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Reduction of inflammation by high-dose methylprednisolone does not attenuate oxidative stress in children undergoing bidirectional Glenn procedure with or without aortic arch or pulmonary arterial repair

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Abstract

Objective: Corticosteroids attenuate inflammatory reaction in pediatric heart surgery. Inflammation is a source of free oxygen radicals. Children with a cyanotic heart defect are prone to increased radical stress during heart surgery. We hypothesized that high-dose methylprednisolone reduces inflammatory reaction and thereby also oxidative stress in infants with a univentricular heart defect undergoing bidirectional Glenn procedure.

Design: A double-blind, placebo-controlled, randomized clinical trial.

Setting: Operation theatre and pediatric intensive care unit of a university hospital.

Participants: Twenty-nine infants undergoing bidirectional Glenn procedure with or without aortic arch or pulmonary arterial repair.

Interventions: After anesthesia induction, the patients received intravenously either 30 mg/kg of methylprednisolone (n=15) or the same volume of saline as placebo (n=14).

Measurements and Main Results: Plasma interleukin-6, interleukin-8 and interleukin-10 (biomarkers of inflammation) as well as 8-hydroxydeoxyguanosine concentrations (a biomarker of oxidative stress) were measured at four different time points: preoperatively, during CPB, after protamine administration, and six hours postoperatively. The study parameters did not differ between the study groups preoperatively. Methylprednisolone reduced the pro-inflammatory cytokines interleukin-6 and interleukin-8 and increased the anti-inflammatory cytokine interleukin-10 postoperatively. Despite reduced inflammation, there were no differences in 8-hydroxydeoxyguanosine between the methylprednisolone and placebo groups.

Conclusions: Pro-inflammatory reaction and increase in free radical stress were not interrelated during congenital heart surgery in cyanotic infants with a univentricular heart defect undergoing bidirectional Glenn procedure. High-dose methylprednisolone was ineffective in attenuating free radical stress.

Key words: infant, congenital heart defect, bidirectional Glenn procedure, methylprednisolone, radical stress, inflammation

Introduction

Corticosteroids are used in pediatric cardiac surgery to attenuate the systemic inflammatory reaction caused by cardiopulmonary bypass (CPB) and surgery.[1] In randomized studies, corticosteroids have also been reported to have cardioprotective effects.[2-4] On the other hand, steroids have many side effects and may even be associated with increased morbidity, particularly in lower-risk patients.[5] The overall benefit of perioperative steroids remains controversial. Biochemical evaluation of corticosteroid effects in pediatric heart surgery is mainly based on measurement of pro- and anti-inflammatory cytokines. Cytokines, however, are merely mediators/regulators but not effectors of pathophysiological processes. Biochemical mechanisms relevant to tissue destruction and organ dysfunction should be studied for better understanding of corticosteroid effects in pediatric heart surgery. We have previously shown that high-dose methylprednisolone (MP) reduces activation of neutrophils, which are important first line actors in ischemia/reperfusion injury.[6] Furthermore, we have observed that MP conserves endothelial glycocalyx, a major determinant of capillary permeability and tissue edema.[7]

The overall prognosis for congenital heart diseases has improved. Children with the single ventricle circulation treated with palliative surgery are still those associated with significant morbidity and considerably higher risk of mortality than children who undergo other types of cardiac surgery.[8] As one pathophysiological mechanism of morbidity, CPB is associated with production of free oxygen radicals that cause degradation of macromolecules and tissue destruction.[9] The radical stress during cardiac surgery is stronger in children with cyanotic than non-cyanotic cardiac defects.[10,11] In children with a univentricular heart defect, the extent of lipid peroxidation correlates inversely

with postoperative lung function.[12] In cyanotic patients, compared to hyperoxia, normoxia during CPB reduces not only lipid peroxidation but also biomarkers of heart, brain and liver damage.[10,11,13]

Free oxygen radicals are derived from molecular oxygen with one unpaired electron, and can oxidize mitochondrial and nuclear DNA during ischemic conditions.[14] When guanine is oxidized to 7,8-dihydro-8-oxoguanine it can cause carcinogenic transversion mutations, as it can pair up with both adenine and cytosine.[15] Utilizing rapid repair mechanisms to maintain their genomic integrity, cells recognize and excise 7,8-dihydro-8-oxoguanine by OGG1 gene-encoded 8-oxoguanine DNA glycosylase. As a result, DNA is repaired. The stable end-product, 8-hydroxydeoxyguanosine (8-OHdG), can be reliably measured as it is transferred out of the cell and ultimately to the urine.[16]

Inflammation is also associated with increased oxidative stress. Unlike mitochondrial free radicals produced in electron transport chain during ischemia/reperfusion injury, immune activated cells actively generate reactive oxygen species *via* the nicotinamide adenine dinucleotide phosphate oxidase system.[17] Surgical repair for congenital cardiac malformations results in vascular inflammation, endothelial dysfunction, and subsequent imbalance in cellular redox regulating enzymes leading to increased oxidative stress.[18,19] MP reduces the pro-inflammatory reaction in pediatric heart surgery.[4,20] In experimental conditions, MP increases cardiac anti-oxidative capacity.[21] We hypothesized that high-dose MP reduces inflammatory reaction and thereby also oxidative stress in children with a univentricular heart defect undergoing bidirectional Glenn procedure.

Methods

Ethics and Informed Consent

The study protocol was approved by the Ethics Committee of Helsinki University Hospital and by the Finnish Medicines Agency. It was registered in the European Union Drug Regulating Authorities Clinical Trials (Eudra-CT 2008-007413-76). The study was conducted according to the declaration of Helsinki. Written informed consent was obtained from the parents of each participating patient before the study commenced.

Study Design

In this randomized (sealed envelope) double-blind study, thirty infants of 2-12 months of age undergoing bidirectional Glenn procedure for palliation of a univentricular heart defect were administered with either 30 mg/kg of intravenous MP or an equal volume of saline as placebo at anesthesia induction. There was one dropout because of cancellation of surgery after randomization. Exclusion criteria were preoperative steroid treatment and prematurity under gestational age of 36 weeks. The study drug was administered after anesthesia induction and collection of the first study plasma sample. A pharmacist, not otherwise involved in the patient care, prepared the study drug solutions. All personnel at the operation theatre, intensive care unit (ICU) and ward were blinded against the allocation of the patients to the study groups. The syringes of the study drug were covered with non-transparent paper foil. No additional perioperative steroids were given to study patients.

Intraoperative Management

Balanced general anesthesia was attained with sufentanil, pancuronium, propofol or s-ketamine and sevoflurane. All operations were undertaken using CPB. Myocardial protection and CBP were accomplished by using the methods described previously.[22] During CPB, partial oxygen tension (pO2) in the arterial line was maintained above 12 kPa. In the pediatric intensive care unit (PICU), arterial oxygen saturation (spO2) was either above 80% or achieved the presumed optimal target of the corrected cardiac defect. However, both during CPB and in the PICU, hyperoxia above the target value was avoided. Eight patients (four in both study groups) received cardioplegia following aortic cross clamp. In these patient, 1 ml/kg of mannitol was administered at the time of aortic declamping. Antegrade cerebral perfusion was used for two patients (one in each study groups) during aortic arch correction. Aortic cross clamp was not used for 21 patients. Four mg/kg of sodium heparin was used for anticoagulation before CPB and the Hepcon HMS Plus (Medtronic, Minneapolis, MN) was used to obtain the target heparin concentration of 6 IU/ml during CPB. Protamine sulphate was used to reverse the anticoagulant effect of heparin after weaning from CPB. All study patients received a bolus of 30000 IU/kg of aprotinin before CPB and a continuous infusion of 30000 IU/kg/hour during CPB.

Milrinone was used as the first line inotropic drug. Epinephrine and norepinephrine were added to milrinone for hemodynamic support if needed. Levosimendan was used in two patients in the MP group and in one patient in the placebo group. One patient in both groups received phenylephrine and one patient in the MP group received vasopressin for unstable hemodynamics. The inotropic score was calculated as previously described.[22] Inhaled nitric oxide was administered to five patients in the MP and to seven patients in the placebo group. Insulin was administered at ICU according to the decision of the clinician on duty. In general, insulin infusion was started when blood glucose was more than 12 mmol/l in two consecutive measurements.

Blood samples

Five ml of arterial blood were collected into tubes containing sodium citrate at four different time points: T1) after anesthesia induction before administration of study drug ("preop"), T2) 30 minutes after initiation of CPB ("on CPB"), T3) five minutes after administration of protamine ("after CPB") and T4) six hours after cessation of CPB ("6 h"). Plasma was separated immediately by centrifugation and stored at –70 ºC until analysis. At time points T1 to T4, Interleukin-6 (IL-6), IL-8 and IL-10 (Quantikine, R&D Systems, Abington, UK) and 8-hydroxydeoxyguanosine (Highly Sensitive 8-OHdG Check ELISA kit, The Japan Institute for the Control of Aging, Fukuroi, Japan) were determined using commercial ELISA kits following manufacturer's instructions. Blood glucose was measured for study purposes at time points T1 to T4 and at 6 a.m. on the first postoperative morning.

Statistical analysis

At the time of the study design, previous data were available on corticosteroid treatment in pediatric congenital heart surgery using IL-6 as an index of the inflammatory reaction.[23] In that study, IL-6 was measured using a commercial ELISA kit of the same manufacturer as in the present study. As in two previous MP trials of our research group,[4,24] power analysis indicated that 12 patients would be required to demonstrate a 20% difference in IL-6 values in either direction between the two study groups (α=0.05, 1-β=0.80). The applied power analysis is for t-test and assumes normal distribution. For the Mann-Whitney test 10% increase in the patient number is needed.[25] We used non-parametric tests because of the small sample size. Changes as a function of time within a group were tested with Friedman's test. Differences between the groups were tested with Mann-Whitney U test. The χ^2 test was used for comparison of frequencies between the groups. In comparison of cytokine and 8-hydroxydeoxyguanosine concentrations between the study groups, Bonferroni-Holm correction was used due to the three (interleukins and 8-

hydroxydeoxyguanosine) or four (blood glucose) postoperative sampling time points. Otherwise, p-values less than 0.05 were considered as statistically significant. Data are expressed as medians and interquartile ranges (IQR) or patient numbers or depicted as box plots. SPSS 25 for Windows (SPSS Inc., Chicago, IL, USA) was used for data analyses.

Results

There were no significant differences in the demographic and operative data between the MP and placebo groups (Table 1). Insulin infusion was started in 3 patients in the MP group and no patients in the control group (p=0.08). Blood glucose levels were significantly higher in the MP than the placebo group at time points T2 [10.0 (8.4-11.7) mmol/L vs. 7.2 (6.2-8.5) mmol/L, p=0.001], T3 [11.7 (8.3-12.9) mmol/L vs. 6.6 (5.5-8.8) mmol/L, p=0.001], T4 [7.8 (6.1-10.8 mmol/L vs.5.6 (5.0-6.3) mmol/L, p=0.006] and the first postoperative morning [12.8 (10.8-15.5) mmol/L vs. 10.1 (8.9-11.9) mmol/L, p=0.006] but not preoperatively [4.7 (4.6-5.3) mmol/L vs. 4.7 (4.5-4.9) mmol/L, p=0.40]. Also, leukocyte levels were higher in the MP group at PICU arrival and the first postoperative morning (Table 2). There were no differences in other clinical and physiological parameters between the study groups (Table 2).

There were no statistically significant differences in plasma cytokine concentrations preoperatively (Table 3). Plasma concentrations of all cytokines increased significantly in both study groups (all p<0.001 in Friedman's test, Table 3). Plasma concentrations of the pro-inflammatory cytokines IL-6 and IL-8 were higher and those of the anti-inflammatory cytokine IL-10 were lower in the placebo than the MP group (Table 3).

There were no statistically significant differences in plasma 8-hydroxydeoxyguanosine concentrations preoperatively (Fig. 2). 8-hydroxydeoxyguanosine concentrations increased significantly as a function of time in both study groups (p<0.001 for MP and p=0.002 for placebo in Friedman's test, Fig. 1). At six hours postoperatively, 8-hydroxydeoxyguanosine concentrations tended to be higher in the MP than the placebo group (p=0.027) but the difference did not reach statistical significance level of p<0.017 after Bonferroni-Holm correction that was conducted due to three repeated postoperative measurements.

Additionally, we conducted statistical analyses among patients with isolated BDG (13 patients in MP group and 8 patients in placebo group, Table 3). The results of cytokine concentrations as well as blood glucose and leukocyte levels in the isolated BDG patients were comparable with the results of all the patients. Likewise, there were no statistically significant differences in plasma 8-hydroxydeoxyguanosine concentrations between the study groups although the concentrations were slightly higher in the MP groups (data not shown).

Discussion

In terms of pro- and anti-inflammatory cytokines, high dose MP resulted in a substantially reduced inflammatory reaction in our patients undergoing BDG procedure. Reduced inflammation, however, did not lead to decreased radical stress. The observation is contradictory to our original hypothesis but eventually not surprising in the light of existing literature. Dissociation of radical stress from inflammation has previously been reported in pediatric cardiac surgical patients. In cyanotic children, hyperoxia, compared with normoxia, increased radical stress measured as 8-isoprostane while cytokines IL-6, IL-8 and IL-10 as well as the complement degradation product C3a remained unchanged.[13] In oxidative stress, ascorbate/dehydroascorbate redox state is shifted towards oxidized dehydroascorbate,[26] and malondialdehyde as a toxic, reactive compound is produced from polyunsaturated fatty acids.[27] In children with various cyanotic and non-cyanotic cardiac defects, decreased ascorbate, increased dehydroascorbate and increased malondialdehyde plasma concentrations occurred at the time of aortic cross-clamp removal several hours before the peak of the pro-inflammatory cytokines IL-6 and IL-8.[28] Taken together, the association of radical stress with inflammation is not straightforward.

There are only few studies on the effects of corticosteroids on radical stress in cardiac ischemia/reperfusion injury. Administration of MP before cardiac ischemia improved tissue antioxidative capacity during reperfusion in the *ex vivo* model of Langendorff perfused heart.[21] The results in a clinical scenario have been more modest. In adult patients undergoing coronary artery bypass grafting, MP reduced only cardiac lipid peroxidation measured as the trans-coronary difference in concentrations of plasma malondialdehyde but did not have any effect on oxidative state of glutathione, oxidative modification of proteins or postoperative occurrence of arrhythmias.[29] In our patients, MP did not reduce radical stress measured by plasma concentrations of 8-hydroxydeoxyguanosine, a specific and stable marker of hydroxyl radical mediated DNA damage.[30] Conspicuously at six hours postoperatively there was a non-significant tendency of radical stress to be higher in the MP group than the placebo group. In accordance, an extensive meta-analysis

showed increased oxidative stress in vertebrates after glucocorticoid treatment.[31]

As the weaknesses of the present study, the power analysis was based on plasma IL-6 concentrations. There are not applicable published data on 8-hydroxydeoxyguanosine concentrations in the present clinical context. Thus, the obtained results of 8-hydroxydeoxyguanosine may be hampered by insufficient statistical power. Still, because 8-hydroxydeoxyguanosine tended to be higher in the MP than the control group, it can fairly safely be concluded that with the used biomarker, MP was at least ineffective in reducing radical stress in our patients. Due to small patient number, we could not evaluate the effect of MP on meaningful clinical outcomes. Furthermore, aprotinin was administered in every patient. Because aprotinin possesses anti-inflammatory properties besides the anti-fibrinolytic effect, it may have influenced on the results. Relatively homogenous patient selection is a strength of this double-blinded, placebo-controlled, randomized clinical study. Most clinical trials on corticosteroids in pediatric heart surgery are hampered with vast age range and combination of various congenital heart defects of the patients. The age range (2-12 months) of our patients was fairly narrow. There was, however, heterogeneity also in the present study, because half of the patients underwent either aortic arch or pulmonary arterial correction beyond isolated bidirectional Glenn procedure Still, the intervention arms were well in balance with major surgical and therapeutic procedures, such as the use of aortic cross clamp, antegrade cerebral perfusion and vasoactive drugs. Finally, children with a cyanotic heart defect are especially susceptible to radical stress in heart surgery.[10,11] Thus, the chosen patient cohort is relevant for the present study.

As a conclusion, despite substantial reduction of the pro-inflammatory reaction, high-dose MP was ineffective in attenuating free radical stress in a patient group prone to oxidative stress, i.e. cyanotic infants undergoing bidirectional Glenn procedure. According to the present results, reduced free radical stress probably is not a potential therapeutic biochemical mechanism of corticosteroid treatment. The overall benefit of corticosteroids in pediatric heart surgery is controversial. Two ongoing large-scale trials (Clinicaltrials.gov: NCT01579513 and NCT03229538) will provide us more information to this intriguing question.

References

1. Checchia PA, Bronicki RA, Costello JM, et al. Steroid use before pediatric cardiac operations using cardiopulmonary bypass: an international survey of 36 centers. Pediatr Crit Care Med 2005;6:441-444.
2. Checchia PA, Backer CL, Bronicki RA, et al. Dexamethasone reduces postoperative troponin levels in children undergoing cardiopulmonary bypass. Crit Care Med 2003;31:1742-1745.
3. Malagon I, Hogenbirk K, van Pelt J, et al. Effect of dexamethasone on postoperative cardiac troponin T production in pediatric cardiac surgery. Intensive Care Med 2005;31:1420-1426.
4. Keski-Nisula J, Suominen PK, Olkkola KT, et al. Effect of timing and route of methylprednisolone administration during pediatric cardiac surgical procedures. Ann Thorac Surg 2015;99:180-185.
5. Pasquali SK, Hall M, Li JS, Peterson ED, et al. Corticosteroids and outcome in children undergoing congenital heart surgery: analysis of the Pediatric Health Information Systems database. Circulation 2010;122:2123-2130.
6. Pesonen EJ, Suominen PK, Keski-Nisula J, et al. The effect of methylprednisolone on plasma concentrations of neutrophil gelatinase-associated lipocalin in pediatric heart surgery. Pediatr Crit Care Med 2016;17:121-127.
7. Pesonen E, Keski-Nisula J, Andersson S, et al. High-dose methylprednisolone and endothelial glycocalyx in paediatric heart surgery. Acta Anaesthesiol Scand 2016;60:1386-1394.
8. Pagel C, Brown KL, Crowe S, et al. A mortality risk model to adjust for case mix in UK paediatric cardiac surgery. Southampton (UK): NIHR Journals Library; 2013 May, Health Services and delivery research, No. 1.1

9. Pesonen EJ, Korpela R, Leijala M, et al. Prolonged granulocyte activation, as well as hypoxanthine and free radical production after open heart surgery in children. Intensive Care Med 1996;22:500-506.
10. Allen BS, Rahman S, Ilbawi MN, et al. Detrimental effects of cardiopulmonary bypass in cyanotic infants: preventing the reoxygenation injury. Ann Thorac Surg 1997;64:1381-1387.
11. Bulutcu FS, Bayindir O, Polat B, et al. Does normoxemic cardiopulmonary bypass prevent myocardial reoxygenation injury in cyanotic children? J Cardiothorac Vasc Anesth 2002;16:330-333.
12. Albers E, Donahue BS, Milne G, et al. Perioperative plasma F(2)-Isoprostane levels correlate with markers of impaired ventilation in infants with single-ventricle physiology undergoing stage 2 surgical palliation on the cardiopulmonary bypass. Pediatr Cardiol 2012;33:562-568.
13. Caputo M, Mokhtari A, Rogers CA, et al. The effects of normoxic versus hyperoxic cardiopulmonary bypass on oxidative stress and inflammatory response in cyanotic pediatric patients undergoing open cardiac surgery: a randomized controlled trial. J Thorac Cardiovasc Surg 2009;138:206-214.
14. Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol 2003;552:335-344.
15. Greco NJ, Sinkeldam RW, Tor Y. An emissive C analog distinguishes between G, 8-oxoG, and T. Org Lett 2009;11:1115-1118.
16. Ba X, Aguilera-Aguirre L, Rashid QT, et al. The role of 8-oxoguanine DNA glycosylase-1 in inflammation. Int J Mol Sci 2014;15:16975-16997.
17. Lam GY, Huang J, Brumell JH. The many roles of NOX2 NADPH oxidase-derived ROS in immunity. Semin Immunopathol 2010;32:415-430.
18. Siti HN, Kamisah Y, Kamsiah J. The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review). Vascul Pharmacol 2015;71:40-56.
19. Sznycer-Taub N, Mackie S, Peng YW, et al. Myocardial oxidative stress in infants undergoing cardiac surgery. Pediatr Cardiol 2016;37:746-750.

20. Keski-Nisula J, Pesonen E, Olkkola KT, et al. Methylprednisolone in neonatal cardiac surgery: reduced inflammation without improved clinical outcome. Ann Thorac Surg 2013;95:2126-2132.
21. Valen G, Kawakami T, Tähepôld P, et al. Pretreatment with methylprednisolone protects the isolated rat heart against ischaemic and oxidative damage. Free Radic Res 2000;33:31-43.
22. Suominen PK, Keski-Nisula J, Tynkkynen P, et al. The effect of tepid amino acid-enriched induction cardioplegia on the outcome of infants undergoing cardiac surgery. Perfusion 2012;27:338-344.
23. Schroeder VA, Pearl JM, Schwartz SM, et al. Combined steroid treatment for congenital heart surgery improves oxygen delivery and reduces postbypass inflammatory mediator expression. Circulation 2003;107:2823-2828.
24. Keski-Nisula J, Pesonen E, Olkkola KT, et al. High-dose Methylprednisolone has no benefit over moderate dose for the correction of tetralogy of Fallot. Ann Thorac Surg 2016;102:870-876.
25. Lehmann E. Nonparametrics: Statistical methods based on ranks. New York: McGraw-Hill, 1975, pp 69-71.
26. Do H, Kim IS, Jeon BW, et al. Structural understanding of the recycling of oxidized ascorbate by dehydroascorbate reductase (OsDHAR) from Oryza sativa L. japonica. Sci Rep 2016;6: 19498.
27. Gaschler MM, Stockwell BR. Lipid peroxidation in cell death. Biochem Biophys Res Commun. 2017;482:419-425.
28. Christen S, Finckh B, Lykkesfeldt J, et al. Oxidative stress precedes peak systemic inflammatory response in pediatric patients undergoing cardiopulmonary bypass operation. Free Radic Biol Med 2005;38:1323-1332.

29. Volk T, Schmutzler M, Engelhardt L, et al. Effects of different steroid treatment on reperfusion-associated production of reactive oxygen species and arrhythmias during coronary surgery. Acta Anaesthesiol Scand 2003;47:667-674.
30. Chiou CC, Chang PY, Chan EC, et al. Urinary 8-hydroxydeoxyguanosine and its analogs as DNA marker of oxidative stress: development of an ELISA and measurement in both bladder and prostate cancers. Clin Chim Acta 2003;334:87-94.
31. Costantini D, Marasco V, Møller AP. A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. J Comp Physiol B 2011;181:447-456.

Figure legend

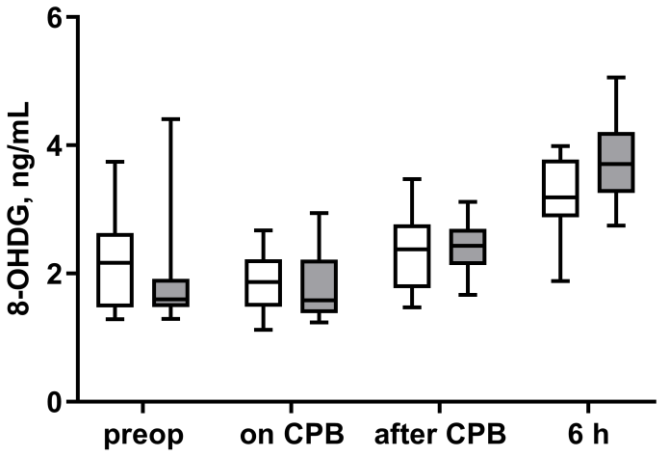

Figure 1. Plasma concentrations of 8-hydroxydeoxyguanosine in the control (white bars) and the MP (grey bars) groups preoperatively (preop), at 30 min after start of CPB (on CPB), 5 minutes after protamine (after CPB) and 6 hours postoperative (6 h). No statistically significant differences were observed between the study groups after Bonferroni-Holm correction of repeated postoperative measurements (see the Results). Data are expressed as box plots (median and interquartile range) with whiskers (minimum and maximum).

Table 1. Patient demographic and operative data.

	MP 30 mg/kg **N=15**	**Control** **N=14**	**p-value**
Age (months)	6.0 (4.8-8.3)	6.7 (5.6-8.1)	0.74
Weight (kg)	6.8 (6.1-7.8)	7.2 (5.8-8.4)	0.41
Male/female (n)	10/5	7/7	0.36
CPB support time (min)	55 (46-103)	66 (59-81)	0.20
Aortic cross clamp (n)	4	4	1.0
Aortic cross clamp time (min)	20 (7-64)	49 (22-89)	0.31
ACP (n)	1	1	1.0
ACP time (min)	19	57	
Lowest temperature (°C)	34.0 (32.0-34.0)	34.0 (31.5-35.0)	0.79
Cardiac defects			
HLHS	8	8	
TA	1	1	
Other UVH	6	5	
Surgical correction			0.07
BDG (n)	13	8	
BDG and aortic arch reconstruction (n)	2	1	
BDG and LPA / RPA reconstruction (n)	0	4	

BDG and CoA angioplasty (n)	0	1	
Primary sternal closure (n)	14	13	0.85
Late re-sternotomy (n)	1	0	0.33

Values are medians and interquartile ranges. Abbreviations: ACP, antegrade cerebral perfusion; BDG, bidirectional Glenn procedure; CoA, coarctation of the aorta; CPB, cardiopulmonary bypass; HLHS, hypoplastic left heart syndrome; MP, methylprednisolone; LPA, left pulmonary artery; RPA, right pulmonary artery; TA, tricuspid valve atresia; UVH, univentricular heart.

Table 2. Clinical data.

	MP 30 mg/kg **N=15**	**Control** **N=14**	**p-value**
Arrival to PICU			
Lactate (mmol/L)	1.3 (0.9-1.8)	1.1 (0.9-1.5)	0.25
White blood cell count (E^9/L)	9.6 (7.8-12.3)	7.65 (6.0-9.5)	0.05
Rectal temperature (^{o}C)	36.3 (35.6-36.5)	36.0 (35.5-36.5)	0.26
Inotropic score	15.0 (13.0-25.0)	17.0 (11.8-22.5)	0.79
Central venous saturation (%)	55.9 (45.3-57.7)	46.4 (37.5-59.3)	0.62
The first postoperative day #			
Lactate (mmol/L)	1.0 (0.7-1.4)	1.1 (0.7-1.5)	0.60
White blood cell count (x10^9/L)	12.8 (10.8-15.5)	10.1 (8.9-11.9)	0.01
Rectal temperature (^{o}C)	37.6 (37.3-38.2)	38.1 (37.5-38.5)	0.17
Inotropic score	5.0 (5.0-7.0)	5.0 (5.0-6.8)	0.91
Central venous saturation (%)	55.8 (46.0-59.9)	59.7 (45.9-63.2)	0.37

NT-proBNP (ng/L)	4332 (1952-11172)	2362 (1518-9735)	0.33
Ventilatory treatment (days)			
PICU stay (days)	2 (2-4)	2 (2-5)	0.96
PICU mortality	1	0	0.33

#Measured on the first POD at 6:00 a.m., except inotropic score was calculated at 12:00 a.m.

Values are medians and interquartile ranges. Abbreviations: NT-proBNP, N-terminal pro-B-type natriuretic peptide; PICU, Pediatric Intensive Care Unit.

Table 3. Plasma cytokine concentrations.

	MP 30 mg/kg n=15	**control n=14**	**p-value**
IL-6 (pg/mL)			
T1 (preop)	2.6 (1.8-3.0)	2.8 (2.2-6.7)	0.35
T2 (on CPB)	2.3 (1.8-4.8)	5.5 (2.7-8.3)	0.03*
T3 (after CPB)	2.6 (0.7-5.6)	5.4 (3.3-19.4)	0.02*
T4 (6 h)	38.9 (31.2-53.8)	448.1 (140.4-627.2)	<0.001*
IL-8 (pg/mL)			
T1 (preop)	14.0 (11.7-17.2)	14.1 (10.7-18.5)	0.91
T2 (on CPB)	18.3 (13.7-21.0)	19.9 (17.2-23.7)	0.11
T3 (after CPB)	34.4 (25.0-45.3)	62.7 (41.8-119.5)	0.02*
T4 (6 h)	24.5 (22.5-46.5)	59.6 (50.1-113.9)	<0.001*
IL-10 (pg/mL)			
T1 (preop)	0.5 (0.0-1.7)	0.5 (0.0-1.7)	0.85
T2 (on CPB)	16.2 (7.6-45.1)	1.7 (0.9-5.6)	<0.001*
T3 (after CPB)	342.0 (183.6-990.4)	25.7 (9.4-57.4)	<0.001*
T4 (6 h)	82.2 (27.4-97.0)	20.6 (7.6-35.6)	0.001*

*, statistically significant after Bonferroni-Holm correction of repeated measure at the three postoperative time points.