Dexamethasone Pretreatment Provides Antiinflammatory and Myocardial Protection in Neonatal Arterial Switch Operation

Ruth Heying, MD, PhD, Edith Wehage, PhD, Katharina Schumacher, MD, Peter Tassani, MD, PhD, Felix Haas, MD, PhD, Rudiger Lange, MD, PhD, John Hess, MD, PhD, and Marie-Christine Seghaye, MD, PhD

Department of Pediatric Cardiology, University Hospital Aachen, Aachen, Germany; Department of Pediatric Cardiology, UZ Leuven, Leuven, Belgium; Department of Pediatrics, University Hospital Liège, Liège, Belgium; and Departments of Anesthesiology, Cardiac Surgery, and Pediatric Cardiology, German Heart Centre at the Technical University, Munich, Germany

Background. **This prospective double-blinded randomized study tested the hypothesis that preoperative treatment with dexamethasone would attenuate inflammatory priming of the myocardium, reduce the systemic inflammatory reaction upon cardiac operation, and provide organ protection in neonates.**

Methods. **Twenty neonates (age, 8 to 21 days) with transposition of the great arteries scheduled for arterial switch operation were included. Nine received dexamethasone (1 mg/kg body weight) 4 hours before cardiopulmonary bypass, and 11 received natrium chloride. We studied intramyocardial messenger RNA expression of interleukin (IL)-6, IL-8, IL-1β, and tumor necrosis factor-α (TNF-α), as well as IL-10 and expression of TNF-α on protein level in right atrial tissue taken before institution of CPB. We measured plasma levels of IL-6, IL-10, lipopolysaccharide binding protein, and cardiac troponin T. Cytokine expression was related to postoperative outcome.**

Results. **Pretreatment with dexamethasone led to a significant decrease in myocardial expression of IL-6, IL-8, IL-1β, and TNF-α messenger RNA and to a decrease in protein synthesis of TNF-α. Plasma concentrations of IL-6 were significantly lower and those of IL-10 significantly higher in pretreated patients. This was associated with lower cardiac troponin T values and lower dobutamine requirement. Levels of lipopolysaccharide binding protein were significantly higher postoperatively in pretreated neonates.**

Conclusions. **Dexamethasone administration before arterial switch operation leads to a shift in the myocardial and systemic cytokine expression profile in neonates with transposition of the great arteries, with downregulation of proinflammatory and upregulation of antiinflammatory cytokines. Lower myocardial cell damage and lower catecholamine requirement suggest myocardial protection in treated patients.**

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Cardiac surgical intervention with cardiopulmonary bypass (CPB) leads to an acute systemic inflammatory reaction involving stimulation of inflammatory and noninflammatory cells that release proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α (TNF-α), into the circulation [1, 2]. There are several stimuli for the proinflammatory reaction, including production of lipopolysaccharide binding protein (LBP) that, in concert with CD14, serve as binders for lipopolysaccharide and enhance the proinflammatory reaction in its early phase [3–5]. Noninflammatory cells, such endothelial cells, hepatocytes, and myocardial cells [1, 6–9], not only contribute to the systemic inflammatory reaction but also act as autocrine mediators and induce local inflammatory cell damage and cell death, resulting in organ dysfunction [8, 10].

In children with congenital cardiac diseases, conditions such as hypoxemia or heart failure induce intramyocardial cytokine synthesis, as shown by our group [10, 11]. This inflammatory priming, which is already present before the operation, might exacerbate the systemic inflammatory response to the cardiac operation as well as intraoperative and postoperative myocardial cell damage.

The proinflammatory response to cardiac operations is counterbalanced by a complex system of inhibitors comprising the production of the antiinflammatory and monocyte inhibitory cytokine IL-10 [12, 13]. IL-10 is induced in response to several proinflammatory signals, including TNF-α and IL-6, and represses proinflammatory cytokine-signalling molecules, such as nuclear factor-κB, and might be protective by limiting, in turn, the production of harmful proinflammatory cytokines [12, 14, 15].

Lipopolysaccharide binding protein and soluble CD14 play a dual role in the inflammatory reaction. In concert they serve as binders for lipopolysaccharide, leading to enhancement of the inflammatory reaction in the early phase. Lipopolysaccharide-activated macrophages re-

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Address correspondence to Dr Heying, Pediatric Cardiology, UZ Leuven, Herestraat 49, 3000 Leuven, Belgium; e-mail: ruth.heying@uzleuven.be.

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lease proinflammatory cytokines, such as IL-6, IL-1, and TNF-α, and therefore induce an inflammatory reaction [3, 16]. Furthermore, negative feedback mechanisms lead to an additional protective effect of LPB and soluble CD14 by preventing the lethal side effects of an increased inflammation in its late phase [17, 18].

Immunomodulating strategies, such as the use of hypothermia during cardiac operations, are related to upregulation of IL-10 with downregulation of TNF-α and postoperative organ protection, as our group showed previously [8, 10]. Besides hypothermia, the preoperative administration of glucocorticosteroids also induces a shift of the inflammatory balance toward the antiinflammatory reaction with increased levels of IL-10 that are the result of increased intrahepatic production, as suggested in adults undergoing cardiac operations [19, 20].

Despite this, the evidence of a beneficial effect of pretreatment with glucocorticosteroids is under discussion in adults [21–23]. In children it has been suggested to ameliorate postoperative outcome [24]. However, the nonhomogeneity of the patients studied with respect to age, diagnosis, and severity of operative procedures renders it difficult to draw definitive conclusions concerning glucocorticosteroid use in pediatric cardiac surgery. We therefore tested the hypothesis that preoperative treatment with dexamethasone would attenuate the myocardial and systemic inflammatory reaction and improve postoperative outcome in a homogenous group of newborns undergoing arterial switch operation for transposition of the great arteries (TGA) in a prospective randomized, double-blinded, placebo-controlled study.

Patients and Methods

This study was approved by the Ethical Medical Committee of the Technical University Munich, and written informed parental consent was obtained.

Clinical Data

Twenty neonates (age, 8 to 21 days) diagnosed with TGA with or without ventricular septum defect were scheduled for arterial switch operation and enrolled in this prospective randomized double-blinded study. Patients were randomized into two groups. Group I (n = 9) received dexamethasone at a dosage of 1 mg/kg in 1 mL isotonic natrium chloride intravenously. Group II (n = 11) received 1 mL of isotonic natrium chloride. Placebo or verum was given on the ward 4 hours before the scheduled start of CPB. The randomization was done by the pharmacy, and the randomization code was broken after data acquisition was completed. Patient clinical data are summarized in Table 1.

Anesthesia

Conventional general anesthesia consisted of midazolam, fentanyl sulphate, and pancuronium bromide. Premedication was not given. After induction of anesthesia, nasotracheal intubation was performed, and central venous and peripheral arterial catheters were inserted.

CPB Technique

The CPB protocol was uniform for all children and was performed with a roller pump (Stoeckert, Munich, Germany), a disposable membrane oxygenator (Liliput, Dideco, Mirandola, Italy), and an arterial filter. The priming solution consisted of packed red cells (250 mL) and fresh frozen plasma (250 mL) added to lactated Ringer's solution (100 mL) along with heparin (1,000 IU) sodium bicarbonate 4.2% (2.5 mL/kg), and mannitol 20% (3 mL/kg). CPB was instituted with a perfusion index of 2.4 L/min/m^2 body surface area.

Anticoagulation was achieved by heparin sulphate (300 U/kg body weight). Cooling was performed with a heat exchanger to a rectal temperature of 24°C. A stable flow of 1.2 L/min/m^2 was maintained during the period of intracardiac correction. After the aorta was cross-clamped, cold crystalloid cardioplegic solution (30 mL/kg; Bretschneider, Custodiol, Köhler, Alsbach-Hähnlein, Germany) was injected through the aortic root.

Anatomic correction consisted of arterial switch procedure with Lecompte maneuver and closure of the ventricle septum defect in 3 patients. To close the atrial septal defect, a short period of total circulatory arrest was instituted in all patients. During rewarming, the flow rate was gradually increased to 2.4 L/min/m^2. Modified ultrafiltration was used immediately after weaning from CPB in all patients.

Postoperative Care

Standardized postoperative care was provided. Postoperative monitoring included continuous registration of heart rate and rhythm, arterial blood pressure, central venous pressure, diuresis, and blood gases. Inotropic support consisted in all cases of dopamine (5 μg/kg/min) and, if necessary, dobutamine (5 to 7 μg/kg/min). Diuretics (furosemide, single dosage 0.1 to 1 mg/kg) and volume substitution, consisting of fresh frozen plasma or human albumin 5%, were administered according to the hemodynamic variables. Postoperative clinical end point variables were mean arterial blood pressure, heart rate, central venous pressure, diuresis, fluid balance, need of inotropic support, and oxygenation index, calculated as partial pressure of arterial oxygen/fraction of inspired oxygen (Pa_{O_2}/Fi_{O_2}).

Laboratory Tests

Blood samples were taken 10 different times before, during, and after the operation as follows: in the operating theater after anesthesia and before sternotomy, 10 minutes after onset of CPB, end of CPB, 1 hour, 4 hours, and at 1, 2, 3, 5, and 10 days postoperatively. Samples (1.5 mL ethylenediaminetetraacetic acid) were immediately centrifuged for 10 minutes (3,000 rpm) at 4°C, and plasma was stored at −80°C until analysis.

Determination of Cytokines, Cardiac Troponin T, and LBP

Serum IL-6, IL-10 and LBP concentrations were measured with a commercially available solid-phase enzyme-

Table 1. Patient Characteristics[a]

Variables	Control Group (n = 11)	Dexamethasone (n = 9)	*p* Value
Age at operation, days	11 (8–17)	9 (7–21)	0.46
Body weight, kg	3.2 (2.8–4.3)	3.5 (2.3–4.1)	0.68
Ventricular septal defect	2	1	...
Rashkind balloon dilation	6	5	...
Pre-op PGE_1 administration	11	9	...
Pre-op mechanical ventilation	2	3	...
CPB time, min	138 (123–145)	124 (119–133)	0.25
Aortic cross-clamp duration, min	72 (67–78)	64 (53–68)	0.05
Arrest duration, min	0 (0–3)	0 (0–3)	0.92
Minimal esophageal temp, °C	24.4 (21.7–25.7)	24.0 (19.9–25.3)	0.79
Mean arterial blood pressure, mm Hg			
4 hours after CPB	55 (53–58)	57 (49–61)	0.89
1 day post-op	51 (48–57)	54 (50–66)	0.31
Heart rate, beats/min			
4 hours after CPB	159 (143–166)	158 (129–169)	0.96
1 day post-op	146 (125–150)	145 (136–160)	0.30
Central venous pressure, mm Hg			
4 hours after CPB	8.0 (7.0–8.75)	6.5 (6.0–8.8)	0.26
1 day post-op	7.5 (6.0–9.0)	7.0 (6.0–7.0)	0.17
Diuresis, mL/kg/h			
4 hours after CPB	8.6 (3.5–12.8)	7.1 (3.9–10.7)	0.79
1 day post-op	3.8 (2.8–5.9)	5.4 (4.1–9.4)	0.17
Renal fluid balance, mL/kg/h			
4 hours after CPB	1.30 (−0.40 to 2.80)	1.60 (−2.20 to 4.40)	0.82
1 day post-op	−0.03 (−0.90 to −1.80)	−1.90 (−2.40 to −0.01)	0.11
Oxygenation index, Pao_2/Fio_2 mm Hg			
4 hours after CPB	117.5 (99.5–156.8)	122.5 (104.3–140.0)	0.93
1 day post-op	152.0 (124.0–250.0)	162.5 (135.3–213.8)	0.87
Dopamine dosage, μg/kg/min			
4 hours after CPB	3.0 (3.0–5.0)	3.0 (3.0–5.0)	0.87
1 day post-op	3.0 (3.0–3.0	3.0 (2.6–3.0)	0.25
Dobutamine dosage, μg/kg/min			
4 hours after CPB	7.0 (5.0–7.3)	5.0 (3.5–5.0)	0.003[b]
1 day post-op	4.0 (3.0–13.2)	4.0 (2.3–5.0)	0.36

[a] Values are expressed as numbers of patients or median (interquartile range), except for age and body weight where median (range) is shown. [b] $p < 0.05$ significant difference between control and dexamethasone treated group.

CPB = cardiopulmonary bypass; Fio_2 = fraction of inspired oxygen; Pao_2 = partial pressure of arterial oxygen; PGE_1 = prostaglandin E_1.

labelled chemiluminescent sequential immunometric assay with an Immulite analyzer (DPC Biermann, Bad Naunheim, Germany). The limits of detection are 2 pg/mL for IL-6, 1 pg/mL for IL-10, and 0.2 μg/mL for LBP. Serum cardiac troponin T (cTnT) concentrations were measured by an electrochemiluminescence immunoassay using the Elecsys 1010 system (Roche Diagnostic, Mannheim, Germany). The limit of detection is 0.03 ng/mL.

Myocardial Biopsy Specimens

A biopsy specimen was taken from the right atrial appendage immediately before connection to CPB. Samples were immediately snap-frozen in liquid nitrogen and stored at −80°C until processed. The interval between administration of dexamethasone and sampling of atrial biopsies averaged 4 hours and was similar in both groups.

RNA Isolation and Quantitative Reverse Transcription Polymerase Chain Reaction

Total RNA was extracted from the atrial myocardium in all patients using RNeasy Mini Kit (Qiagen Inc, Hilden, Germany). RNA (100 ng) in a total volume of 20 μL was reverse transcribed to complementary DNA (cDNA) using the iScript cDNA Synthesis Kit (BioRad, Munich, Germany). Primers were used for:

IL-6 (sense 5′-AAAGAGGCACTGGCAGAAAA-3′ and antisense 5′-AGCTCTGGCTTGTTCCTCAC-3′),

IL-8 (sense 5′-CAGGAATTGAATGGGTTTGC-3′ and antisense 5′- AAACCAAGGCACAGTGGAAC −3′),
IL-10 (sense 5′-TGCAAACCAAACCACAAGA-3′ and antisense 5′-TCTCGGAGATCTCGAAGCAT-3′),
TNF-α (sense 5′-GGAGCCAGCTCCCTCTATTT-3′ and antisense 5′-GGCTACATGGGAACAGCCTC-3′),
IL-1β (sense 5′-CTGTCCTGCGTGTTGAAAGA-3′ and antisense 5′-TTCTGCTTGAGAGGTGCTGA-3′), and the housekeeping gene 18sRNA (sense 5′-AAACGGCTACCACATCCAAG-3′ and antisense 5′-CCTCCAATGGATCCTCGTTA-3′).

A standard for each primer set was generated for quantitative real-time reverse transcription-polymerase chain reaction (PCR) by cloning PCR products in pBluescript (Agilent Technologies, Santa Clara, CA) and the identity was verified by sequencing. A cDNA sample (2 μL) was incubated with QuantiTect Mix (20 μL) (Qiagen GmbH, Hilden, Germany) containing fluorescence dye SYBR Green (Qiagen) and 0.6 μmol/L of each primer pair. PCR amplification was performed after initial denaturation at optimized annealing temperatures for each primer pair using MJ Research Opticon 2 (Biozym Scientific GmbH, Hessisch Oldendorf, Germany). Melting curves were acquired by stepwise increase of the temperature from 55°C to 95°C. Threshold cycles (CTs) of real-time PCR curves were determined by Opticon Monitor software. The difference of the CTs (ΔCT) of targets and the 18 sRNA housekeeping control gene reflected the amount of target mRNA in each sample. Target mRNA was quantified according to the standard curve and normalized to levels of 18 sRNA.

Western Blotting

Total protein homogenates (100 μg) from the atrial myocardium taken in all patients were denatured and separated on 12% polyacrylamide gels by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Primary antibodies in immunoblotting were monoclonal mouse antihuman TNF-α (R&D Systems, Wiesbaden, Germany) and monoclonal mouse antihuman β-actin (Sigma, St. Louis, MO). The bands were detected by a chemiluminescence system according to the manufacturer's instructions (Amersham-Pharmacia, Freiburg, Germany). Protein signals for TNF-α were normalized for β-actin signals that were developed on the same blotting.

Statistical Analysis

Results are expressed as median values and interquartile ranges. Mann-Whitney *U* tests were used to compare patient characteristics between groups. Associations were evaluated using Spearman correlations. Linear models for repeated measures were used to compare the evolution of IL-6, IL-10, cTnT, and LBP between the control and the dexamethasone group. Using a direct likelihood approach, the variance is allowed to differ between the points in time. The between-group (difference between control and dexamethasone), within-group (evolution over time), and interaction effects are reported. The groups are also compared at each interval with a (stepdown) Bonferroni approach to correct for multiple testing. Values have been transformed (natural logarithm) when needed to obtain a more symmetric distribution of the model residuals. Values of $p < 0.05$ are considered significant. All analyses have been performed using SAS 9.2 software (SAS Institute, Cary, NC).

Results

Clinical Results

Epidemiologic data were similar between the groups. Durations of CPB were similar in both groups, but duration of aortic cross-clamping tended to be longer in the control group than in the dexamethasone-treated group ($p = 0.05$). There was no significant difference in the duration of complete circulatory arrest and minimal difference in esophageal temperature between the groups. All neonates were uneventfully weaned from CPB.

Clinical outcome variables (arterial blood pressure, heart rate, central venous pressure, diuresis, fluid balance and oxygenation index) recorded 4 and 24 hours after CPB were not significantly different between the groups. Patients who received dexamethasone required a lower dosage of dobutamine 4 hours postoperatively ($p < 0.01$). All patients survived. Patient characteristics are reported in Table 1.

Laboratory Results

ITERLEUKIN-6. IL-6 concentrations before sternotomy were within the normal reference range and not significantly different in either group. All patients showed a steady increase of IL-6 during and after CPB, with a maximum observed 4 hours postoperatively in the control patients and 24 hours postoperatively in the pretreated patients. Patients pretreated with dexamethasone had significantly lower IL-6 concentrations than control patients 10 minutes after onset of CPB, 1 hour and 4 hours after CPB, and also 1 day postoperatively (Table 2). There was no correlation between IL-6 levels and the CPB or aortic cross-clamp time in the groups.

CARDIAC TROPONIN T. Preoperative cTnT concentrations were within the normal reference range in all patients. Concentrations of cTnT increased from the end of CPB to reach maximal concentrations 2 days after the operation in all children. From day 2 postoperatively, cTnT decreased to the preoperative level. Levels of cTnT did not differ between the groups before sternotomy but were significantly lower in patients pretreated with dexamethasone 1 hour postoperatively than in the control group ($p < 0.05$, Table 2).

ITERLEUKIN-10. Preoperative IL-10 concentrations before sternotomy were within normal reference ranges and not significantly different in either group. The overall course of IL-10 concentrations showed a steady increase during and after CPB to reach maximal values 1 hour after CPB in all patients. Pretreated patients showed higher IL-10 concentrations 10 minutes after onset of CPB, at the end of CPB, and 1 hour after CPB than the control patients.

Table 2. Plasma Levels of Interleukin-6, Cardiac Troponin T, Interleukin-10, and Lipopolysaccharide Binding Protein[a]

Times	Interleukin-6	cTnT	Interleukin-10	LBP
Preop				
Control group	5.0 (4.7–7.7)	0.03 (0.01–0.05)	4.9 (4.9–4.9)	6.5 (4.7–8.9)
Dexamethasone	5.0 (1.9–9.4)	0.03 (0.01–0.05)	4.9 (4.9–13.5)	13.9 (9.3–23.3)
10 min after CPB				
Control group	5.5 (4.9–9.6)	0.07 (0.03–0.09)	4.9 (4.9–4.9)	3.5 (3.3–4.1)
Dexamethasone	2.9 (1.9–4.3)[b]	0.04 (0.01–0.06)	7.0 (4.9–10.6)[c]	5.7 (4.5–9.3)
End of CPB				
Control group	16.2 (7.8–51.5)	0.76 (0.20–0.76)	33.2 (10.2–78.3)	1.7 (1.3–1.9)
Dexamethasone	11.9 (4.9–23.1)	0.91 (0.45–0.97)	631.0 (35.6–1794.0)[d]	2.6 (1.6–4.9)
1 hour after CPB				
Control group	122.0 (47.8–165.0)	0.89 (0.77–0.94)	169.0 (99.2–234.0)	2.9 (2.1–3.8)
Dexamethasone	26.2 (22.3–47.1)[e]	0.48 (0.33–0.70)[f]	847.0 (571.5–2,021.0)[g]	6.1 (4.6–8.7)[h]
4 hours after CPB				
Control group	244.0 (88.8–395.0)	0.90 (0.72–1.16)	14.1 (7.9–43.1)	9.6 (7.6–10.2)
Dexamethasone	36.8 (23.3–60.8)[i]	0.81 (0.61–0.92)	26.0 (15.2–55.1)	12.6 (11.8–15.2)
1 day postop				
Control group	124.0 (62.9–174.0)	1.54 (1.32–1.90)	10.0 (5.6–12.3)	17.8 (16.8–32.6)
Dexamethasone	61.3 (30.6–79.8)[j]	1.54 (0.83–1.73)	5.4 (4.9–8.7)	28.0 (23.1–34.1)
2 days postop				
Control group	46.2 (36.7–66.2)	2.20 (1.74–2.81)	5.0 (4.9–7.9)	37.1 (18.8–44.2)
Dexamethasone	30.4 (25.6–54.5)	2.23 (1.99–2.54)	4.9 (4.9–6.1)	43.8 (30.7–52.1)
3 days postop				
Control group	31.3 (15.4–43.5)	2.00 (1.73–2.81)	4.9 (4.9–4.9)	30.1 (24.3–40.5)
Dexamethasone	25.8 (18.6–36.8)	1.90 (1.42–2.91)	4.9 (4.9–4.9)	40.0 (31.4–46.7)
5 days postop				
Control group	13.7 (9.6–29.4)	1.09 (0.58–1.64)	4.9 (4.9–4.9)	17.6 (8.4–27.2)
Dexamethasone	12.7 (12.2–23.2)	0.89 (0.72–1.13)	4.9 (4.9–4.9)	25.4 (17.8–32.3)
10 days postop				
Control group	8.1 (7.2–15.5)	0.23 (0.15–0.63)	4.9 (4.9–4.9)	24.8 (17.5 32.9)
Dexamethasone	5.3 (1.9–13.6)	0.26 (0.20–0.60)	4.9 (4.9–8.4)	17.1 (16.6–32.3)
Effect within (time)	<0.0001	<0.0001	<0.0001	<0.0001
Effect between (group)	0.0006	0.4718	0.0017	0.0784
Effect (interaction)	0.0021	0.0938	<0.0001	0.0024

[a] Values are expressed as median (interquartile range). Linear models for repeated measures are used to compare the evolution of interleukin 6, cardiac troponin T (cTnT), interleukin-10, and lipopolysaccharide binding protein (LBP) between the control and the dexamethasone group. The between-group (difference between control and dexamethasone), within-group (evolution over time) and interaction effects (residual) are calculated. Measurements are correct for multiple testing using a (stepdown) Bonferroni approach. P values < 0.05 are significant. [b] $p = 0.0086$; [c] $p = 0.0293$; [d] $p = 0.0485$; [e] $p = 0.0086$; [f] $p = 0.0178$; [g] $p < 0.0001$; [h] $p = 0.0117$; [i] $p < 0.0001$; [j] $p = 0.0272$ vs control.

CPB = cardiopulmonary bypass; cTnT = cardiac troponin T; LBP = lipopolysaccharide binding protein; postop = postoperative; preop = preoperative.

IL-10 concentrations decreased thereafter to reach normal values 10 days postoperatively (Table 2).

LIPOPOLYSACCHARIDE BINDING PROTEIN. Before sternotomy, LBP concentrations were not different between the groups. The course of LPB values during and after CPB was similar in both groups, with a decrease after onset of CPB. LBP reached minimal concentrations at the end of CPB. Postoperatively, LBP values increased to reach maximal values 2 days after CPB and remained elevated (Table 2). Pretreated patients showed significantly higher levels 1 hour postoperatively compared with the control group (Table 2).

INTRAMYOCARDIAL EXPRESSION OF PROINFLAMMATORY AND ANTIINFLAMMATORY CYTOKINES. Expression of mRNA encoding for the proinflammatory cytokines IL-6, IL-8, IL-1β, TNF-α, and the antiinflammatory cytokine IL-10 could be detected with quantitative reverse transcription PCR in the right atrium of all neonates. In the control group, the ratio of the cytokine/18 sRNA cycle threshold value showed a significant lower cycle threshold value, which means a higher amount of proinflammatory cytokine mRNA encoding for IL-6 (3.60 ± 0.09 vs 3.91 ± 0.07, $p = 0.001$), IL-8 (3.41 ± 0.05 vs. 3.50 ± 0.12, $p = 0.004$), TNF-α (4.17 ± 0.06 vs. 4.44 ± 0.1, $p < 0.05$), and IL-1β (3.90 ± 0.07 vs. 4.23 ± 0.08, $p = 0.006$) than in the dexamethasone recipients (Figure 1). In contrast, there was no significant difference in the ratio of the cycle threshold value for the mRNA encoding for the antiinflammatory cytokine IL-10 between the groups (Fig 1).

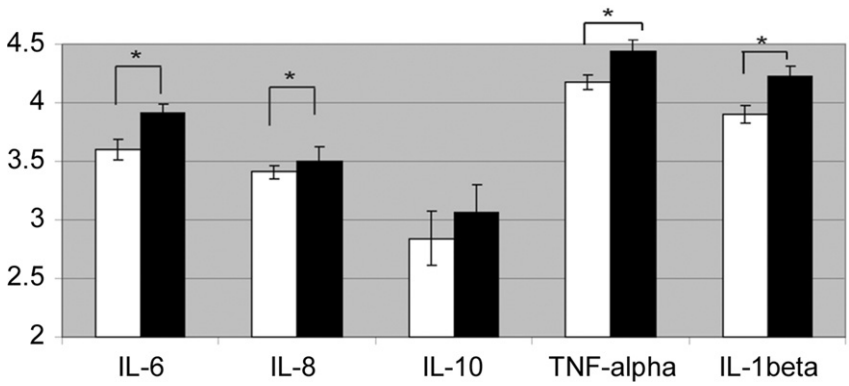

*Fig 1. Intramyocardial messenger RNA expression of proinflammatory and antiinflammatory cytokines. Ratio of cytokine/housekeeping gene (18sRNA) cycle threshold values of real-time polymerase chain reaction are shown. A lower cycle threshold is based on a higher amount of messenger RNA expressed. Values are shown as means and standard error of the mean (error bars). Significant differences between both groups were analyzed by Mann-Whitney U test. *p < 0.05. Results of the control group are shown in the open boxes, and results of the dexamethasone-treated group in the hatched boxes. (IL = interleukin; TNF = tumor necrosis factor.)*

TNF-α protein was expressed to a lesser degree after pretreatment with dexamethasone (Fig 2).

Comment

A systemic inflammatory reaction develops in neonates and infants undergoing cardiac operations for congenital cardiac defects that is related to postoperative complications, in particular, myocardial dysfunction [10, 25]. There is currently no established antiinflammatory strategy to improve postoperative outcome. We therefore examined the effect of preoperative administration of one dose of dexamethasone on myocardial expression of proinflammatory and antiinflammatory cytokines on the systemic inflammatory response to a cardiac operation and on postoperative outcome in a randomized, prospective, double-blinded study of neonates with TGA.

We report two major results: First, the administration of dexamethasone before TGA shifts the inflammatory balance toward the antiinflammatory reaction in the neonate myocardium after the CPB circuit is connected. Second, the myocardial and systemic antiinflammatory reaction induced by dexamethasone is related to less myocardial damage.

In detail, we observed that mRNA, encoding for IL-8, IL-6, TNF-α, and IL-1β, is significantly reduced by dexamethasone in the myocardium of neonates with TGA before the onset of CPB (4 hours after dexamethasone administration, Fig 2). In line with others, we also confirmed that the plasma cytokine balance changed in favor of the antiinflammatory reaction, expressed by higher IL-10 to IL-6 ratio levels after administration of dexamethasone [24, 26]. We did not find any significant effect of dexamethasone on clinical parameters, but neonates receiving steroids required less dobutamine infusion 4 hours postoperatively.

The inflammatory response after CPB is complex and related to various cascades and acute-phase reactions mediating pathophysiologic changes that compromise the maintenance of normal postoperative organ function [2, 25, 27]. Corticosteroids given before CPB minimized the inflammatory reaction and improved the postoperative course after CPB [22, 24, 26].

In particular, dexamethasone decreased the proinflammatory and antiinflammatory cytokine ratio in adults undergoing CPB with a combined administration preoperatively and intraoperatively and therefore was suggested to improve outcome [21, 26, 28]. Although the clinical benefit is controversial, a recent meta-analysis in adults showed a reduced risk of atrial fibrillation, a shorter intensive care unit stay, and no increased infections, with a side effect of increased incidence of insulin

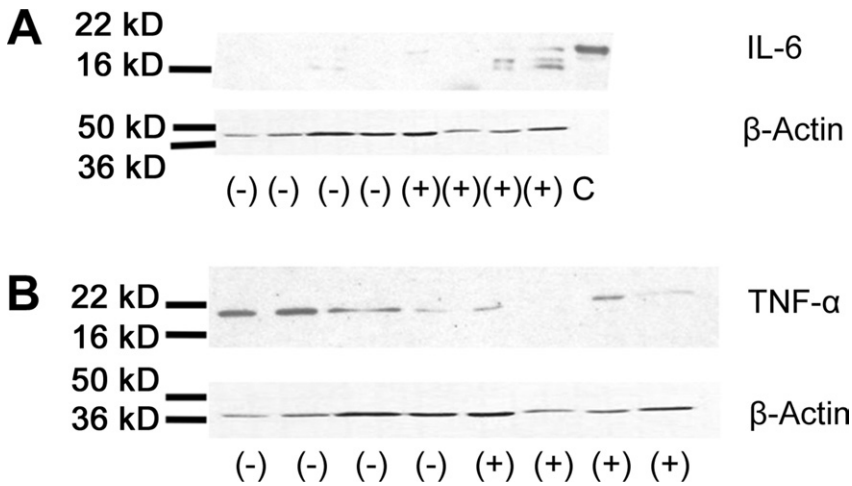

Fig 2. Intramyocardial protein expression of interleukin (IL)-6 and tumor necrosis factor (TNF)-α. (A) Expression of IL-6 on protein level (Western blotting) showed a weak expression without any differences between both groups. (B) TNF-α was detected in the protein level in all patients, with lower expression levels in the dexamethasone-pretreated group (+) compared with the control group (–). (C = positive control.)

required for hyperglycemia in the postoperative course [22, 23].

Increased IL-6 and TNF-α are known players in the perioperative course of children undergoing cardiac operations, with neonates being even more susceptible for an increased inflammatory reaction [11, 27, 29, 30]. An abnormal IL-10/IL-6 ratio expressing an abnormal cytokine balance and accelerated IL expression are associated with prolonged intubation and pediatric intensive care unit stay in neonates with TGA and hypoplastic left heart syndrome undergoing CPB [29].

A reduced myocardial expression of IL-6 and IL-8, as well as other proinflammatory cytokines before CPB, as shown by our data, could lead to myocardial protection because IL-6 and IL-8 were associated with postoperative myocardial ischemia and segmental wall abnormalities [31].

Our data demonstrated plasma IL-10 values were already higher in the dexamethasone-treated group after anesthesia and before connection to CPB. We assume that dexamethasone induced a shift in inflammatory priming in these patients. In general, children with complex cardiac lesions are known to have higher plasma proinflammatory cytokines (IL-6) as well as a remarkable expression of proinflammatory and antiinflammatory cytokines in their myocardium [10].

Changes of myocardial IL-10 expression were not seen in our study when biopsy occurred (Fig 2). This is rather unexpected, and we might speculate that dexamethasone administration has an immediate effect on the systemic inflammatory response of blood monocytes and lymphocytes and, in contrast, a delayed effect on the myocardial synthesis of IL-10, which might occur at a later time.

Maintenance of postoperative organ function, especially myocardial function, is likely to depend on the balance between proinflammatory and antiinflammatory cytokine syntheses. Myocardial function is well assessed by cTnT levels expressing myocardial cell damage, which are known to peak at the end of CPB through 24 hours postoperatively [11, 31]. Importantly, peak cTnT levels as well as IL-6 values are increased in neonates developing postoperative myocardial dysfunction, and IL-6 values 4 hours postoperatively are even an independent predictive risk factor [11].

Dexamethasone pretreatment achieved postoperative myocardial protection shown by significantly lower cardiac cTnT levels 1 hour after bypass in the dexamethasone-treated neonates compared with the control group. Our results suggest that dexamethasone provides myocardial protection in the most vulnerable postoperative period. This goes along with a previous study reporting significantly lower postoperative cTnT values in treated patients [32].

In our two groups of neonates we observed an immediate elevation of plasma cTnT levels within 10 minutes after onset of CPB, before aortic cross-clamping. This might be the result of early myocardial cell damage due to systemic or myocardial inflammation, or both. Neonates are likely to develop myocardial cell damage much earlier than older children undergoing CPB [27].

Others have shown that cTnT elevation correlates to the aortic cross-clamp time (ischemia and reperfusion) and the duration of bypass (systemic inflammatory response) [33]. For that reason, longer aortic cross-clamping time in the pretreated patients of our series resulted in a bias. However, because pretreated patients had a proinflammatory cytokine balance in the blood and myocardium before aortic cross-clamping commenced, and a previous study of our group showed a relationship between IL-6 levels and myocardial cell damage in neonates [11], we suggest that dexamethasone led to myocardial protection throughout its antiinflammatory effect. Interestingly, a correlation between cTnT levels on day 1 postoperatively and aortic cross-clamp time existed in our control patients but not in the pretreated group.

Pretreatment with dexamethasone was also associated with a lower catecholamine requirement postoperatively, suggesting a clinical benefit of the pretreatment.

LBP is an acute-phase protein with antiinflammatory capacity [13, 17]. The transcriptional regulation of the LBP gene is induced by IL-1 alone or synergistically by IL-1 and IL-6 leading to a maximal LBP concentration within 24 to 48 hours after stimulation [17, 34, 35]. In our series, patients who received dexamethasone were observed with significantly higher levels of LPB 1 hour postoperatively. More important, the protective effect of LPB on lethal side effects of an increased inflammation in its late phase is mediated by negative feedback mechanisms [17, 18]. LBP levels in our study seem therefore to reflect the antiinflammatory counterbalance in the acute-phase reaction to CPB.

In conclusion, this study demonstrates that a single dose of dexamethasone in neonates before CPB is safe and induces a shift in the inflammatory response to the cardiac operations toward an antiinflammatory reaction. In addition, myocardial mRNA expression of proinflammatory cytokines is significantly reduced at the time of the operation. Dexamethasone administration is associated with a significant reduction in postoperative production of cTnT and increased myocardial protection. These results are conclusive to recommend to a single dose dexamethasone before CPB in neonates.

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INVITED COMMENTARY

The systemic inflammatory response syndrome is, at least partly, responsible for complications in children undergoing cardiac surgery with cardiopulmonary bypass (CPB), and has been the target of the prophylactic use of corticosteroids. The potential benefits and risks of corticosteroids remain controversial despite a widespread use for more than 30 years. Even though several trials demonstrated the ability of corticosteroids to modulate the inflammatory response related to CPB in children [1, 2], a pediatric meta-analysis published in 2007 [3] found no clinical benefit, and highlighted the paucity of data on adverse events, in particular sepsis and bleeding. A very recent publication by Clarizia and associates [4] has reported a significant reduction in length of stay when 30 mg/kg methylprednisolone was given intraoperatively, and a further reduction in the duration of mechanical ventilation when additional corticosteroids were given preoperatively. These were consistent with

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