flow:

\[
\text{Urinary production} = \frac{\text{measured urine concentration}}{(\text{time interval for urine collection} \times \text{urinary creatinine concentration})}
\]

Blood sampling was performed before induction of anesthesia (preinduction), 5 minutes after Aortic cross clamp release (Ao clamp release), 6 h postoperative (6 h ICU), and 24 h postoperative (24 h ICU). Urine and plasma were stored at −20° C until assay.

**(Bio)markers**

*Inflammatory biomarkers*

- Interleukin–6 (IL–6), Interleukin–8 (IL–8), Interleukin–10 (IL–10) – solid–phase, enzyme–labelled, chemiluminescent sequential immunometric assay (IMMULITE, EURO/DPC Ltd, USA)
- *C Reactive protein* – high sensitive ELISA (HaemoScan, Groningen, The Netherlands).
- *Serum glucose* concentration was determined using a Vitros analyzer (Ortho Clinical Diagnostics; Beerse, Belgium).

*Myocardial injury biomarkers*

- *cardiac Troponin I* (cTnI–myofibrillar protein released from injured myocytes) – microparticle enzyme immunoassay (AxSYM, ABBOT Laboratories, USA).
- *Creatine kinase MB (CK–MB)* activity – Vitros analyzer (Ortho Clinical Diagnostics; Beerse, Belgium).

*Kidney injury biomarker*
• **Urine N-acetyl-glucosaminidase** (NAG–enzyme released from injured proximal renal tubules) – modified enzyme assay according to Lockwood\textsuperscript{16} at pH 4.5 and corrected for non–specific conversion (HaemoScan, Groningen, The Netherlands).

**Intestinal injury biomarkers**

• **Intestinal/Liver-type fatty acid binding proteins** (I/L–FABP–cytosolic proteins in the enterocytes released into the blood stream and excreted by kidney early in the course of intestinal ischemia\textsuperscript{17}) ELISA kit (HyCult Biotechnology BV, Uden, The Netherlands).

**Hepatic injury biomarkers**

• **αGlutathione S-transferase** (αGST – enzyme released from centrolobular and periportal damaged hepatocytes reported as having uniform hepatic distribution, high cytosol concentration, and short half–life\textsuperscript{18})– enzyme immunoassay (Biotrin International Ltd., Dublin, Ireland).

**Statistical Analysis**

The statistical analysis was performed using SPSS (Statistical Package for the Social Sciences). A power analysis, based on previous studies of IL–6 and IL–8 plasma levels in this population suggested that at least 20 patients have to be studied in order to detect a 1 SD difference between the two groups, with a reliability of 5\% and a power of 80\%. Before analysis, the data was tested for distribution according to Kolmogorov–Smirnov goodness of fit test. The variation of the urinary and plasma markers over the study period and the differences between groups were investigated using repeated measures ANOVA. A total area under curve (AUC) was calculated for all plasma biomarkers. Continuous variables were compared by means of parametric (Student T Test) or nonparametric tests (Mann–Whitney). Fishers exact test was used to compare discrete variables. Correlation analysis between variables was tested using Spearman correlation test. Regression analysis was used to detect predictors for organ injury. Statistical significance was accepted at \( p < 0.05 \). Results are presented as mean±SEM (unless stated otherwise).

2.3 Results

All twenty patients included completed the study and survived the hospital stay. The following complications were observed: revision for bleeding (n=2); perioperative myocardial infarction (n=1); atrial fibrillation (n=1); nosocomial pneumonia (n=1). Seven patients in the dexamethasone group and two patients in the placebo group (Fischer’s exact test \( p=0.025 \)) received dopamine less than 5 µg.kg\textsuperscript{-1}.min\textsuperscript{-1} because of low cardiac index. Six patients in the dexamethasone group and one patient in
the placebo group received insulin to regulate serum glucose in the postoperative period (Fischer’s exact test \( p=0.057 \)). Fever (highest measured rectal temperature) was more prominent in the placebo group during the first 24 hours postoperatively. Additional patients’ characteristics and operative data are shown in Table 2.1. The patients in the dexamethasone group were slightly older than the patients in placebo group. However, the age did not prove to be a predictor for any of the biomarkers tested. Marked blood loss occurred in one patient in the dexamethasone group, who received 13 units of blood more than 6h after bypass. As this would affect only the last time point of the study, this patient was included in the analysis.

<table>
<thead>
<tr>
<th></th>
<th>Placebo group Mean±SEM</th>
<th>Dexamethasone group Mean±SEM</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>10</td>
<td>10</td>
<td>/</td>
</tr>
<tr>
<td>Male/Female</td>
<td>9/1</td>
<td>8/2</td>
<td>/</td>
</tr>
<tr>
<td>Age 95%CI (mean) (y)</td>
<td>53.4-65.5 (59.5)</td>
<td>63.4-72.1 (67.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>Body surface area (( \text{m}^2 ))</td>
<td>1.96±0.07</td>
<td>1.92±0.03</td>
<td>0.58</td>
</tr>
<tr>
<td>Perfusion time (min)</td>
<td>95.9±7.6</td>
<td>115.7±8.8</td>
<td>0.10</td>
</tr>
<tr>
<td>Aorta clamping time(min)</td>
<td>61.7±5.7</td>
<td>74.8±6.9</td>
<td>0.16</td>
</tr>
<tr>
<td>MAP Preoperative</td>
<td>90.4±5.6</td>
<td>96.1±4.8</td>
<td>0.52</td>
</tr>
<tr>
<td>MAP ICU admission</td>
<td>73.7±4.8</td>
<td>74.2±4.4</td>
<td>0.79</td>
</tr>
<tr>
<td>MAP 24h ICU (mmHg)</td>
<td>80.9±3.4</td>
<td>93.8±4.5</td>
<td>0.053</td>
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<tr>
<td>SVRI Preoperative</td>
<td>2263±212</td>
<td>2429±251</td>
<td>0.85</td>
</tr>
<tr>
<td>SVRI ICU admission</td>
<td>2134±283</td>
<td>1889±181</td>
<td>0.57</td>
</tr>
<tr>
<td>SVRI 24h ICU (dynes/cm(^5)/m(^2))</td>
<td>1630±151</td>
<td>2030±239</td>
<td>0.35</td>
</tr>
<tr>
<td>CI Preoperative</td>
<td>2.6±0.2</td>
<td>2.6±0.4</td>
<td>0.79</td>
</tr>
<tr>
<td>CI ICU admission</td>
<td>2.8±0.3</td>
<td>2.8±0.3</td>
<td>0.63</td>
</tr>
<tr>
<td>CI 24h ICU (L/min/m(^2))</td>
<td>3.7±0.3</td>
<td>3.6±0.3</td>
<td>0.96</td>
</tr>
<tr>
<td>preoperative ( \text{PaO}_2/\text{FiO}_2 )</td>
<td>52.68±4.91</td>
<td>51.65±4.54</td>
<td>0.63</td>
</tr>
<tr>
<td>extubation ( \text{PaO}_2/\text{FiO}_2 ) (kPa)</td>
<td>37.17±1.8</td>
<td>29.95±2.1</td>
<td>0.009</td>
</tr>
<tr>
<td>Intubation time (h)</td>
<td>15.01±0.99</td>
<td>18.86±1.13</td>
<td>0.02</td>
</tr>
<tr>
<td>Highest measured</td>
<td>37.9±0.8</td>
<td>37.2±0.3</td>
<td>0.02</td>
</tr>
<tr>
<td>rectal temperatures (°C)</td>
<td>2.2±3.6</td>
<td>1.3±1.6</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Table 2.1: Patient characteristics and operative data (mean ± standard error of the mean). \( \text{MAP} = \text{Mean arterial pressure}, \text{SVRI} = \text{systemic vascular resistance index}, \text{CI} = \text{cardiac index}, \text{PaO}_2/\text{FiO}_2 = \text{oxygen index}. \) Statistics: Continuous variables where compared by means of parametric (Student T Test) or nonparametric tests (Mann-Whitney). Fisher’s exact test was used to compare discrete variables.
**Inflammatory biomarkers**

*Plasma levels of pro-inflammatory cytokines IL-6 and IL-8* increased significantly (Wilks Sig. < 0.001) in both groups, with a lower response in the dexamethasone group (lower total AUC in dexamethasone group for both IL-6 and IL-8, p < 0.001). The peak values were measured at 6 h ICU for IL-6, and during the sternum closure for IL-8 (Fig. 2.1a, b). The IL-6 values were significantly lower in the dexamethasone group at 6 h and 24 h ICU (p < 0.001). IL-8 was significantly lower in the dexamethasone group after aortic clamp release (p = 0.023), during sternum closure (p < 0.001), at 6 h ICU (p = 0.003), and total AUC (p < 0.001). IL-6 values at 24 h ICU were higher than baseline values in both groups (p < 0.001). IL-8 values returned to baseline values after 24 hours in both groups.

*Plasma IL-10* increased significantly (Wilks Sig. < 0.001) in both groups. In the dexamethasone group plasma IL-10 had a ≈4 fold higher peak at sternum closure. The differences between groups were statistically significant after aortic clamp release and sternum closure (p < 0.001), 6 h ICU (p = 0.029), and total AUC (p < 0.001) (Fig. 2.1c). The IL-10 values returned to baseline values after 6 h ICU in both groups.

*Plasma levels of CRP* did not increase during the operation. The differences between groups on their overall plasma CRP were statistically significant (p = 0.048). The dexamethasone group had lower total AUC (p = 0.028), and lower CRP levels at 6 h ICU (4.9 ± 1 µg/ml in dexamethasone group versus 39.5 ± 24.9 µg/ml in the placebo group, p = 0.043), at 24 h ICU (842.7 ± 524 µg/ml in dexamethasone group versus 2463.5 ± 968 µg/ml in the placebo group, p = 0.028).

*Tryptase* increased significantly during operation in both groups (Wilks Sig. = 0.018) (Fig. 2.1d). In the dexamethasone group, tryptase concentrations increased only moderately with peak values at sternum closure. In the placebo group, the values rose abruptly reaching peak values immediately after releasing the aortic cross-clamp, and decreased after sternum closure. The tryptase values were significantly lower in the dexamethasone group after aortic clamp release (p = 0.015), during sternum closure (p = 0.009), and total AUC (p = 0.05). Tryptase values returned to baseline values after 6 h ICU in both groups.

**Myocardial injury biomarkers**

The release patterns of the myocardial damage markers had a different time course. Plasma H-FABP (Fig. 2.2a) started to rise directly after aortic cross clamp release, reaching peak values after 1.23 hours (95% CI = 0–2.66 h), which was significantly earlier (p < 0.001) than the peak values of cTnI and CK-MB (cTnI: mean = 14.1 h, 95% CI = 6.36–21.84 h; CK-MB: mean = 16.35, 95% CI = 9.23–23.47 h). The only difference between the treatment groups, was observed at 6 h ICU, with a lower value of cTnI in the dexamethasone group (p = 0.009) (Fig. 2.2b).
Figure 2.1: Pro-inflammatory interleukins (Interleukin–6, Interleukin–8), anti-inflammatory interleukin (Interleukin–10), and mast cell degranulation product (Tryptase). The values are represented as mean (symbols) and standard error of the mean (bars). **, * Differences between groups are significant at the .01, .05 level, respectively.

Renal injury biomarkers

Glomerular and tubular function in this very group of patients was recently described elsewhere\textsuperscript{19}. Briefly, urinary NAG increased significantly in time (Wilks p=0.009),
Chapter 2

Figure 2.2: Myocardial injury biomarkers: Heart–type fatty acid binding protein (H–FABP) and cardiac Troponin I (cTnI). The values are represented as mean (symbols) and standard error of the mean (bars). ** Differences between groups are significant at the .01 level.

reaching peak values at 2 h ICU, with no significant effect for the dexamethasone treatment (Fig. 2.3).

Microalbuminuria increased during CPB, with peak values in the urine collected during CPB for both groups (mean 7.9 mg/mmol creatinine, 95%CI=(4.8–10.9)).

Intestinal injury biomarkers

Urinary I–FABP and L–FABP (Fig. 2.4a,b) increased significantly during CPB (Wilks’ p=0.02 I–FABP, and p=0.013 L–FABP) in both groups, reaching peak values in the urine collected during the first postoperative 2 h and 6 h, respectively. The change in mean urinary L–FABP production was significantly dependent upon dexamethasone treatment (Wilks’ p=0.026), with higher values in the dexamethasone group. Analyzing each individual time point, no statistical significant differences between groups were detected for I–FABP and L–FABP.

Hepatic injury biomarkers

$\alpha$GST increased promptly after initiation of CPB in both groups, with peak values during sternum closure (Wilks’ p<0.001) (Fig. 2.5). There were no differences between the groups (time points and total AUC). ALT remained constant for the duration of the investigation. AST increased moderately in both groups with maximum values
Figure 2.3: Renal injury biomarker: urine $N$–acetyl–glucosaminidase (NAG). The values are represented as mean (symbols) and standard error of the mean (bars)\textsuperscript{19}.

Figure 2.4: Intestinal injury biomarkers: urine intestinal–type (I–FABP) and liver–type (L–FABP) fatty acid binding proteins. The values are represented as mean (symbols) and standard error of the mean (bars).

at 24 h ICU (58.9±10.8 U/L).

Serum glucose (Fig. 2.6). Dexamethasone treatment increased significantly the serum
glucose levels (p=0.009). During sternum closure the values reached the peak values of 10.7±0.6 mmol/L in the dexamethasone group, and 7.4±0.5 mmol/L in the placebo group. The glucose values in the dexamethasone group were significantly higher than in the placebo group during sternum closure (p=0.005), 6 h ICU (p=0.007) and 24 h ICU (p=0.023).

**Figure 2.5:** Hepatic injury biomarker: plasma αGlutathione S–transferase (αGST). The values are represented as mean (symbols) and standard error of the mean (bars). NS=not significant.

**Figure 2.6:** Serum glucose level. The values are represented as mean (symbols) and standard error of the mean (bars). **, * Differences between groups are significant at the .01, .05 level, respectively.

**Predictors of organ injury**

*Peak serum glucose values* (mmol/L) were significant independent predictors for urine I–FABP peak values (R²=42.5%, regression coefficient B=114.4±31.4, Sig.=0.002), urine L–FABP peak values (R²=47.3%, regression coefficient B=7714.1±1920.9, Sig.=0.001), urine H–FABP peak values (R²=48%, regression coefficient B=2829.5±694.5, Sig.=0.001) and urine NAG peak values (R²=32.1%, regression coefficient B=0.21±0.07, Sig.=0.009).

*Perfusion duration* (minutes) was a significant independent predictor for urine I–FABP peak values (R²=22%, regression coefficient B=6.7±3, Sig.=0.03), urine L–FABP peak values (R²=26.6%, regression coefficient B=476±186.4, Sig.=0.02), and urine H–FABP (R²=37.7%, regression coefficient B=206.3±62.5, Sig.=0.004).
**Correlations**

**Inflammatory biomarkers:** The statistical correlations found between the inflammatory markers are shown in Table 2.2. CRP at 6 h ICU correlated positively with peak cTnI concentrations (corr. 0.49, p=0.02). Tryptase peak values correlated negatively with peak plasma I–FABP (corr. –0.445, p=0.04), peak urinary I–FABP (corr. –0.474, p=0.03), peak urinary L–FABP (corr. –0.647, p=0.002), peak urinary H–FABP (corr. –0.60, p=0.005), peak urinary NAG (corr. –0.609, p=0.004), peak microalbuminuria (corr. –0.559, p=0.01).

<table>
<thead>
<tr>
<th>Spearman’s corr.</th>
<th>CRP 6h ICU</th>
<th>Peak Tryptase</th>
<th>Peak IL–6</th>
<th>Peak IL–8</th>
<th>Peak IL–10</th>
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<tbody>
<tr>
<td>Peak IL–6</td>
<td>.568**</td>
<td>.474*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak IL–8</td>
<td>.498*</td>
<td>.520*</td>
<td>.555*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak IL–10</td>
<td>ns</td>
<td>-.411 (p=0.072)</td>
<td>-.412 (p=0.071)</td>
<td>-.630**</td>
<td></td>
</tr>
</tbody>
</table>

*Correlation is significant at the .05 level (2–tailed), ** Correlation is significant at the .01 level (2–tailed), ns=not significant.

**Myocardial biomarkers:** cTnI AUC correlated significantly with plasma H–FABP (AUC corr. 0.469, p=0.03; peak corr. –0.444, p=0.05) and CK–MB (AUC corr. 0.80, p<0.001, peak corr. 0.77, p<0.001).

**Intestinal biomarkers:** Urine I–FABP correlated significantly with urine L–FABP (peak corr. 0.81, p<0.001).

**Renal biomarkers:** the urinary peak of H–FABP correlated strongly and significantly with the urinary peak of NAG (corr. 0.65, p=0.002) and peak microalbuminuria (corr. 0.66, p=0.001). In addition, the peaks of intestinal damage markers correlated significantly with the peak values of renal damage markers (I–FABP~NAG: corr. 0.55, p=0.01; I–FABP~H–FABP corr. 0.77, p<0.001; L–FABP~Microalbuminuria corr. 0.57, p=0.009).
2.4 Discussion

In the present study we have found that administration of dexamethasone inhibited the SIRS in patients undergoing elective on-pump CABG. However, administration of dexamethasone did not offer protection against pulmonary, renal and intestinal perioperative damage. Even more, dexamethasone-induced hyperglycemia was found as a strong independent predictor of intestinal and renal perioperative damage. Postoperative pulmonary function was adversely affected by dexamethasone, with decreased PaO$_2$/FiO$_2$ ratio and prolonged time to tracheal extubation in the dexamethasone group of patients.

Myocardial injury
Dexamethasone seemed to offer, to a small extent, myocardial protection during the first 6 h of reperfusion as shown by lower concentration of cardiac Troponin I, but with no further protection after 24 h of reperfusion. Additionally, the protective effect was not noticeable when estimating the myocardial damage by the plasma concentration of heart-type fatty acid binding protein. The recently introduced marker H–FABP is a cytosolic protein abundant in the myocardium, with a 10 fold lower expression in the skeletal muscles, kidney (distal tubules), lung, brain and endothelial cells$^{20,21}$. H–FABP has been introduced as a plasma marker for an early assessment of myocardial tissue injury with a peak as early as 3 h after acute myocardial infarction and 2 h post–reperfusion after CABG$^{22,23}$. The early plasma peak also present in our study promotes H–FABP as a valuable myocardial injury marker, since peak levels of the Troponine T and I occur only much later, around 18 hours post reperfusion$^{24}$.

Pulmonary Injury
Dexamethasone treatment resulted in more pronounced postoperative pulmonary dysfunction and prolonged time to tracheal extubation. The detrimental consequence of dexamethasone on lung function was clinically relevant in terms of significantly lower PaO$_2$/FiO$_2$ ratio immediately after extubation, and the significantly prolonged time to tracheal extubation in the patients in the dexamethasone group. These adverse effects of dexamethasone treatment on pulmonary function confirm the findings reported recently by Chaney et al.$^{12,13}$, after treatment with methylprednisolon in a similar group of patients.

Renal Injury
Urinary N–acetyl–glucosaminidase (enzyme released from injured proximal renal tubules) and microalbuminuric increased significantly during CPB, with no effect of dexamethasone. Measurements of urinary H–FABP proved to be a better indication of kidney damage than of myocardial damage, because the urinary peak of H–FABP did not correlate with the other cardiac markers, but correlated strongly and significantly with the urinary peak of NAG (proximal tubules injury) and peak
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microalbuminuria (glomerular injury). This measurement might be explained by a urinary release of H–FABP from the damaged distal renal tubules. H–FABP has been associated before with early release following injury of the distal renal tubules\textsuperscript{25,26}

**Intestinal injury**
I/L–FABP are cytosolic proteins readily released into the circulation following enterocytes damage, with a 40 fold higher content of L–FABP, reported as useful urine markers for the detection of intestinal injury\textsuperscript{27–29}. Elevated I–FABP in relation to gastrointestinal complications following cardiopulmonary bypass was described earlier\textsuperscript{29}. The increased values of I/L–FABP during CPB reported in our study confirm the indirect line of evidence suggesting mucosal integrity loss during CPB reported previously as a reduction in intramucosal pH, increase in gut permeability and endogenous endotoxemia\textsuperscript{30–32}. Significantly elevated I–FABP urine levels in critical ill patients correlated with clinical development of the systemic inflammatory response syndrome\textsuperscript{33}. In our study, 20% in the variation of intestinal injury markers and 30% in the variation of renal injury markers were explained by the CPB duration.

**Hepatic injury**
\(\alpha\)Glutathione S–transferase (\(\alpha\)GST) increased promptly after initiation of CPB in both groups, with peak values during sternum closure, without effect for the dexamethasone treatment. Increased levels of \(\alpha\)GST as indication of hepatocytes injury were reported before in patients undergoing CPB\textsuperscript{34}.

**Inflammatory response**
The release of pro–inflammatory interleukins was inhibited by dexamethasone, while the anti–inflammatory interleukin IL–10 was increased. The acute phase protein CRP was found in lower concentrations during the first day postoperative in the plasma of the patients receiving dexamethasone. These data confirmed that the administered dose of dexamethasone (1 mg/kg before induction of anesthesia and 0.5 mg/kg after 8 hours) was therapeutically effective. In the first 24 postoperative hours, rectal temperature was moderately but significantly higher in the placebo group. In a recent study postoperative temperature was controlled by active surface cooling to prevent cerebral damage\textsuperscript{35}. The present study demonstrates that temperature can be controlled as effectively with medication. The modulation of the humoral inflammatory response and lower postoperative rectal temperatures as a result of dexamethasone treatment observed in this study are in agreement with previous studies published on the subject. Gluocorticoid administration prior to CPB was shown to attenuate inflammatory response, as based on biochemical analysis of serum inflammatory mediators, to reduce the incidence of postoperative febrile episodes in pediatric cardiac surgery\textsuperscript{36} and to decrease incidence of postoperative hyperthermia in adult surgery\textsuperscript{11}. Only limited amount of data characterizing mast cell activation with subsequent
tryptase release during CPB is available in the literature\textsuperscript{37,38}. The present study reports an important mast cell degranulation (activation), with a peak in the systemic release of tryptase as early as the release of the aorta cross clamp. Dexamethasone was effective in inhibiting tryptase release. Tryptase is a serine proteinase with trypsin–like properties, being released in peripheral blood subsequent to mast cell activation in lungs, heart, stomach, spleen, skin, colon and kidneys\textsuperscript{39,40}. Extracellular release of tryptase is known to recruit inflammatory cells, to induce IL–8 secretion from airway epithelial cells, and to promote airway inflammation\textsuperscript{41}. In our study, tryptase correlated positively and significantly with IL–6 and IL–8 (Table 2.2). Surprisingly, we also found a negative correlation between tryptase and the organ damage markers. Lower levels of tryptase correlated significantly with higher levels of intestinal injury (plasma I–FABP, urinary I–FABP, urinary L–FABP), and high levels of proximal tubular (urine NAG), distal tubular (urine H–FABP) and glomerular (microalbuminuria) renal damage during the first two hours of reperfusion post CPB.

These data support the hypothesis of a preconditioning effect of tryptase: early release of tryptase might offer protection against perioperative intestinal and renal damage. By amplifying the signal for histamine release\textsuperscript{42}, and thus inducing an endothelial–NO dependent vasodilator effect, tryptase might counteract the vasoconstriction induced by the hyperglycemia and ischemia/reperfusion injury. This hypothesis is supported by data showing that histamine–induced vasodilatation mediated by endothelial derived NO was attenuated under hyperglycemic conditions\textsuperscript{43}. In our study, we found high serum glucose levels in patients undergoing CPB receiving dexamethasone. Serum glucose levels had strong positive predictive value for the postoperative intestinal and renal damage. The variation in serum glucose concentration explained more than 40\% in the variation of intestinal damage biomarkers (I–FABP and L–FABP) and more than 30\% in the variation of renal tubules damage markers (NAG and urine H–FABP). In addition, the patients in the dexamethasone treated group tended to require more insulin treatment.

To explain our results on the effect of acute hyperglycemia on organ injury, we refer to the recent published results of Vanhorebeek et al.\textsuperscript{44}, showing in a study on critically ill patients that hyperglycemia was associated with organ injury, as demonstrated by mitochondrial ultrastructural abnormalities with increased production of reactive oxygen species in the hepatocyte of hyperglycemic patients (10–11.1 mmol.L\textsuperscript{−1}). Using animal experiments, Bohlen and colleagues\textsuperscript{45,46} demonstrated that oxygen radicals formed during acute hyperglycemia affect flow–mediated endothelium regulation in the intestinal vasculature due to depression of nitric oxide, resulting in reduced blood flow.

Our data quantifies for the first time the effect of hyperglycemia on organ injury. These results might provide an explanation for the increased morbidity and mortality among critically ill patients in the surgical intensive care unit when blood glucose
level is above 6.1 L$^{-1}$.

A limitation of this study is that, despite randomization, patient characteristics were slightly different. In the dexamethasone group patients were slightly older and therefore the possibility of confounding exists. However, this influence seems limited because age did not prove to be a predictor for any of the biomarkers tested. Moreover, baseline values of a large number of sensitive markers were similar in both groups, and there was no correlation between age and the baseline levels of the tested markers. The patients in this study had little co-morbidity and thus belong to the “healthy” CABG group. Dexamethasone in patients of a higher risk profile could have different effects on inflammatory response and organ injury. Finally, this study was not powered to analyze effects on mortality, or possible differences in wound healing and postoperative infections.

2.5 Conclusions

Dexamethasone, as administered in this study, offered no protection against transient, perioperative renal, intestinal and hepatic injury in patients undergoing on-pump CABG. Dexamethasone treatment resulted in more pronounced postoperative pulmonary dysfunction, prolonged time to tracheal extubation and initiated postoperative hyperglycaemia. Given the strong positive predictive value of hyperglycaemia for renal and intestinal tissue injury, a stricter management of serum glucose may offer beneficial effects.

As a contribution to the efforts made for understanding the complex pathophysiologic mechanism of the “post–CPB” syndrome, this study verified theories existent in the literature and brought under attention new essential aspects: (1) higher glycemic values as strong predictors for higher intestinal and renal damage; (2) preconditioning effect of mast cells activation and tryptase release for the subsequent postoperative intestinal and renal injury.

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References


