Research article

Gender-related plasma levels of progesterone, interleukin-8 and interleukin-10 during and after cardiopulmonary bypass in infants and children

Andreas Trotter*, Kristina Mück*, Hans-Jörg Grill†, Uwe Schirmer‡, Andreas Hannekum§ and Dieter Lang*

*Section of Pediatric Cardiology, Children's Hospital, University of Ulm, Ulm, Germany
†Department of Obstetrics and Gynecology, University of Ulm, Ulm, Germany
‡Department of Cardiac Anesthesia, University of Ulm, Ulm, Germany
§Department of Cardiac Surgery, University of Ulm, Ulm, Germany

Correspondence: Andreas Trotter, andreas.trotter@medizin.uni-ulm.de

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Abstract

Background It is known that proinflammatory and anti-inflammatory cytokines are released during and after cardiopulmonary bypass (CPB) in infants and children. Sex steroids are known to have immunomodulatory functions, and release of the anti-inflammatory cytokine IL-10 is stimulated by progesterone *in vitro*. The purpose of the present study was to investigate the plasma levels of progesterone, IL-8 (proinflammatory cytokine) and IL-10, and to relate them to sex and postoperative morbidity.
Method Eighteen infants and children (eight female) undergoing CPB were prospectively studied. Plasma levels of progesterone, IL-8 and IL-10 were determined before and 10 min after the start of CPB, and immediately after CPB; and 6 h, 24 h, 3 days and 7 days postoperatively. Organ dysfunction was identified on the basis of arbitrarily defined criteria.
Results After CPB, all patients showed significant increases in plasma levels of progesterone, IL-8 and IL-10. Plasma levels of IL-10 were significantly higher in female patients, except for during the immediate postoperative period. According to the criteria used, six out of 10 male patients, but none of the female patients developed multiple organ dysfunction (MOD).
Conclusion The present study shows that CPB induces a significant and marked increase in plasma levels of progesterone in infants and children. Studies of administration of progesterone-blocking substances to male and female animals may help to elucidate the roles of sex and progesterone in the setting of CBP.

Keywords bypass, children, gender, infants, interleukin, progesterone

Introduction

It is known that IL-8 (a proinflammatory cytokine) is released during and after CPB in adults [1], and in infants and children [2]. It has recently been shown [2,3] that IL-10 is released during and after CPB in infants and children. IL-10 serves as an anti-inflammatory agent and acts via suppressed macrophage production of proinflammatory mediators [4]. Sex steroids are known to have immunomodulatory functions. *In vitro* data [5] has shown that the production of proinflammatory cytokines by fibroblasts can be suppressed by progesterone. The release of T-helper-2-type cytokines, which are predominantly anti-inflammatory cytokines, appears to be stimulated by progesterone [6–8]. In a pilot study conducted

CPB = cardiopulmonary bypass; IL = interleukin; MOD = multiple organ dysfunction.

Table 1

Diagnosis, type of operation, sex and age at operation of the 18 infants and children studied

Patient number (sex)	Diagnosis	Type of operation	Age (months)
1 (female)	ASD II	Closure	55
2 (female)	ASD II, AP window	Closure, transaortic patch	8
3 (male)	PA + IVS, PDA, AS	Valvulotomy of PV and AV, closure of PDA, AP shunt	2
4 (female)	VSD, PAB	Closure, debanding	42
5 (male)	VSD	Closure	19
6 (male)	SAS	Resection	182
7 (female)	VSD	Closure	30
8 (female)	VSD	Closure	24
9 (male)	Fallot, ASD II	Correction*	16
10 (male)	VSD, SAS, pulmonary hypertension	Closure, resection	15
11 (male)	VSD, PAB, corrected CoA	Closure, debanding	16
12 (female)	Fallot, FO	Correction	30
13 (female)	ASD II, CCAVB	Closure	24
14 (male)	Fallot, BTA	Correction, BTA closure	18
15 (male)	Fallot	Correction	10
16 (male)	Fallot (VACTERL)	Correction	20
17 (female)	Fallot	Correction	17
18 (male)	SAS	Resection†	185

*Second cardiopulmonary bypass run because of a residual shunt. †Resternotomy because of bleeding. AP = aortopulmonary; AS = aortic valve stenosis; ASD II, secundum atrioventricular defect; AV = aortic valve; BTA = Blalock–Taussig anastomosis; CCAVB = congenital complete atrioventricular block; CoA = coarctation of the aorta; Fallot = tetralogy of Fallot; FO = foramen ovale; IVS = intact ventricular septum; PA = pulmonary atresia; PAB = pulmonary artery banding; PDA = persistent ductus arteriosus; PV = pulmonary valve; SAS = subaortic stenosis; VACTERL = association of vertebral, anal, cardiac, tracheoesophageal, renal and limb defects; VSD = ventricular septal defect.

in 11 adults (three female) undergoing CPB for coronary–aortic bypass grafting [9], plasma levels of progesterone before and after the bypass were measured. Mean progesterone levels rose significantly from 0.13 to 0.90 ng/ml.

The purpose of the present study was to investigate the plasma levels of progesterone, IL-8 and IL-10 during and after CPB in infants and children, in relation to sex and postoperative morbidity.

Patients and method

Patients

The study was conducted at the University of Ulm. Infants and children were consecutively enrolled and underwent CPB if informed consent had been given by the parents. A total of 18 were included (median age 19 months, range 2 months to 15 years). On the day before the CPB, all patients had negative plasma levels of C-reactive protein and were not believed to have infections. Before the elective operation, the patients were in a haemodynamically stable condition and none of them had signs of heart failure. Diagnosis, type of operation, sex and age at operation of every patient are summarized in Table 1. None of the patients was pretreated with β-blockers. Patient 3 (pulmonary atresia with intact ventricular septum) was on prostaglandin and furosemide treatment, and patient 8 (ventricular septal defect) received digoxin. Patients 3 and 15 were cyanotic, with preoperative arterial oxygen saturations of 80 and 72%, respectively. Age, body weight and length, and body surface area were not significantly different between male and female patients (Table 2). The present study was approved by the Institutional Review Board of the University of Ulm.

Procedure

Anaesthesia was induced and maintained with fentanyl, midazolam (except in patients 6 and 18, who received clorazepate) and vecuronium bromide. Further anaesthetics were used in five male patients (enflurane in patients 9–11 and propofol in patients 6 and 18) and in three female patients (enflurane in patients 1, 8 and 13, and propofol in patient 1). A tri-lumen central venous line was inserted into the internal jugular vein. For the monitoring of arterial blood pressure, an arterial line was placed into the radial artery. Volume-controlled ventilation was applied (SERVO 900C respirator; Siemens, Munich, Germany). A transurethral catheter was inserted in all patients in order to monitor fluid balance.

The CPB was performed using a CAPS-roller pump system (Stöckert Instruments, Munich, Germany) with continuous flow. The system was connected to a membrane oxygenator (Dideco, Sorin Biomedica, Puchheim, Germany; Cobe Optima, Cobe Laboratories, Planegg-Martinsried, Germany) and an arterial filter. The priming solution consisted of ringer solution and sodium bicarbonate, and contained 2 IU/ml heparin-sodium and 1.5% human albumin. If the haematocrit was expected to drop to below 25% after the patient was connected to the bypass circuit, then red packed cells were added (which contained no detectable levels of progesterone).

After the sternotomy had been conducted (patients 1 and 13 had a right-sided thoracotomy for atrial septal defect type II closure), 30,000 IU/kg aprotinin were administered over a period of 20 min and the administration was continued with 7500 IU/kg per h throughout the operation. Before cannulation of the patient's heart, 300 IU heparin-sodium/kg was given intravenously in order to achieve an activated clotting time of greater than 400 s. No corticosteroids were given before or during the operation. After the caval veins, the left atrium and the aorta had been cannulated, hypothermia was induced by cooling down the priming solution (median oesophageal temperature 28.5°C; minimum 28°C and maximum 33°C). A secundum type atrial septal defect in patients 1 and 13 was closed under normothermic conditions. After electrical fibrillation of the heart, 30 ml/kg cold Bretschneider solution was infused into the cross-clamped aortic bulbus. The perfusion index was adjusted to 2.5 ml/kg per m^2 with the CPB system. At the end of the procedure rewarming was started. Catecholamines were used whenever systemic perfusion was impaired. Heparinization was countered using 300 IU/kg protamine-hydrochloride. The ventilated patients were transferred to the cardiac intensive care unit.

The patients were weaned from the respirator as soon as possible. Crystalloid fluid management consisted of 40 ml/kg per day during the first postoperative day, increasing by approximately 10 ml/kg every other day. Fresh frozen plasma or thrombocytes were given if the patient had postoperative bleeding (>3 ml/kg per h), or if the prothrombin time was below 40% of normal or the thrombocyte count was below 50,000/µl, respectively. Furosemide (0.5–1 mg/kg per dose) was used to facilitate diuresis. Blood pressure was maintained within age-specific ranges with catecholamines (dopamine, dobutamine, adrenaline, noradrenaline).

Laboratory data

Blood samples were taken immediately before the operation; after induction of anaesthesia and administration of heparin; 10 min after the commencement of CPB; after disconnection from the circuit and administration of protamine; and 6 h, 24 h, 3 days and 7 days postoperatively. Additional blood samples were taken when this was clinically indicated.

Blood cell counts, electrolytes, urea nitrogen, creatinine, protein, creatine kinase, heart-specific creatine kinase, aspartate aminotransferase, bilirubin, prothrombin time, partial thromboplastin time, fibrinogen and antithrombin III were measured using standard methods. Plasma levels of progesterone were measured in EDTA-medium using a RIA-kit (Coat-A-Count Progesterone; Diagnostic Products Corporation, Los Angeles, CA, USA). The detection limit was 0.02 ng/ml. The intra- and inter-assay coefficient of variation was 4.0 and 5.3% at 1.5 ng/ml, respectively. The test has no detectable cross-reactivity with aldosterone. IL-8 plasma levels were determined immediately after the blood was drawn (EDTA-coated tubes) using a chemiluminescence immunoassay (Immulite; DPC-Biermann, Bad Nauheim, Germany). The lower detection limit

Table 2

Physical characteristics of the patients, and operative and outcome measures

Parameter	Male ($n = 10$)	Female ($n = 8$)	P
Age (months)	17 (2–185)	27 (8–55)	0.21
Body weight (kg)	9.6 (4.0–68)	10.4 (5.7–16)	0.72
Body length (cm)	80 (54–169)	81 (65–102)	0.76
Body surface area (m^2)	0.45 (0.23–1.78)	0.46 (0.30–0.68)	0.72
Cardiopulmonary bypass time (min)	103 (79–218)*	89 (27–131)	0.08
Cross-clamping (min)	71 (31–129)*	46 (13–93)	0.10
Mechanical ventilation (h)	17 (2–111)	4.8 (2–18)	0.20
Intensive care unit (days)	2 (1–20)	2 (1–5)	0.51
Multiple organ dysfunction (*n*)	6	0	0.01

Median (minimum, maximum) age, body weight and length, body surface area at operation and duration of cardiopulmonary bypass, aortic cross-clamping time, duration of mechanical ventilation, and days on the intensive care unit for male and female patients (Mann–Whitney U-test). The incidence of multiple organ dysfunction is also shown (Fisher's exact test). *Times for the patient who underwent a second cardiopulmonary bypass run because of a residual shunt are cumulative.

Table 3

Definition of organ dysfunction

Dysfunctional organ system	Criteria
Renal	Urine output ≤1 ml/kg per h or serum creatinine ≥100 μmol/l
Respiratory	Ventilatory support with inspired oxygen fraction ≥0.4 for more than 5 days postoperatively
Hepatic	Aspartate aminotransferase ≥500 IU/L or prothrombin time* ≤50%
Cardiac	Need for catecholamines >24 h postoperatively†
Haematological	Thrombocytes <100,000/μl
Neurological	Seizures or coma

*Patient/control value. †Dopamine <5 μg/kg per min excluded.

for this assay was 5 pg/ml. The assay was calibrated up to 10,000 pg/ml. Intra- and inter-assay coefficient of variation was below 5% and below 2% at 95 pg/ml, respectively. All IL-10 plasma samples (EDTA-coated tubes) were frozen at −70°C and then measured using an enzyme-linked immunoassay (LD Zytokit IL-10 ELISA; LD Labordiagnostica, Heiden, Germany). The assay had a lower limit of detection of 15 pg/ml. Intra- and inter-assay coefficient of variation was 4.0 and 5.5% at 130 pg/ml, respectively. The IL-10 levels at 6 h postoperatively could not be measured because immediate workup of the samples was not possible.

Clinical criteria for multiple organ dysfunction

The different organ systems considered and the criteria used to define the dysfunction of an organ system are summarized in Table 3. MOD was diagnosed if two or more organ systems were affected. The occurrence of organ dysfunction was monitored for as long as the patient was in the intensive care unit.

Statistical analysis

The nonparametric Mann–Whitney U-test and the Wilcoxon test were used to analyze the results. Medians with interquartile ranges were used in the graphs. Correlations were calculated using Spearman's method. Differences in outcome were analyzed using the Fisher's Exact Test for categorical variables. $P < 0.05$ was considered statistically significant. Because of the small sample size, no multivariate regression analysis was performed. This was an explorative study without confirmatory design, and therefore no correction for repeated comparisons was performed.

Results

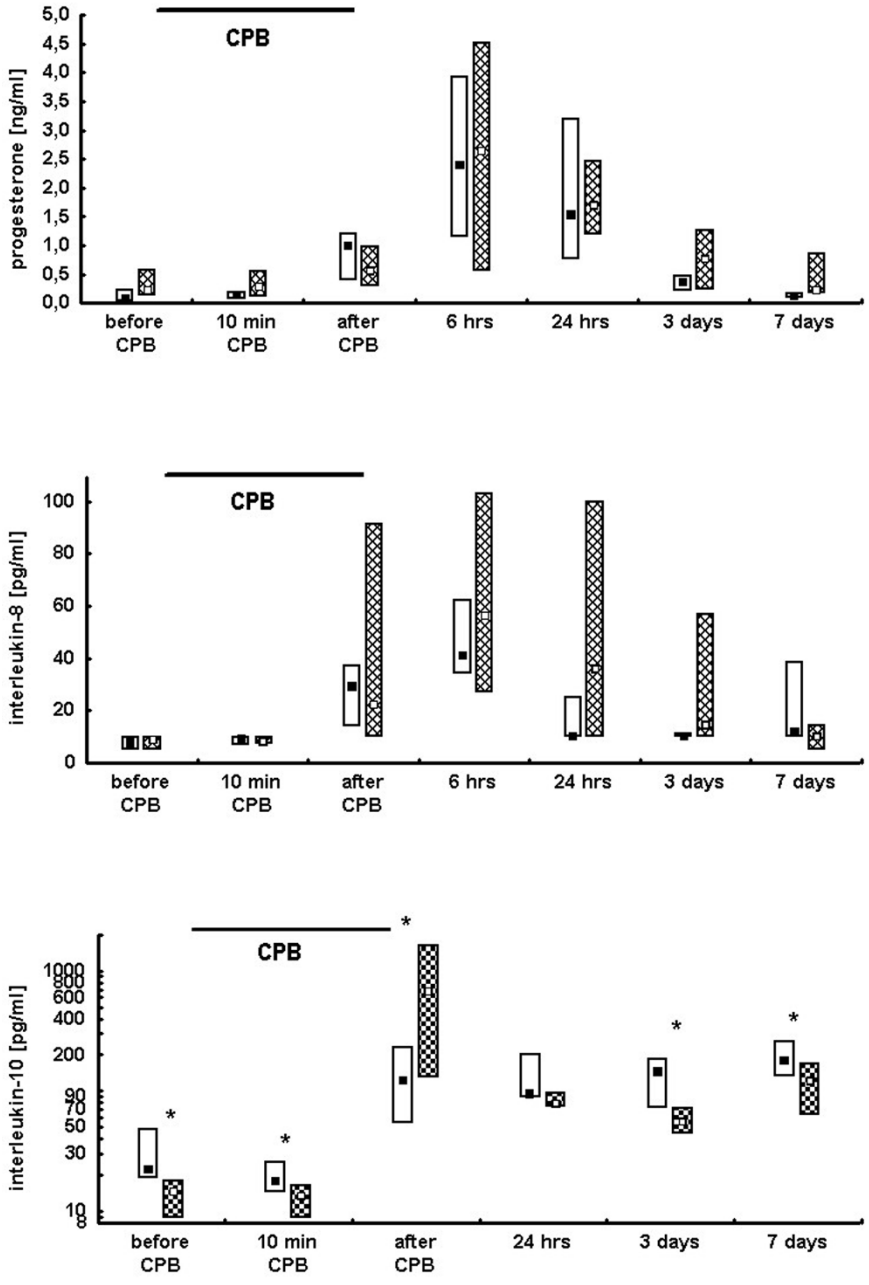
After CPB, all patients showed a significant increase in plasma levels of progesterone, IL-8 and IL-10 (Wilcoxon test). Plasma levels of progesterone and IL-8 showed no differences between male and female patients (Fig. 1; Mann–Whitney U-test). Plasma levels of IL-10 were significantly greater in female patients, except for during the immediate postoperative period (Fig. 1). Progesterone plasma levels were not correlated to patient age. No correlation between progesterone and IL-10 plasma levels in female patients was found at any sample time. After disconnection from the bypass circuit, plasma levels of IL-8 and IL-10, but not that of progesterone, were significantly correlated to the duration of the CPB ($r = 0.54$ and 0.60, $P < 0.05$ and $P < 0.01$ Spearman's method, respectively).

CPB time, aortic cross-clamping time, duration of mechanical ventilation and days on the intensive care unit for the male and female patients are summarized in Table 2. There were no significant differences between the two groups. According to the defined criteria for organ dysfunction, six out of 10 male patients but none of the female patients developed MOD ($P = 0.01$). The haematological system was most frequently affected (83%, $n = 15$), followed by the cardiac and renal systems (22% each), and hepatic and neurological systems (6% each). None of the patients needed ventilatory support for more than 5 days postoperatively, and all patients survived.

Discussion

Normative data for the prepubertal age show no sex-specific differences in plasma levels of progesterone [10], and this was found for the preoperative values in the predominantly prepubertal patients included in the present study. During CPB plasma progesterone levels increased in all patients, with no correlation to patient age or sex. It is unlikely that the progesterone increase is of gonadal origin. We did not determine the level of gonadotrophic hormones during and after bypass. No changes in the secretion rate of luteinizing hormone were found during and after CPB in adults [11]. An increase in aldosterone level after open heart surgery has been described in children [12] and adults [13]. For adult patients, it has been suggested that activation of the renin–angiotensin–aldosterone axis may be responsible for the observed changes in plasma aldosterone [13]. Progesterone is a precursor of aldosterone synthesis in the adrenal cortex. Whether the increase in plasma levels of progesterone during and after CPB is caused by an activated renin–angiotensin–aldosterone axis or whether the increase is the result of a CPB-related diminished metabolic rate for progesterone remains unknown.

Figure 1



Plasma levels of progesterone (upper panel), IL-8 (middle panel) and IL-10 (lower panel; semi-logarithmic Y-scale) at different sample times for female ($n = 8$, open bars) and male ($n = 10$, hatched bars) patients (*$P < 0.05$, medians with interquartile ranges). The horizontal bar represents the period of cardiopulmonary bypass (CPB).

It has been suggested that IL-10 may play a protective role by downregulating the production of proinflammatory cytokines after CPB [2]. We identified an increase in IL-10 levels, peaking after the disconnection from the bypass circuit, which is in accordance with recently published data [3]. *In vitro* data [14,15] showed that, after administration of exogeneous progesterone, β_2-adrenoreceptors on lymphocytes are upregulated in females, but not in males. The β_2-adrenoreceptor is known to mediate anti-inflammatory effects (e.g. the release of IL-10 [16]). On the basis of these *in vitro* data, it can be speculated that even identical amounts of progesterone may result in different anti-inflammatory effects in females as compared with males. No correlation was found between plasma levels of progesterone and IL-10 in the female patients included in the present study. In recent studies addressing human sepsis [17,18], women had a

significantly better prognosis than did men, and this was related to increased levels of IL-10 in the women. In contrast to those findings, female sex was identified as a risk factor for mortality after cardiac surgery with CPB [19].

Evaluation of the clinical course following cardiac surgery was conducted by defining organ dysfunction. There is some controversy in the literature that is relevant to the definition of organ dysfunction in infants and children. If we apply the MOD criteria defined by Wilkinson *et al*. [20] to the present data, the incidence of MOD would have been 50% ($n = 9$). Wilkinson *et al*. found an incidence of MOD of 27% in 831 paediatric intensive care unit patients, but they did not distinguish between postoperative cardiac surgery patients and others. Seghaye *et al*. [21] used a different definition of MOD for patients following cardiac surgery. If we apply those criteria to our patients, the incidence of MOD would have been only 6% ($n = 1$). We arbitrarily defined the criteria for organ dysfunction that fitted best the postoperative morbidity of our patients. All patients included in the present study who developed MOD were male. This would suggest that sex is the main factor in determining postoperative morbidity in infants and children. However, our arbitrary definition of organ dysfunction has limitations. Thrombocytopenia was the most common finding, the sample size was small, and the patients had various diagnoses and types of operation.

To our knowledge, this is the first study in which progesterone and the changes that occur in its plasma levels during and after CPB have been followed. After CPB a significant and marked increase in plasma levels of progesterone was identified. We were also able to confirm the known CPB-induced release of IL-8 and IL-10. Female patients had significantly higher plasma levels of IL-10 even preoperatively, and none of them developed MOD. Sex-related differences in postoperative morbidity cannot be supported by these preliminary data. Limitations include the small and very inhomogeneous group of patients with regard to age and diagnosis, and the arbitrary definition of MOD. Studies of administration of progesterone-blocking substances in male and female animals may help to elucidate the role of sex and progesterone in the setting of CBP.

Competing interests

None declared.

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