Gut permeability and myocardial damage in paediatric cardiac surgery

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Dedicated to Minke, Clara and Elias

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CHAPTER 1

INTRODUCTION

The use of steroids has attracted considerable attention in several areas of acute medicine, including acute septic shock and cardiopulmonary bypass. There are remarkable similarities in the pathophysiology of these two events, and after nearly forty years, the use of steroids for both indications remains highly controversial. Steroids have proven to be beneficial in only a few specific pathologies, bacterial meningitis in children[1], meningitis in adults[2], severe typhoid fever[3], late acute respiratory distress syndrome[4], *Penumocystis carinii* pneumonia in acquired immunodeficiency syndrome[5] and adrenal insufficiency.[6]

Cardiopulmonary bypass (CPB) induces a systemic inflammatory response syndrome (SIRS) in patients following cardiac surgery that can lead to major organ injury and postoperative morbidity. Initiation of CPB sets in motion an extremely complex and multifaceted response involving complement activation along with activation of platelets, neutrophils, monocytes and macrophages. These changes initiate the coagulation, fibrinolytic and kallikrein cascades, increasing blood levels of various endotoxins, cytokines and increasing endothelial cell permeability.

The physiological insults caused by CPB have been associated with major postoperative morbidity, including neurological, pulmonary and renal dysfunction, and/or haematological abnormalities. Additional clinical manifestations associated with the SIRS include increased metabolism (fever), fluid retention, myocardial oedema and detrimental haemodynamic changes.

The use of steroids began in the mid 1960s, following reports of their beneficial effects in septic shock. In 1974, Weitzman and Berger[7] systematically reviewed 32 original clinical investigations published between 1950 and 1971 that addressed the use of corticosteroids in bacterial infections. Of the 12 studies involving septic shock, nine advocated the use of corticosteroids and three concluded that steroids were not beneficial.

Two years later, Schumer[8] published a report that demonstrated very impressive reductions in mortality in patients with septic shock treated with corticosteroids. Mortality rates of 11.6% and 9.3% were reported for patients treated with methylprednisolone (30 mg/kg iv) and dexamethasone (3 mg/kg iv) respectively, versus 38.4% for patients receiving placebo. Based on this report, it became standard practice in the late 1970s and early 1980s to administer high-dose corticosteroids at the onset of septic shock.

In the 1980s there were several well-designed single centre studies as well as two large multicentre clinical trials. In an open label trial, Sprung and colleagues[9] randomized 59 patients to receive methylprednisolone, 30 mg/kg iv, dexamethasone 6 mg/kg iv or placebo. Objective criteria for septic shock closely approximated current consensus criteria, and outcome measures were well established. They demonstrated more rapid shock reversal as well as improved survival at 6 days after drug administration with corticosteroids. This survival benefit disappeared, however, when patients were followed up beyond 10 days. In another single centre study, Luce and colleagues[10] found no improvement in survival or ARDS in a double blind comparison of methylprednisolone versus placebo in 75 patients with septic shock.

The results of two large multicentre clinical trials of steroids in sepsis were published in 1987. In the Veteran Administration trial,[11] 233 patients were randomized to receive methylprednisolone (30 mg/kg iv) or placebo within three hours of diagnosis. No difference in 14-day mortality or complications was demonstrated. In the largest clinical trial, Bone and colleagues[12] randomized 381 patients to receive methylprednisolone (30 mg/kg iv) or placebo. Patients who received methylprednisolone had a higher mortality rate than those in the placebo group. As a result of these multicentre trials, the use of corticosteroids for septic shock fell out of favour. Reviews and critical care textbooks in the 1990s have generally cautioned against the use of supraphysiological doses of corticosteroids in septic shock.

In 2003, critical care and infectious disease experts representing 11 international organizations developed management guidelines for the use of corticosteroid therapy in patients with sepsis and septic shock that would be of practical use for the bedside clinician.[13] *Among their recommendations it is clearly stated that the use of corticosteroids in high doses is strongly discouraged.*

Interestingly, the use of steroids in cardiac surgery developed in a similar pattern to that in sepsis. Following animal studies carried out in the 1960s, methylprednisolone became the drug of choice because of its anti-inflammatory potency and minimal tendency to induce sodium and water retention. An intravenous dose of 30 mg/kg was at that time considered optimal because this had been shown to be beneficial in clinical shock studies,[14] yet caused no detrimental systemic effects when administered to a small group of healthy volunteers.[15]

A seminal study published in 1970 by Dietzmanand and colleagues[16] reported that methylprednisolone (30 mg/kg) was effective in treating the low output syndrome in dogs and humans following cardiac surgery. Specifically, in 98 dogs, methylprednisolone administration decreased systemic vascular resistance, increased cardiac index, improved tissue perfusion, and increased survival from 22 to 65%. In 19 humans following cardiac valve replacement the same beneficial haemodynamic effects were observed.

In the early 1980s the pivotal role that complement activation played in the basic physiological insults caused by CPB was demonstrated. This triggered a number of investigations focusing on the effect of steroids on post-bypass complement activation and cytokines production. An overwhelming number of these studies demonstrated that the use of steroids was associated with a considerable reduction of proinflammatory cytokines production in the postoperative period.

However we had to wait another decade to see investigators moving away from biochemical parameters and focusing on clinical outcome. Tassani and colleagues[17] showed in 1999 that patients given methylprednisolone had better haemodynamic parameters in the postoperative period although extubation time was not affected. More recently Yared and colleagues[18] reported, in a study involving more than 200 patients, that giving dexamethasone before CPB started was associated with earlier tracheal extubation than the placebo group. In contrast to these two previous studies Chaney and colleagues[19,20] demonstrated in two prospective randomized trials that the use of methylprednisolone was associated with delayed tracheal extubation and was not associated with any haemodynamic improvements.

In paediatric cardiac surgery the use of steroids has not been investigated to the same extent as in adults, and its use is as controversial. Lindberg and colleagues[21] considered that it was unethical not to use dexamethasone in children weighing less than 10 kg scheduled for cardiac surgery. Schroeder and colleagues[22] accept that the lack of a control group without methylprednisolone is a limiting factor in their study. According to them it is standard practice to use steroids in these patients. Dexamethasone appears to reduce postoperative troponin I production.[23] It has also been shown to reduce the production of C-reactive protein without any effect on the release of protein S100B and Von Willebrand factor.[21] The concentration of proinflammatory cytokines decreases when steroids are used before CPB starts.[24] The reduction is even more remarkable if steroids are given before and during CPB.[22]

Oxygen delivery and cardiac output improve faster when steroids were used in an animal model.[25] Even the timing of the administration seems to be relevant.[26] However, when clinical end points have been used to test the benefits of steroids the results are not so impressive.[27]

Our aim in this thesis was to investigate how dexamethasone could influence the side effects associated with CPB in two organs, the small intestine and the heart. To that purpose we chose two surrogate markers, gut permeability and cardiac troponin T production.

The dual sugar permeability test to assess gut permeability was introduced in the 1970s to overcome the problems associated with the use of single markers. After thirty years it has stood the test of time and remains in use for clinical and research purposes. Gut permeability had not been investigated in paediatric patients undergoing cardiac surgery. Before examining the effect of dexamethasone on gut permeability we first had to evaluate the changes in intestinal permeability during the perioperative period in patients undergoing surgery with and without cardiopulmonary bypass. This study is presented in chapter two.

Proinflammatory cytokines have a deleterious effect on the intestinal barrier when studied *in vitro*.[28] In the same *in vitro* model, when the intestinal mucosa is exposed to anti-inflammatory cytokines, the gap between the epithelial cells of the intestinal mucosa improves.[29] Animal studies have shown that steroids accelerate the maturation and stimulate the growth of the intestinal mucosa in ex-premature animals and it has long been accepted that steroids given to the pregnant mother reduce the risk of necrotizing enterocolitis in the premature baby.

Chapter three describes the effect of dexamethasone on gut permeability when administered during induction of anaesthesia in paediatric patients undergoing cardiac surgery with cardiopulmonary bypass. The results of this study confirm what has been already shown in *in vitro* studies; although the mechanism is not clear, dexamethasone reduces gut permeability when given before CPB starts.

Neonates with a hypoplastic left heart syndrome undergoing stage I of the Norwood operation are at higher risk of intestinal ischaemia in the perioperative period. In these patients the systemic and pulmonary circulations depend on one functioning ventricle.

This can expose the patient to periods when there is an imbalance between the systemic and pulmonary circulations, with excessive pulmonary flow to the detriment of mesenteric perfusion. The surgical procedure requires a period of circulatory arrest, adding an extra insult to the mesenteric circulation. Necrotizing enterocolitis is relatively uncommon in paediatric patients undergoing cardiac surgery, however mortality is nearly 100% in patients with HLHS.[30] It has been common practice in our institution to use steroids in these occasions only. We felt that preoperative and intraoperative insults to the intestinal mucosa in this group of patients warranted a separate investigation. The results are presented in chapter four.

Sometimes research produces totally unexpected results. Rhamnose is one of the saccharides used in the dual sugar permeability test. For the last thirty years it has been assumed that rhamnose was an inert sugar, which did not undergo any metabolism in humans. However we found increased concentrations of rhamnitol, a metabolite of rhamnose, in the urine of patients undergoing the DSPT. This is evidence that rhamnose is indeed metabolized by the human body and the results are presented in chapter five.

Cardiac troponin T (cTnT) is a specific marker of myocardial infarction.[31] It is also a reliable marker of myocardial injury in the paediatric population.[32] Before we investigated the effect of dexamethasone on postoperative release of cTnT another issue had to be addressed.

The anaesthetic agent used during the surgical procedure could influence cTnT concentrations postoperatively. Several studies have demonstrated that sevoflurane and other volatile anaesthetics reduce the postoperative production of cardiac troponin I when compared to other anaesthetic agents in adult patients undergoing coronary graft surgery.[33,34]

The theoretical explanation for this benefit can be found in the process of anaesthetic preconditioning. Exposing the adult myocardium to brief periods of ischaemia and reperfusion induces greater tolerance to a subsequent, more prolonged ischaemic insult, a phenomenon known as ischaemic preconditioning (IP). Animal experiments have shown that inhalational anaesthetics, morphine, and possibly other opioids mimic the effects of ischaemic preconditioning. This is often referred to as anaesthetic preconditioning. The results of our study and further discussion over the subject are presented in chapter six.

A possible consequence of the SIRS related to the use of CPB is myocardial damage. This can manifest itself as a low output syndrome with a need for high inotropic support in the postoperative period. cTnT concentrations rise up to three fold postoperatively in children undergoing cardiac surgery when compared to adults undergoing coronary bypass surgery.[35]

Human and animal studies have shown a reduction in cardiac troponin I concentrations after CPB related to the use of steroids.[36,37] The explanation for this is not totally clear. The use of cardiac troponin I in paediatric cardiac surgery is limited by the fact that for up to two years after birth in infants with congenital heart disease and for up to nine months after birth in healthy infants, troponin I is produced not only by myocardial muscle but also by skeletal muscle. Slow twitch skeletal muscle troponin I is expressed in variable amounts in these infants.[38]

When cTnT was used as an end point to test the effect of dexamethasone on myocardial protection, patients receiving dexamethasone before CPB had lower concentrations of cTnT in the postoperative period. However, these changes were short-lived and 24 h after admission to the intensive care unit there were no differences between the two groups. The subject is further discussed in chapter seven. In the same study we have shown that dexamethasone did not improve morbidity. Sixty-eight patients were needed to demonstrate a 50% reduction in the postoperative use of inotropic support or an 18 hours reduction in ventilator hours. However, the use of dexamethasone was not associated with any changes in postoperative ventilator hours or the amount of inotropic support needed in the postoperative period.

As one could expect this thesis provides no definitive answer to the use of dexamethasone (1 mg kg^{-1}) before CPB starts. On the one hand dexamethasone does not seem to provide any benefits in terms of lowering postoperative morbidity. On the other hand it does clearly reduce gut permeability in the postoperative period. Since intestinal complications in this group of patients are relatively rare, a sufficiently powered study to test the benefits of dexamethasone using intestinal complications as an end point would be difficult, if not impossible. However, mortality related to intestinal complications in this setting is so high that it is perhaps unethical to even consider further research.

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SECTION 1

Gut permeability and paediatric cardiac surgery

CHAPTER 2

Gut permeability in paediatric cardiac surgery

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Background: Intestinal mucosal ischaemia can occur in infants and children during and after cardiac surgery. Severe decreases in mucosal perfusion may be a causative factor for postoperative mortality or complications such as necrotizing enterocolitis. We have investigated gut permeability in paediatric patients undergoing cardiac surgery using the dual sugar permeability test and absorption of two other saccharides.

Methods: Thirty-four patients undergoing palliative or corrective surgical procedures with and without cardiopulmonary bypass were investigated. Intestinal permeability was measured using 3-O-methyl-D-glucose, D-xylose, L-rhamnose and lactulose, given orally after induction of anaesthesia and 12 and 24 hours later.

Results: Lactulose/Rhamnose ratios were increased from the outset, 0.39 (0.07-1.8) (median CI) for patients undergoing operations without cardiopulmonary bypass and 0.30 (0.02-2.6) with cardiopulmonary bypass. The highest Lactulose/Rhamnose ratios were recorded 12 hours after surgery 0.32 (0.07-6.9), when cardiopulmonary bypass was used. This is approximately seven times the value expected in healthy children. There was an improvement in patients not undergoing cardiopulmonary bypass: 0.22 (0.03-0.85) 12 hours and 0.11 (0-0.48) 24 hours after induction of anaesthesia. Patients undergoing repair of aortic coarctation showed the fastest recovery: 0.09 (0.03-0.31) 12 hours and 0.07 (0.04-0.35) 24 hours after induction of anaesthesia.

Conclusions: Patients with congenital heart defects have abnormal gut permeability when compared to healthy children of similar age. Cardiopulmonary bypass seems to affect the intestinal barrier morphologically (lactulose and rhamnose absorption) and functionally (3-O-methyl-D-glucose and D-xylose absorption).

Intestinal mucosal ischaemia, although transient, can occur in infants and children during and after cardiopulmonary bypass (CPB).[1] Severe decreases in mucosal perfusion may be a causative factor for postoperative mortality or complications such as necrotizing enterocolitis (NEC). NEC can have devastating consequences, e.g. in patients undergoing repair of hypoplastic left heart syndrome[2] (mortality more than 90%). Neonates with aortic arch anomalies and infants subjected to CPB-induced profound hypothermia may be at particular risk of developing splanchnic ischaemia in the perioperative period.[3] These studies used indirect indicators of intestinal mucosal perfusion (e.g. laser Doppler probe or gastric tonometry). Patients with coarctation of the aorta may, on the other hand, be exposed to reperfusion injuries after the surgical repair, manifesting as the postcoarctectomy syndrome.[4]

Intestinal permeability can be evaluated noninvasively by measuring the urinary excretion of orally administered water-soluble, non-degradable test molecules.[5,6] This barrier function test is based on the comparison of intestinal permeation of larger molecules with that of smaller molecules by measuring the ratio of their urinary excretion. These two types of molecules follow different routes of intestinal permeation: the larger molecules are assumed to permeate paracellularly, and the smaller molecules transcellularly.

Preabsorption factors such as gastric emptying, dilution by secretion and intestinal transit time, and post-absorption factors such as systemic distribution and renal clearance are assumed to affect both molecules equally. Four saccharides, 3-O-methyl-D-glucose (molecular weight 194 Da), D-xylose (molecular weight 150 Da), L-rhamnose (R, molecular weight 164 Da) and lactulose (L, molecular weight 342 Da) are employed to assess active carrier-mediated, passive carrier-mediated, transcellular, and paracellular transport, respectively in the small intestine.

Intestinal permeability is considered to be normal if the lactulose (% recovery)/rhamnose (% recovery) (L/R) ratio is below 0.05.[5] Intestinal absorptive capacity for saccharides is considered to be normal when the recoveries of D-xylose (passive carrier-mediated transport) and 3-O-methyl-D-glucose (active carrier–mediated transport) are around 10% and 30% respectively.[7]

The aim of our study was to observe the changes in gut permeability in paediatric patients undergoing cardiac surgery using the dual sugar permeability test (DSPT).

Materials and methods

After approval from the local ethics committee and informed consent from the parents or legal guardians, 34 patients were enrolled in this prospective, non-randomized observational study. Patients received a premedication consisting on oral atropine (0.02 mg kg^{-1}) and midazolam (0.5 mg kg^{-1}) 30 min before induction of anaesthesia. Anaesthesia was induced with sevoflurane followed by a bolus of sufentanil (1 μg kg^{-1}) and pancuronium (0.2 mg kg^{-1}). Maintenance of anaesthesia consisted on a combined continuous infusion of midazolam (0.2 mg kg^{-1} h^{-1}) and sufentanil (2 μg kg^{-1} h^{-1}). The lungs of the patients were mechanically ventilated with a mixture of oxygen and air. Mechanical ventilation was maintained until cardiopulmonary bypass (CPB) commenced. After heparin administration (3 mg kg^{-1}) and aorta cannulation, CPB was instituted with a Dideco hollow fibre oxygenator with a blood flow between 200 and 300 ml kg^{-1} min^{-1}. The prime volume, between 325 and 750 ml according to the patient's weight, contained lactate-free Ringer's solution, albumin, mannitol, blood and heparin. Patients underwent modified ultra-filtration at the end of the surgical procedure. The effect of heparin was reversed with protamine sulphate at a ratio of 1 mg protamine for 1 mg heparin.

After induction of anaesthesia, 2 ml kg^{-1} of the sugar solution was administered through a nasogastric tube. Urine was subsequently completely collected through a urinary catheter for 3 h, the total volume was recorded and samples were stored at -20° C until analysis. This process was repeated 12 and 24 h after induction of anaesthesia.

The sugar solution, prepared by the hospital pharmacy, contained 3-O-methyl-D-glucose (2 g $litre^{-1}$), D-xylose (5 g $litre^{-1}$), L-rhamnose (10 g $litre^{-1}$) and lactulose (50 g $litre^{-1}$). The osmolarity of the solution is approximately 240 mOsm l^{-1}.

Sugar concentrations in urine were determined by gas chromatography following a slight modification[8] of the procedure described by Jansen and colleagues.[9] Briefly, to an aliquot of urine corresponding to 0.5 μmol of creatinine 30 μg of ribitol and 10μg of trehalose (Sigma-Aldrich, St. Louis, USA) were added as internal standards (ribitol for the determination of 3-O-methyl-D-glucose, D-xylose and L-rhamnose and trehalose for lactulose) for the determination of 3-O-methyl-D-glucose, D-xylose, L-rhamnose and lactulose.

The sample was dried, derivatized with 300µl Tri-Sil TBT (Pierce, Rockford, USA) at 100° C and partly hydrolyzed with water. Subsequently the intact sugar trimethylsilyl (TMS) derivatives were extracted with hexane and after concentrating, gas chromatographic analysis was performed on a 30 m capillary fused silica HP-1 column (Agilent, Palo Alto, USA) using split injection. Quantification was performed after the construction of standard addition calibration curves.The type of vasoactive drugs and their amount were recorded after admission to the intensive care unit and 24 h later. To quantify inotropic support, inotrope scores were calculated as the sum of all inotrope doses correcting for potency (dopamine, dobutamine =1, milrinone = 15, epinephrine = 100).[10,11] Fluid intake (including crystalloids, colloids and blood products), output (urine, blood and serous fluid loss) and balance were recorded over a 36 h period following admission to the intensive care unit.

Statistical analysis

Data were analysed with the statistical package SPSS 10. Data are presented as median (95% confidence intervals). The data were not normally distributed and we used Mann-Whitney U-test for unpaired data and the Friedman test for sequential data. Values of $P < 0.05$ were considered significant. Based on a previous study[6] that found a mean (SD) L/R ratio of 0.047 (0.018), a prospective analysis showed that we needed a sample size of 34 to detect a difference in the L/R ratio of 0.02, with $\alpha = 0.05$ and a power of 80%.

	No CPB	CPB	P value
Number of patients	17	17	
Age (months)	2 (0.2-24)	5 (2-47)	0.17
Sex (M/F)	8/9	6/11	0.72
Weight (kg)	4.8 (2.5-15)	6 (4-14)	0.24
Surgery time (min)	83 (45-160)	73 (176-360)	0.01
Bypass time (min)	0	105 (73-202)	
Aortic clamp time (min)	0 (0-21)	73 (0-150)	
Ventilator hours	24 (6-144)	48 (24-168)	0.11

Table 1: Patient characteristics. Values expressed as median (95% confidence intervals).

Results

Table 1 shows patients characteristics. The groups were comparable with respect to age, sex and weight. The type of operations performed in each group is shown in table 2. Table 3 shows the L/R ratios and percentage recovery of the four sugars throughout the study period. Figure 1 is a graphic representation of the L/R changes in both groups. Patients undergoing repair of aortic coarctation showed the fastest improvement in L/R ratios 0.52 (0.21-1.01) at T0, 0.09 (0.03-0.31) at T12 and 0.07 (0.04-0.35) at T24 ($P < 0.01$). Those patients receiving a Blalock-Taussig shunt or banding of the pulmonary artery had the following L/R ratios: 0.37 (0.06-1.81) at T0, 0.27 (0.12-0.85) at T12 and 0.14 (0-0.48) at T24 ($P< 0.04$). Inotropic scores (median 95% confidence intervals) on admission to the ICU 5 (0-59.7) were not significantly different from those at 24 h after admission 5 (0-54) in the CPB group. In the group without CPB inotropic scores were 0 (0-10) after admission and 0 (0-15) at 24 h.

No CPB		CPB	
Coarctation of the aorta	7	Aortic stenosis	1
Banding pulmonary artery	3	AVSD	2
Blalock-Taussig shunt	7	Glenn	2
		MVA	2
		TAPVC	2
		ToF	1
		VSD	7
Total	17		17

Table 2: Type of operations performed in each group. Atrioventricular septal defect (AVSD). Mitral valve anuloplasty (MVA). Total anomalous pulmonary venous connection (TAPVC). Tetralogy of Fallot (ToF). Ventricular septal defect (VSD).

Patients operated without CPB had a fluid intake of 79 ml kg^{-1} (39-251) and output of 70 ml kg^{-1} (22-176), with an overall fluid balance of 18 ml kg^{-1} (-97 - 92). For those patients undergoing operations with CPB the fluid intake was 86 ml kg^{-1} (41 - 147) and output 58 ml kg^{-1} (23 - 110) with an overall balance of 31 ml kg^{-1} (-41 - 121). Values between the two groups were not significantly different.

		T0	T12	T24
L/R	No CPB	0.39 (0.07-1.8)	0.22 (0.03-0.85)	0.11 (0-0.48)
	CPB	0.30 (0.02-2.6)	0.32 (0.07-6.9)	0.24 (0.05-3.2)*
Lactulose	No CPB	0.18 (0.02-0.73)	0.35 (0.01-1.2)	0.41 (0.2-0.92)†
	CPB	0.03 (0.01-0.3)*	0.29 (0.07-1.76)	0.82 (0.05-3.32) †
Rhamnose	No CPB	0.29 (0.04-1.56)	1.56 (0.12-11.1)	3.64 (1.2-17.6) †
	CPB	0.08 (0.03-1.14)*	1.17 (0.06-4.23)	3.8 (0.04-18.1) †
3OMG	No CPB	0.64 (0.11-9.21)	3.9 (0.11-25.34)	14.1 (2.2-55.3) †
	CPB	0.19 (0.04-1.88)*	1.31 (0.1-12.93)	7.4 (0.03-48.6)†
Xylose	No CPB	0.72 (0.17-2.89)	1.34 (0.27-14.81)	3.3 (0.92-38.31) †
	CPB	0.2 (0.04-1)*	0.73 (0.07-7.3)	2.1 (0.04-18.14) †

Table 3: Lactulose/rhamnose ratios (L/R) and percentage recovery for lactulose, rhamnose, 3-O-methyl-glucose (3OMG) and xylose without and with cardiopulmonary bypass (CPB). Values are expressed as median (95% confidence intervals). * Denotes statistical significance between groups. † Denotes statistical significance within groups.

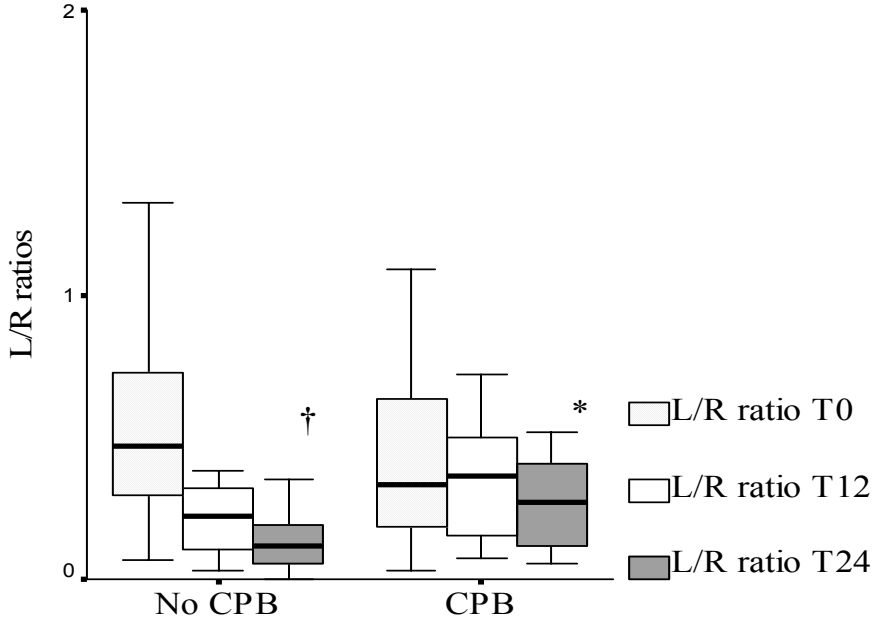

Fig 1: Changes in L/R ratios in both groups at induction of anaesthesia (T0), 12 hours (T12) and 24 hours (T24) after induction of anaesthesia. Values are expressed as median, 25^{th} – 75^{th} interquartile ranges (bars) and 2.5^{th} and 97.5^{th} percentile (error bars). * Denotes statistical significance between groups. † Denotes statistical significance within groups.

Discussion

To our knowledge this is the first report in the literature of changes in gut permeability in paediatric patients with congenital heart defects undergoing cardiac surgery. Our study shows that from the outset L/R ratios were well above the normal values expected in patients of similar age without cardiac defects.

Only patients undergoing repair of coarctation of the aorta had near normal L/R ratios 24 h after the surgical procedure. The DSPT has been used to assess intestinal function in healthy neonates, in whom the L/R ratios were around 0.05.[5] Paediatric patients without intestinal pathology have a similar ratio; range 0.023 – 0.074 and mean (SD) 0.047 (0.018).[6] Several studies have investigated the effect of cardiopulmonary bypass on intestinal permeability in the adult population.[12-14] In all of them the investigators demonstrated an increase in gut permeability that reverted to normal during the postoperative period.
In animals, exposure to CPB induces a transient mesenteric endothelial dysfunction with an increased contractile response to an α1-adrenergic agonist.[15]

Cyanotic patients may be at higher risk of developing intestinal mucosal ischaemia. This may also be true for patients with coarctation of the aorta because of a reduced blood flow through the descending aorta. Infants undergoing cardiac surgery often have chronic low arterial oxygen concentrations due to intra- or extra-cardiac shunting. Neonates with hypoplastic left heart syndrome have at times preferential blood flow to the pulmonary circulation at the expense of the systemic circulation. Those patients operated without CPB had either a total correction of their disease (coarctation of the aorta) or an improvement in the systemic oxygen delivery via a Blalock-Taussig shunt or a pulmonary banding. It is therefore logical in these patients to expect an improvement in the L/R ratios and recovery of single markers.

The accuracy of the DSPT relies on the complete collection of urine samples during the study period. This is a limiting factor on the applicability of the test in non-cooperative patients without a urinary catheter. A high percentage of patients are discharged to the ward the day after surgery with the consequent removal of urinary catheters. For this reason we performed the test during the first 24 hours after the operation.

There has been criticism concerning the interpretation and significance of the DSPT in the literature.[16,17] In an animal model it was shown that fluid loading increased the L/R ratios independent of changes in intestinal permeability. Rats received in an 8-hour period a fluid bolus equivalent to twice the daily fluid oral intake. Put into perspective, this means an infant of 10 kg would receive in an 8-hour period approximately 2 litres fluid intravenously. We carefully documented the fluid balance during the study period. On average patients received less than the daily maintenance fluid expected for their age.

When lactulose and rhamnose are combined in the test solution at a fixed concentration ratio, the effect of preabsorption factors (gastric emptying, dilution by secretions, intestinal transient time) and postabsorption factors (systemic distribution and renal clearance) will apply equally to both. Therefore the L/R ratio is only influenced by the difference in gut permeability for each molecule.[18] Pre and postabsorption factors may influence single markers such as D-xylose and 3-O-methyl-D-glucose. D-xylose is absorbed through a passive carrier-mediated transport and 3-O-methyl-D-glucose through an active carrier-mediated transport. These two single markers provide information about the functional state of the intestinal mucosa while the L/R ratio is a reflection of the mucosal integrity at the morphological level. Results of recovery of single markers should be always interpreted in conjunction with L/R ratios.

From the outset patients operated without CPB had better percentage recovery for both single markers although the differences were significant after induction of anaesthesia only. However the L/R ratio is worse in this group than in the one requiring CPB in the same period. It is difficult to draw conclusions at this point in time. Nevertheless the trend is towards a faster improvement of single markers in the group without CPB keeping in mind, that 24 h after the operation the values are far from normal.

We assume that the deterioration or lack of improvement in the L/R ratios 12 h after induction of anaesthesia must be related to the use of CPB. Interestingly CPB did not prevent a progressive and significant increase in the percentage recovery of D-xylose and 3-O-methyl-D-glucose. It appears that CPB damages temporarily the intestinal mucosa at the morphological level. At the functional level though, CPB may only delay a process that begins with the surgical correction and consequent improvement in arterial oxygen supply and/or systemic perfusion.

Inotropic support was necessary more often in the group undergoing CPB. It may be argued that this factor alone can explain the differences in L/R ratios between the two groups. However, inotropic support in the CPB group did not change between admission to intensive care and twenty-four hours later, while the L/R ratios did improve, although not significantly.

Used on a regular basis the DSPT may help us to identify the optimal time to reintroduce enteral feeding in the postoperative period. This indeed deserves further research. Novel surgical techniques or drugs aimed at protecting the splanchnic circulation can be tested against the DSPT as an end point. We are at present studying how the use of dexamethasone before CPB starts affects gut permeability in the postoperative period during paediatric cardiac surgery.

In conclusion, we have shown that paediatric patients undergoing cardiac surgery with CPB have median L/R ratios up to seven times the normal values expected in healthy children. Patients undergoing surgical repair of aortic coarctation show a swift return to near normal values twenty-four hours after the operation. From our results we can also conclude that the intestinal barrier is affected both at the morphological and functional levels. Measurement of mesenteric blood and oxygen supply in the paediatric population remains a difficult task. Only by using non-invasive, non-toxic surrogate markers of intestinal perfusion can we investigate and most importantly try to improve oxygen supply to the gut in the perioperative period.

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CHAPTER 3

Dexamethasone reduces gut permeability in paediatric cardiac surgery

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Objectives: Little attention has been paid to the effect of the systemic inflammatory response syndrome on intestinal dysfunction in the postoperative period. Several proinflammatory cytokines have been reported to increase the permeability of intestinal mucosa in vitro. We investigated the effect of dexamethasone on gut permeability in paediatric patients undergoing cardiac surgery using the dual sugar permeability test and absorption of two other saccharides.

Methods: Thirty-four patients scheduled for cardiac surgery with cardiopulmonary bypass were prospectively randomized to either act as controls or receive dexamethasone (1 mg kg^{-1}) during induction of anaesthesia. Intestinal permeability was measured using 3-O-methyl-D-glucose, D-xylose, L-rhamnose and lactulose, given orally after induction of anaesthesia and 12 and 24 hours later.

Results: Lactulose/rhamnose ratios were elevated from the outset in both groups; (mean (95% Confidence intervals)) 0.57 (0.24 – 0.91) for the control group and 0.76 (0.35 – 1.17) for patients receiving dexamethasone. While the ratios decreased 12 h (0.29 (0.17 – 0.42)) and 24 h later (0.17 (0.08 – 0.15)) in the dexamethasone group, in the control group there was a rise at 12 h (0.77 (0 – 1.64)), with a slight reduction 24 h later (0.46 (0.06 – 0.85)).

Conclusions: Infants and children undergoing cardiac surgery with cardiopulmonary bypass show a significant reduction in gut permeability when dexamethasone is used during induction of anaesthesia. Dexamethasone does not affect the intestinal barrier at the functional level as assessed by (3-O-methyl-D-glucose and D-xylose absorption).

Cardiopulmonary bypass (CPB) can trigger a systemic inflammatory response syndrome. This may result postoperatively in fever, fluid retention and pulmonary, renal, cardiac and cerebral dysfunction.[1] Little attention has been paid to the effect of CPB and the associated systemic inflammatory response on intestinal dysfunction in the postoperative period.

The postoperative release of inflammatory mediators after paediatric cardiac surgery has been reviewed extensively elsewhere.[2] Several proinflammatory cytokines have been reported to increase the permeability of intestinal mucosa in vitro.[3] Antiinflammatory cytokines on the other hand ameliorated induced gut epithelial hyperpermeability in vitro.[4] The mechanisms involved in this phenomenon are not totally clear.

Intestinal permeability can be evaluated noninvasively by measuring the urinary excretion of orally administered water-soluble, non-degradable test molecules.[5,6] This barrier function test is based on the comparison of intestinal permeation of larger molecules with that of smaller molecules by measuring the ratio of their urinary excretion. These two types of molecules follow different routes of intestinal permeation: the larger molecules are assumed to permeate paracellularly, and the smaller molecules transcellularly. Preabsorption factors such as gastric emptying, dilution by secretion and intestinal transit time, and post-absorption factors such as systemic distribution and renal clearance are assumed to affect both molecules equally. Four saccharides, 3-O-methyl-D-glucose (molecular weight 194 Da), D-xylose (molecular weight 150 Da), L-rhamnose (R, molecular weight 164 Da) and lactulose (L, molecular weight 342 Da) are employed to assess active carrier-mediated, passive carrier-mediated, transcellular, and paracellular transport, respectively in the small intestine. Intestinal permeability is considered to be normal if the lactulose (% recovery)/rhamnose (% recovery) (L/R) ratio is below 0.05.[5] Intestinal absorptive capacity for saccharides is considered to be normal when the minimum recoveries of D-xylose (passive carrier-mediated transport) and 3-O-methyl-D-glucose (active carrier–mediated transport) are around 10% and 30% respectively.[7] A previous study has shown that preoperative L/R ratios in patients with congenital heart defects are on average up to eight times higher than in healthy neonates.[8]

The possible clinical applications of the dual sugar permeability test (DSPT) have not been explored in this group of patients. Gastrointestinal complications in the postoperative period are rare, but with devastating consequences, such as necrotizing enterocolitis in patients with the hypoplastic left heart syndrome.[9]

The DSPT may be used as a surrogate marker to test the effect of drugs or novel therapies on the splanchnic circulation. The use of steroids in cardiac surgery remains controversial.[10] There is evidence in the literature that steroids, given before the start of CPB, reduce the release of proinflammatory mediators associated with both adult and paediatric cardiac surgery.[11,12] However the benefits of steroids on clinical outcome are not totally proven.[13]

The aim of our study was to observe the effect of a single dose of dexamethasone before CPB starts on the changes in gut permeability in paediatric cardiac surgery using the DSPT.

Materials and methods

After approval from the local ethics committee and informed consent from the parents or legal guardians, 34 patients were enrolled in this prospective, randomized, single blinded interventional study. Patients were allocated to receive either dexamethasone (1 mg kg^{-1} during induction of anaesthesia) or act as controls. The use of placebo is not allowed by our local ethics committee. Patients received a premedication consisting on oral atropine (0.02 mg kg^{-1}) and midazolam (0.5 mg kg^{-1}) 30 min before induction of anaesthesia. Anaesthesia was induced with sevoflurane followed by a bolus of sufentanil (1 μg kg^{-1}) and pancuronium (0.2 mg kg^{-1}). Maintenance of anaesthesia consisted on a combined continuous infusion of midazolam (0.2 mg kg^{-1} h^{-1}) and sufentanil (2 μg kg^{-1} h^{-1}). The lungs of the patients were mechanically ventilated with a mixture of oxygen and air. Mechanical ventilation was maintained until CPB commenced. After heparin administration (3 mg kg^{-1}) and aorta cannulation, CPB was instituted with a Dideco hollow fibre oxygenator with a blood flow between 200 and 300 ml kg^{-1} min^{-1}.

The prime volume, between 325 and 750 ml according to the patient's weight, contained lactate-free Ringer's solution, albumin, mannitol, blood and heparin. No steroids were added to the prime. Patients underwent modified ultra-filtration at the end of the surgical procedure. The effect of heparin was reversed with protamine sulphate in a ratio of 1 mg protamine for 1 mg heparin. After induction of anaesthesia, 2 ml kg^{-1} of the sugar solution was administered through a nasogastric tube. Urine was subsequently completely collected through a urinary catheter for 3 h, the total volume was recorded and samples were stored at -20° C until analysis. This process was repeated 12 and 24 h after induction of anaesthesia.

The sugar solution, prepared by the hospital pharmacy, contained 3-O-methyl-D-glucose (2 g litre^{-1}), D-xylose (5 g litre^{-1}), L-rhamnose (10 g litre^{-1}) and lactulose (50 g litre^{-1}). The osmolarity of the solution is approximately 240 mOsm l^{-1}.

Sugar concentrations in urine were determined by gas chromatography following a slight modification[14] of the procedure described by Jansen and colleagues.[15] Briefly, to an aliquot of urine corresponding to 0.5 μmol of creatinine 30 μg of ribitol and 10μg of trehalose (Sigma-Aldrich, St. Louis, USA) were added as internal standards (ribitol for the determination of 3-O-methyl-D-glucose, D-xylose and L-rhamnose and trehalose for lactulose). The sample was dried, derivatised with 300μl Tri-Sil TBT (Pierce, Rockford, USA) at 100° C and partly hydrolyzed with water. Subsequently the intact sugar trimethylsilyl (TMS) derivatives were extracted with hexane and after concentrating, gas chromatographic analysis was performed on a 30 m capillary fused silica HP-1 column (Agilent, Palo Alto, USA) using split injection. Quantification was performed after the construction of standard addition calibration curves. The type of vasoactive drugs and their amount were recorded after admission to the intensive care unit and 24 h later.

To quantify inotropic support, inotrope scores were calculated as the sum of all inotrope doses correcting for potency (dopamine, dobutamine =1, milrinone = 15, epinephrine = 100).[16] Fluid intake (including crystalloids, colloids and blood products), output (urine, blood and serous fluid loss) and balance were recorded over a 36 h period following admission to the intensive care unit. Urea and creatinine concentrations were measured preoperatively, immediately after admission to the intensive care unit and 24 h later.

Statistical analysis

Data were analyzed with the statistical package SPSS v10. Data are presented as mean (95% confidence intervals). Patients characteristics (age, weight, surgery times and ventilator hours), fluid balance and inotropic scores were analyzed by unpaired t test. Because L/R ratios and percentage recovery of the other two sugars were not normally distributed, the data were first subjected to a natural logarithmic transformation. We used repeated measures ANOVA to compare the two time points (T12 and T24) using dexamethasone as between subject factor.

Based on a previous study[6] that found a mean (SD) L/R ratio of 0.047 (0.018), a prospective analysis showed that we needed a sample size of 34 to detect a difference in the L/R ratio of 0.02, with $\alpha = 0.05$ and a power of 80%.

Results

Table 1 shows patients characteristics. Ventilator hours are similar in both groups. Inotropic scores are not significantly different either between the two groups at the two time periods or within each group. The total fluid balance over the 36 hours study period was not significantly different between the two groups. The type of operations performed in each group is shown in table 2. Table 3 shows the L/R ratios and percentage recovery of the four sugars throughout the study period together with the P value for each parameter. Differences in L/R ratios between the two groups were statistically significant. Differences in percentage recovery for rhamnose and 3-O-Methyl-D-Glucose were also significant between the two groups. There were no significant differences in the percentage recovery of lactulose and D-xylose between the two groups.

	No dexamethasone	Dexamethasone	P values
Number of patients	17	17	
Age (months)	12 (5 - 19)	4 (2 - 6)	0.02
Sex (M/F)	6/11	7/10	0.46
Weight (kg)	7 (5 – 9)	5 (4 - 6)	0.01
Surgery time (min)	181 (152 – 211)	190 (145 – 234)	0.65
Bypass time (min)	118 (100 – 135)	118 (87 – 149)	0.66
Aortic clamp time (min)	64 (46 – 83)	78 (52 – 104)	0.58
Ventilator hours	52 (33 – 71)	81 (27 – 135)	0.79
Inoscore	4 (2 – 6)	7 (2 – 11)	0.83
Inoscore 24 hours	6 (3 – 10)	7 (1 – 13)	0.58
Balance (ml kg^{-1})	29 (3 – 55)	33 (12 – 54)	0.89

Table 1: Patient characteristics. Values expressed as mean (95% confidence intervals).

	No dexamethasone	Dexamethasone
VSD	7	6
ToF	1	1
TAPVC	2	2
Arterial switch	0	4
Rastelli	0	1
PA, MAPCA	0	1
AVSD	2	0
AVA	1	0
BCPA	2	1
MVA	2	1
Total	17	17

Table 2: Surgical procedures. Ventricular septal defect (VSD), Tetralogy of Fallot (ToF), Total anomalous pulmonary venous connection (TAPVC), Atrioventricular septal defect (AVSD), aortic valve anuloplasty (AVA), bidirectional cavopulmonary anastomosis (BCPA), mitral valve annuloplasty (MVA), major aortopulmonary collateral arteries (MAPCA), pulmonary atresia (PA).

Urea and creatinine concentrations remained within the normal clinical range throughout the study period. In the control group urea concentrations (normal range 0.7 to 6.7 mmol l^{-1}) were 4.8 (4 – 5.5) mmol l^{-1} preoperatively, 4.1 (3.4 – 4.8) mmol l^{-1} immediately after the operation and 5.9 (4.9 – 6.8) mmol l^{-1} 24 h later. In the dexamethasone group there were 4 (3.4 – 4.6) mmol l^{-1} preoperatively, 4 (3.3 – 4.7) mmol l^{-1} immediately after the operation and 5.5 (4.7 – 6.3) mmol l^{-1} 24 h later. Creatinine concentrations (normal value less than 70 μmol l^{-1}) were in the control group 41 (37 – 45) μmol l^{-1} preoperatively, 40 (37 – 44) μmol l^{-1} immediately after the operation and 52 (41 – 63) μmol l^{-1} 24 h later.

In the dexamethasone group there were 31 (25 – 38) μmol l^{-1} preoperatively, 31 (26 – 37) immediately after the operation and 34 (28 – 39) μmol l^{-1} 24 h later. None of the patients developed acute renal failure during the study period. Figures 1 to 5 are graphical representations of the changes in L/R ratios, and percentage recovery for lactulose, rhamnose, D-xylose and 3-O-methyl-D-glucose in both groups.

	T0	T12	T24	P value
L/R ratios				
No dexamethasone	0.57 (0.24 – 0.91)	0.77 (0 – 1.64)	0.46 (0.06 – 0.85)	
Dexamethasone	0.76 (0.35 – 1.17)	0.29 (0.17 – 0.42)	0.17 (0.08 – 0.15)	0.019
Lactulose				
No dexamethasone	0.07 (0.02 – 0.12)	0.31 (0.22 – 0.4)	0.87 (0.51 – 1.23)	
Dexamethasone	0.08 (0.02 – 0.14)	0.62 (0.34 – 0.89)	1.07 (0.11 – 2.04)	0.056
Rhamnose				
No dexamethasone	0.16 (0.02 – 0.30)	1.44 (0.68 – 2.2)	4.59 (2.23 – 6.94)	
Dexamthasone	0.16 (0.02 – 0.31)	3.02 (1.36 – 4.69)	6.18 (3.35 – 9)	0.047
D-xylose				
No dexamethasone	0.29 (0.14 – 0.45)	1.58 (0.42 – 2.75)	5.69 (2.41 – 8.96)	
Dexamethasone	0.34 (0.08 – 0.60)	2.96 (1.83 – 4.09)	5.47 (2.59 – 8.34)	0.056
3OMG				
No dexamethasone	0.38 (0.12 – 0.64)	3.37 (1.21 – 5.54)	14.42 (6.19 – 22.6)	
Dexamethasone	0.33 (0.09 – 0.57)	7.32 (3.32 – 11.33)	13 (6.59 – 19.42)	0.041

Table 3: Lactulose/rhamnose ratios (L/R) and percentage recovery for lactulose, rhamnose, D-xylose and 3-O-methyl-glucose (3OMG) without and with dexamethasone. Values are expressed as mean (95% confidence intervals). * Denotes statistical significance between groups

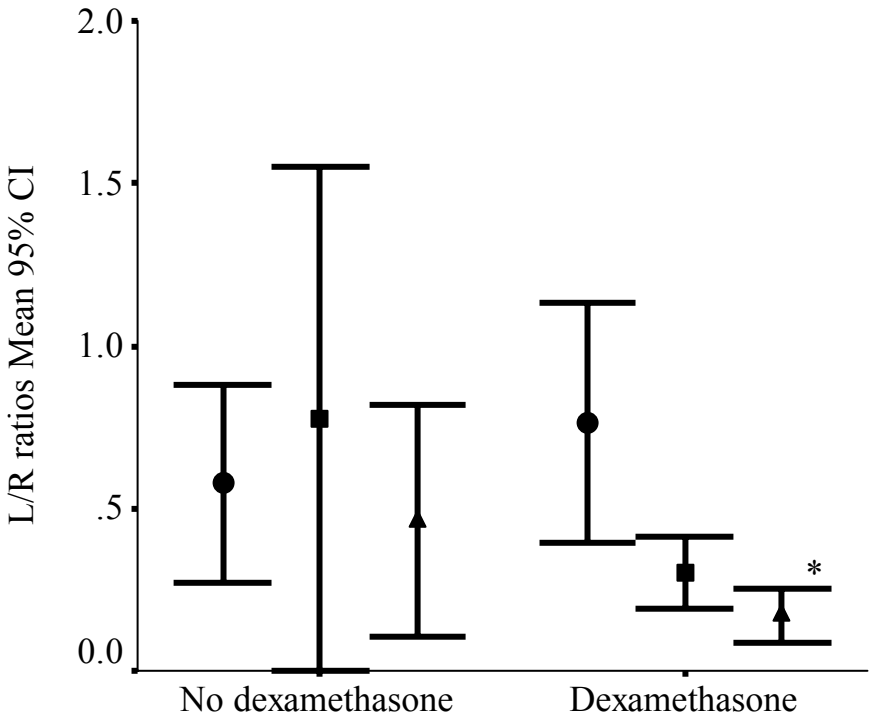

Fig 1: Changes in L/R ratios in both groups. Values expressed as mean 95% CI. (●) T0, (■) T12, (▲) T24.* Denotes statistical significance between groups.

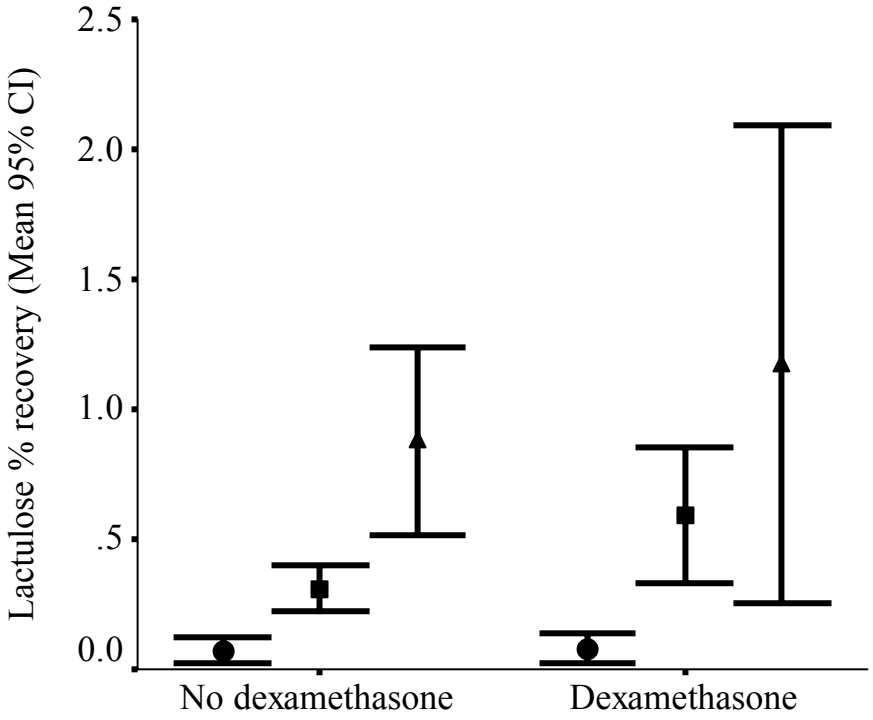

Fig 2: Changes of lactulose percentage recovery in both groups. Values expressed as mean 95% CI. (●) T0, (■) T12, (▲) T24.* Denotes statistical significance between groups.

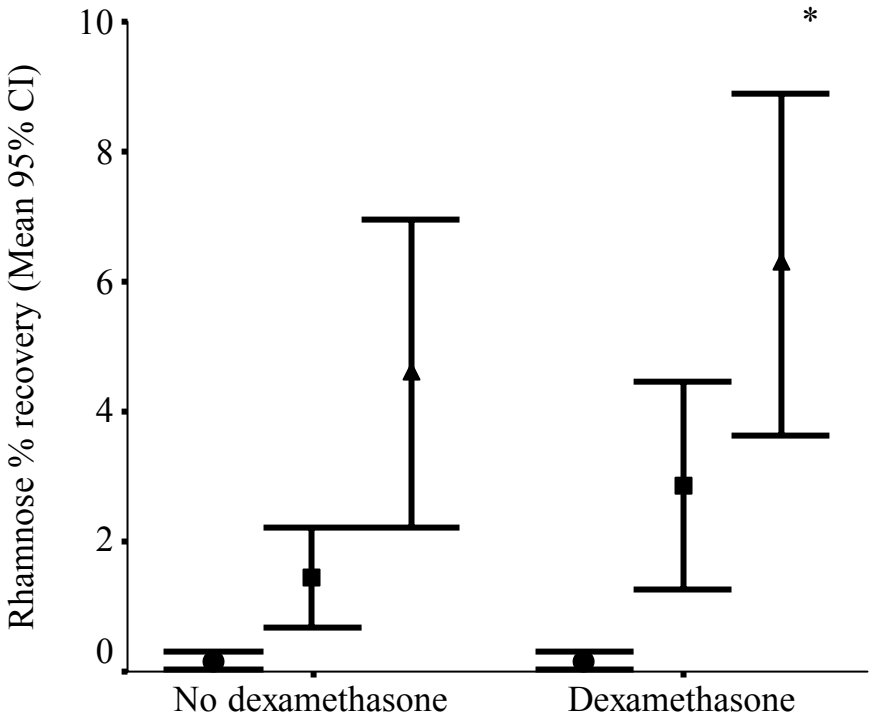

Fig 3: Changes of rhamnose percentage recovery in both groups. Values expressed as mean 95% CI. (●) T0, (■) T12, (▲) T24.* Denotes statistical significance between groups.

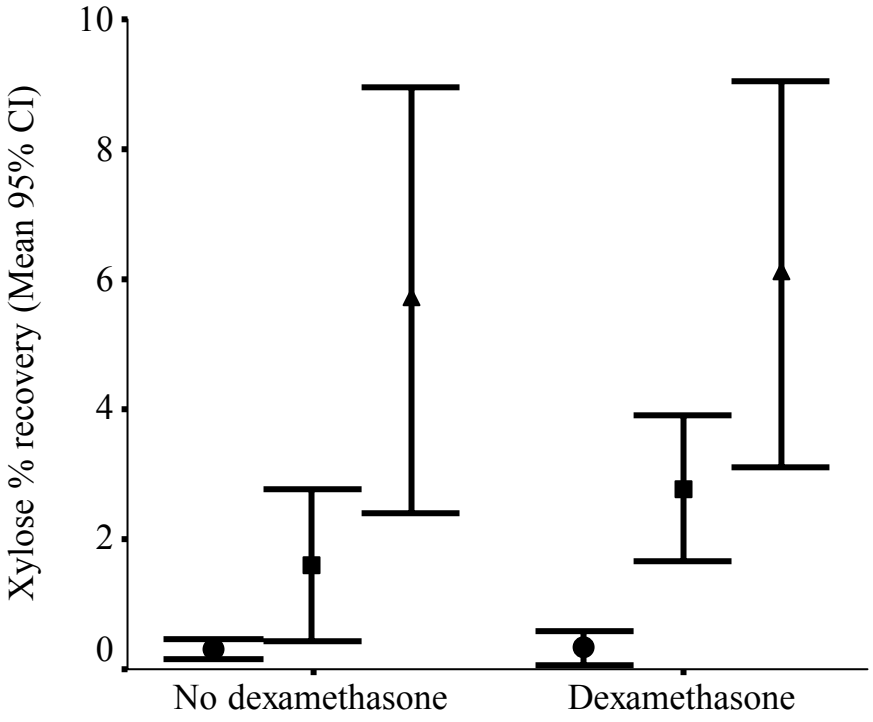

Fig 4: Changes of D-xylose percentage recovery in both groups. Values expressed as mean 95% CI. (●) T0, (■) T12, (▲) T24.* Denotes statistical significance between groups.

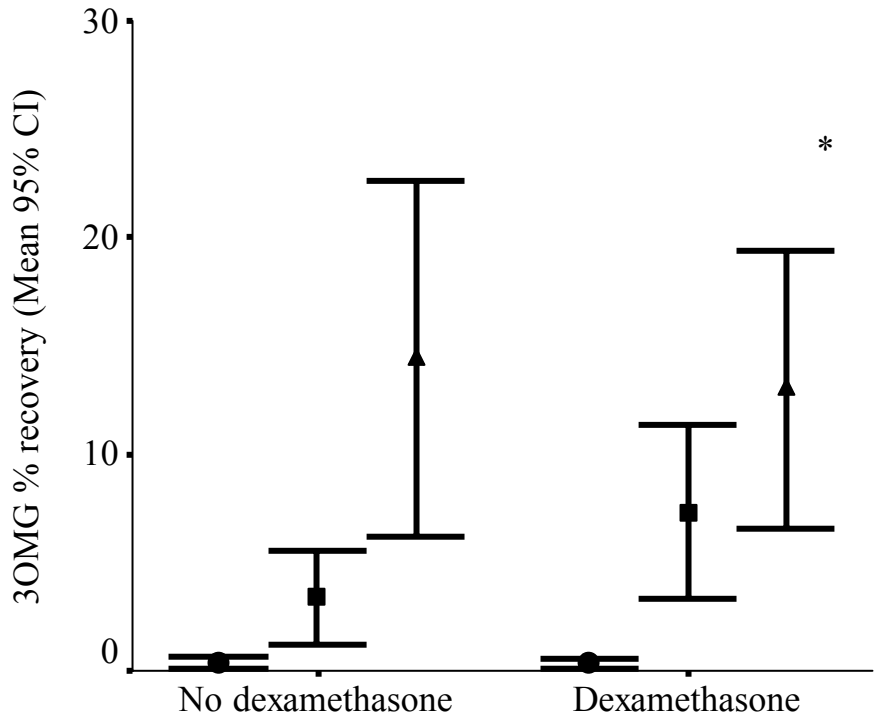

Fig 5: Changes of 3-O-D-Methyl-Glucose percentage recovery. Values expressed as mean 95% CI. (●) T0, (■) T12, (▲) T24.* Denotes statistical significance between groups.

Discussion

We have shown in this study that dexamethasone given before cardiopulmonary bypass starts reduces postoperative gut permeability as assessed by the DSPT. There was an age and weight difference between the two groups. This could be seen as a limiting factor in the interpretation of the results. Univariate analysis showed that both variables did not influence either L/R ratios or percentage recovery of the other two sugars. The DSPT has been used to assess intestinal function in healthy neonates, in whom the L/R ratios were around 0.05.[5] Similar values were found in healthy children; range 0.023 – 0.074 and mean (SD) 0.047 (0.018).[6]

Gut permeability has been investigated in adult patients undergoing coronary artery bypass surgery.[17] L/R ratios increased immediately after surgery returning to normal approximately 48 h after the surgical procedure. The DSPT has also been used to test the beneficial effects of dopexamine over dopamine in the splanchnic circulation during adult coronary bypass surgery.[18]

The accuracy of the DSPT relies on the complete collection of urine samples during the study period. This is a limiting factor on the applicability of the test in non-cooperative patients without a urinary catheter. A high percentage of patients are discharged to the ward the day after surgery with the consequent removal of urinary catheters. For this reason we performed the test during the first 24 hours after the operation.

There has been criticism concerning the interpretation and significance of the DSPT in the literature.[19,20] In an animal model it was shown that fluid loading increased the L/R ratios independent of changes in intestinal permeability. Rats received in an 8-hour period a fluid bolus equivalent to twice the daily fluid oral intake. Put into perspective, this means an infant of 10 kg would receive in an 8-hour period approximately 2 litres fluid intravenously. We carefully documented the fluid balance during the study period. On average patients received less than the daily maintenance fluid expected for their age.

The DSPT relies on the assumption that when lactulose and rhamnose are combined in the test solution at a fixed concentration ratio, the effect of preabsorption factors (gastric emptying, dilution by secretions, intestinal transient time) and postabsorption factors (systemic distribution and renal clearance) will apply equally to both. Therefore the L/R ratio is only influenced by the difference in gut permeability for each molecule.[21] Percentage recovery of single markers may be influenced not only by the permeability of the intestinal mucosa but also by pre and postabsorption factors. D-xylose and 3-O-methyl-D-glucose provide information about the functional state of the intestinal mucosa while the L/R ratio is a reflection of the mucosal integrity at the morphological level. Percentage recoveries for D-xylose and 3-O-methyl-D-glucose did not differ substantially between the two groups.

There is a trend towards normality through the study period although at T24 percentage recoveries for both sugars are far from values expected in healthy children. Our study has shown that the use of dexamethasone improved gut permeability at the morphological level in our patients. However we have no explanation as to why dexamethasone did not affect the intestinal barrier at the functional level. A L/R ratio of 0.46 is nearly ten times the normal value and is a reflection of a highly permeable intestinal mucosa. If this implies that the patient is at higher risk for intestinal bacterial translocation and subsequent sepsis or intestinal ischemia is not clear. The converse is surely true. Patients diagnosed with necrotizing enterocolitis have consistently high L/R ratios.[22]

A likely explanation for our results is the effect that dexamethasone exerts in the postoperative production of proinflammatory cytokines. The concentration of proinflammatory cytokines decreases when steroids are used before CPB starts. This has been demonstrated consistently by a number of studies.[23,11] Several cytokines, including interleukin 1 (IL-1), IL-4, IL-6, IL-10, interferon γ (IFN-γ), and tumour necrosis factor, have been reported to increase intestinal permeability in vitro.[3] IL-6 in particular may be a mediator of intestinal mucosal injury in various conditions associated with transient mesenteric hypoperfusion and subsequent inflammation.[24]

A combined administration of steroids hours before the operation plus its addition to the pump prime appears to be more beneficial.[25] Giving steroids intravenously to patients hours before the surgical procedure is however, often difficult for technical and logistical reasons. It has been standard practice in our institution to use dexamethasone during induction of anaesthesia only in patients undergoing a period of circulatory arrest.

The results of the present study are an encouragement to investigate if adding steroids to the pump prime may provide a better and/or faster improvement in gut permeability. Nevertheless the use of dexamethasone in paediatric cardiac surgery remains a controversial issue. Lindberg and colleagues[26] stated that omitting the use of dexamethasone in children weighing less than 10 Kg scheduled for cardiac surgery is unethical. Schroeder and colleagues[25] accepted that the lack of a control group without methylprednisolone is a limiting factor in their study. It is according to them standard practice to use steroids in these patients. However there are no prospective or retrospective cohort studies that clearly show the influence of steroids on the clinical postoperative course. In the present study none of the patients in the control group developed gastrointestinal complications in the postoperative period and the clinical parameters measured were not affected by dexamethasone.

Using dexamethasone to improve intestinal function is not a new concept. As early as 1984, Bauer and colleagues noticed a reduction in the incidence of necrotizing enterocolitis after prenatal glucocorticoid therapy.[27] Animal models have shown repeatedly that in the immature animal dexamethasone reduces bacterial translocation in the gut and increases the length of the small intestine.[28] The use of dexamethasone (0.5 to 1 mg kg^{-1} per day for several days) to treat or prevent chronic lung disease in preterm infants is discouraged after intensive review of the literature.[29] Our patients were not premature, were exposed to enteral feeding, and their intestinal wall was far from immature.

The mechanisms by which dexamethasone exerts its effects in the intestinal mucosa are not totally elucidated. Further research may be necessary.

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CHAPTER 4

Gut permeability in neonates following a stage I Norwood procedure

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Objective: Intestinal mucosal ischemia can occur during and after cardiac surgery. Severe decreases in mucosal perfusion may be a causative factor for postoperative mortality or complications such as necrotizing enterocolitis (NEC). Mesenteric perfusion is challenged preoperatively due to an imbalance between the systemic and pulmonary circulations, and intraoperatively due to hypothermic circulatory arrest. We have investigated gut permeability in seven patients undergoing stage I of the Norwood procedure, applying the dual sugar permeability test (DSPT) with L-rhamnose and lactulose.

Design: Seven patients with hypoplastic left heart syndrome (HLHS): clinical presentation, gut permeability findings and outcome.

Setting: A ten bed mixed paediatric intensive care unit in a university hospital.

Patients: Seven patients admitted for postoperative care after cardiac surgery.

Interventions: Determination of gut permeability with the dual sugar permeability test using lactulose and rhamnose. Intestinal permeability was measured after induction of anaesthesia and 12 h and 24 h later.

Measurements and Main Results: All patients had abnormal lactulose/rhamnose ratios. One patient, who had a lactulose/rhamnose ratio 12 hours after surgery of 2.3 (46 times normal), developed NEC postoperatively and died three days after surgery.

Conclusions: Gut permeability as assessed by the DSPT is abnormal in patients with HLHS up to 24 hours after surgery. L/R ratios 46 times the normal value reflect a highly permeable small intestine. This may be a sign of a low output state and may help to identify patients at risk of developing NEC.

Intestinal mucosal ischemia can occur in infants and children during and after cardiopulmonary bypass (CPB). Severe decreases in mucosal perfusion may contribute to postoperative mortality or complications such as necrotizing enterocolitis (NEC). Infants exposed to profound hypothermia and circulatory arrest may be at particular risk of developing splanchnic ischemia in the perioperative period. The effects of these insults can be evaluated by measuring gut permeability using the dual sugar permeability test (DSPT).

In the DSPT intestinal permeability is evaluated by measuring the urinary excretion of orally administered water-soluble, non-degradable test molecules. This barrier function test is based on the comparison of intestinal permeation of larger molecules (lactulose) with that of smaller molecules (L-rhamnose) by measuring the ratio of their urinary excretion. These molecules follow different routes of intestinal permeation: the larger molecules are assumed to permeate paracellularly, the smaller molecules transcellularly. Preabsorption factors such as gastric emptying, dilution by secretion and intestinal transit time, and post-absorption factors such as systemic distribution and renal clearance are assumed to affect both molecules equally. Therefore, the urinary excretion ratio is considered a parameter for intestinal permeability per se. Four saccharides, 3-O-methyl-D-glucose, D-xylose, L-rhamnose and lactulose are employed to assess active carrier-mediated, passive carrier-mediated, transcellular, and paracellular transport, respectively, in the small intestine.

Intestinal permeability is considered to be normal if the lactulose (% recovery)/rhamnose (% recovery) (L/R) ratio is lower than 0.05.[1,2] We report here the results of the dual sugar permeability test (DSPT) in seven patients undergoing repair of hypoplastic left heart syndrome (HLHS).

Materials and Methods

After approval from the local ethics committee and written informed consent from parents seven patients were prospectively investigated. All patients presented at term with HLHS. A standard anaesthesia was used and the same surgeon performed all the operations. Enteral feeding was started one day after surgery.

After induction of anaesthesia (T0), 2 mL/kg of the sugar solution was administered through a nasogastric tube. Urine was subsequently completely collected for 3 h, the total volume was recorded and samples were stored at -20° C until analysis.

This process was repeated 12 (T12) and 24 h (T24) after induction of anaesthesia. The sugar solution contained 3-O-methyl-D-glucose (2 g/L), D-xylose (5 g/L), L-rhamnose (10 g/L) and lactulose (50 g/L). The osmolarity was approximately 240 mOsm/L.

Sugar concentrations in urine were determined by gas chromatography following a slight modification[3] of the procedure described by Jansen et al.[4] Briefly, to an aliquot of urine corresponding to 0.5 μmol of creatinine, 30 μg ribitol and 10μg trehalose (Sigma-Aldrich, St. Louis, USA) were added as internal standards for the determination of rhamnose and lactulose respectively. The sample was dried, derivatized with 300μl Tri-Sil TBT (Pierce, Rockford, USA) at 100° C and partly hydrolyzed with water. Subsequently the intact sugar trimethylsilyl (TMS) derivatives were extracted with hexane and after concentrating, gas chromatographic analysis was performed on a 30 m capillary fused silica HP-1 column (Agilent, Palo Alto, USA) using split injection. Quantification was performed after the construction of standard addition calibration curves. Renal function was measured the day before the operation, immediately after the operation and 24 h thereafter.

Statistical analysis

Data were analyzed with the statistical package SPSS v10. Data are presented as mean (95% confidence intervals). Because L/R ratios were not normally distributed, the data were first subjected to a natural logarithmic transformation. We used repeated measures ANOVA to compare the three time points.

Results

Table 1 shows the L/R ratios and durations of cardiopulmonary bypass, circulatory arrest and aortic clamp times. Six patients had uneventful stays in the intensive care unit, and were mechanically ventilated for between two and five days. Patient 4 died of NEC three days after the surgical procedure. Patient 5 was readmitted to the pediatric intensive care unit thirteen days after discharge with severe cardiac failure requiring cardiopulmonary resuscitation, which was unsuccessful. The cause of the cardiac failure was never diagnosed although severe sepsis was suspected. Table 2 shows amount of inotropic support used immediately after admission to the pediatric intensive care unit 12 h and 24 h later.

	L/R Ratios			Bypass (min)	Arrest (min)	Clamp (min)
	T0	T12	T24			
Patient 1	0.18	0.08	0.22	300	80	270
Patient 2	1.55	0.63	0.19	180	80	80
Patient 3	0.04	0.14	0.11	162	65	87
Patient 4	-	2.30	-	238	96	92
Patient 5	27.6	2.37	0.23	345	150	174
Patient 6	0.20	0.57	0.23	140	26	71
Patient 7	0.25	0.33	-	180	88	88

Table 1: Lactulose/Rhamnose (L/R) ratios during the three study periods. Missing values are due to the volume of urine being too small to perform the test. Patient 4 developed NEC postoperatively.

	A	B	C
Patient 1	15	15	19
Patient 2	10	15	5
Patient 3	0	10	10
Patient 4	10	10	10
Patient 5	110	117.5	125
Patient 6	7	7	14
Patient 7	5	5	5

Table 2: Inotropic scores were calculated as the sum of all inotrope doses correcting for potency (dopamine, dobutamine =1, milrinone = 15, epinephrine = 100), immediately after admission to the paediatric intensive care unit (A), 12 h (B) and 24 h (C) later.

L/R ratios were at T0 0.44 (0 – 1.21), at T12 0.91 (0 – 1.83) and at T24 0.19 (0.13 – 0.25). Changes were statistically significant ($P < 0.034$). Subsequent parameter estimate analysis showed a highly significant difference at T24 ($P < 0.002$). Urea concentrations (normal range 0.7 to 6.7 mmol/L) were 5.5 (2.6 – 8.3) mmol/L preoperatively, 3.35 (2 – 4.6) mmol/L immediately after the operation and 5.4 (3.2 – 7.6) mmol/L 24 h later. Creatinine concentrations (normal value less than 70 µmol/L) were 61 (45 – 77) µmol/L preoperatively, 58 (51 – 66) µmol/L immediately after the operation and 76 (69 – 84) µmol/L 24 h later. None of the patients developed acute renal failure during the study period.

Discussion

To our knowledge this is the first time that gut permeability has been investigated in neonates undergoing a period of circulatory arrest. CPB has been shown to temporarily increase gut permeability in adult patients undergoing coronary bypass graft surgery.[5] Changes in gut permeability in the pediatric population have been reported recently in the literature.[6] Thirty four patients undergoing cardiac surgery with and without cardiopulmonary bypass were prospectively investigated. High L/R ratios were present from the outset. L/R ratios decreased over the following 24 h period in patients operated without CPB. L/R ratios increased in patients operated with CPB 12 h after the operation, while 24 h later the differences were significantly higher in the group with CPB.

L/R ratios in the present study were also above the normal value preoperatively and the perioperative changes are significantly different. Insufficient mesenteric oxygen supply due to a combination of cyanosis and preferential flow to the pulmonary circulation instead of the systemic circulation may justify these findings. Table 1 clearly shows that in two of six patients L/R ratios decreased after the operation. However when compared to patients undergoing cardiac surgery without circulatory arrest the L/R ratios tended to be higher before and immediately after the operation. This may be purely coincidental or may be due to the severity of the disease and the use of circulatory arrest during the surgical procedure.

Of the seventeen patients undergoing surgery with CPB[6] only one had L/R ratios similar to those found in patients 4 and 5 of the present study. The patient did not develop NEC but required doses of inotropes in the postoperative period similar to those needed by patient number 5. At the time we conducted the DSPT in patient 4 there were no signs of NEC. However a L/R ratio 46 times the normal value at T12 reflected a highly permeable small intestine. It is unfortunate that in this patient the volume of urine was inadequate for analysis after induction of anaesthesia. The surgical repair in patient 5 was technically difficult resulting in a long circulatory arrest time and considerable inotropic support postoperatively. This could explain the high L/R ratio of 2.37 at T12.

Neonates with congenital heart disease have a high risk of developing NEC, with a reported incidence in patients with HLHS between 7.6% and 10% and mortality above 90%.[7,8]

Enteral feeding has been proposed as a risk factor for NEC in term neonates, although in patients with congenital heart disease this appears not to be the case.[8] The DSPT was performed in our patients before feeding started.

L/R ratios in healthy neonates enterally fed are less than 0.05.[2] Similar values were found in children without intestinal pathology 0.047 (0.018) (Mean (SD)).[1] This is in contrast to what it has been found consistently in patients with congenital heart diseases preoperatively.[6] The DSPT has been previously applied to patients without congenital heart disease already diagnosed with NEC.[9] The DSPT was performed in a number of occasions over a three week period. The highest reported value was 0.98. These patients were starved during the study period and received total parenteral nutrition. In the same study patients undergoing intestinal surgery acted as controls showing elevated L/R ratios as well (highest value 0.32). However L/R ratios returned to normal faster than in the NEC group.

The DSPT has limitations. Gas chromatography is a time consuming procedure, but if important clinical decisions depend on the result, it can be completed in a matter of hours. It can also produce spurious results, such as in the first urine sample of patient 5 in which a very low rhamnose excretion resulted in a L/R ratio of 27.6. Gut permeability has not been investigated in patients suffering from either acute or chronic renal failure. This is a limitation to the applicability of the test. It is assumed that the percentage of both sugars not eliminated in the urine is metabolized by colonic bacteria into short chain fatty acids.[10]

Studies in adult patients investigating the elimination of lactulose and rhamnose have shown that no rhamnose and little lactulose (1.56% of the ingested amount) are present in the urine after 12 h collection.[11] It is unlikely that gut permeability assessment at T12 and T24 may be affected by previous instillations. L/R ratios are not affected if the urinary collection period is three hours or longer.[12]

Conclusions

Gut permeability as assessed by the DSPT is abnormal in patients with HLHS up to 24 hours after surgery. L/R ratios 46 times the normal value reflect a highly permeable small intestine. This may be a sign of a low output state and may help to identify patients at risk of developing NEC. Pre-emptive treatment of NEC before obvious clinical signs appear could have benefits in terms of morbidity and/or mortality.

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We are grateful to our hospital pharmacy for the preparation and supply of the sugar solution.

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CHAPTER 5

Rhamnitol is a metabolite of rhamnose in man

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Submitted

Until now rhamnose has been considered an inert sugar not metabolized by the human body. During an investigation on gut permeability in children undergoing cardiac surgery using the dual sugar permeability test[1] (DSPT), we found increased concentrations of rhamnitol, a metabolite of rhamnose, in their urine. Four saccharides, 3-O-methyl-D-glucose, D-xylose, L-rhamnose and lactulose are used in the DSPT. The identity of rhamnitol was established by mass spectrometry. Rhamnitol also increased when an adult volunteer ingested the sugar solution. Our finding provides evidence that, contrary to accepted opinion, rhamnose is not an inert sugar but is partially metabolized into rhamnitol in humans.

Materials and methods

Our initial study[1] involved 34 paediatric patients undergoing cardiac surgery with (n = 17) or without (n = 17) cardiopulmonary bypass (CPB). Anaesthesia was standard for all patients. Two ml kg^{-1} of a sugar solution were instilled through a nasogastric tube after induction of anaesthesia, and again 12 and 24 h later. The test solution contained 3-O-methyl-D-glucose (2 g l^{-1}), D-xylose (5 g l^{-1}), L-rhamnose (10 g l^{-1}) and lactulose (50 g l^{-1}). After each instillation, urine was completely collected through a urinary catheter for 3 h, and stored at –20 ºC until analysis. One infant undergoing cardiac surgery received the sugar solution only once during induction of anaesthesia and urine was collected for 24 h afterwards. A healthy adult volunteer ingested the sugar solution (2 ml kg^{-1}) after overnight fasting and urine samples were subsequently collected at 4 h, 8 h, 16 h and 24 h.

Sugar concentrations in urine were determined by gas chromatography and a full description of the method can be found elsewhere[1]. The identity of rhamnitol was confirmed by comparison of retention time and electron-ionization mass spectrum with that of authentic rhamnitol (Sigma-Aldrich, St. Louis, USA). Data are presented as mean (95% Confidence Intervals). After a natural logarithmic transformation a paired t test was used for statistical analysis.

Results

Table 1 shows the percentage recovery of rhamnose and the percentage of ingested rhamnose metabolized into rhamnitol in urine in the two groups (Table 2 gives the absolute values). As can be seen from the data both parameters increase in the consecutive 3 h urine collections.

The patient's weight (kg) was 4.8 (2.5-15) for the non-CPB group and 6 (4-14) for the CPB group. Rhamnitol was not detectable in the sugar solution supplied by the pharmacy. Rhamnitol was detected in trace amounts (< 0.03 mg/3 h) in urine of paediatric patients investigated for inborn errors of metabolism. In the patient where the sugar solution was administered only once, 12.2% (9.7 mg) of the ingested rhamnose was recovered unaltered and 1.3% (1.03 mg) was recovered as rhamnitol.

	T0	T12	T24
No CPB			
Rhamnose %	0.34 (0.2 – 0.48)	3.4 (1.4 – 5.4)	4.6 (2.5 – 6.8)
Rhamnitol %	0.03 (0 – 0.48)	0.23 (0.1 – 0.37)	0.68 (0.4 – 1)
CPB			
Rhamnose %	0.16 (0.02 – 0.3)	1.4 (0.68 – 2.2)	2.5 (1.3 – 3.7)
Rhamnitol %	0.02 (0.06 – 0.03)	0.13 (0.08 – 0.2)	0.76 (0.4 – 1.1)

Table 1: Percentage of ingested rhamnose recovered in urine and percentage of rhamnose metabolized into rhamnitol found in urine. Values expressed as mean (95% Confidence Intervals).

	T0	T12	T24
CPB	0.03 (0.01 - 0.06)	0.2 (0.07 - 0.33)	0.89 (0.45 - 1.33)
No CPB	0.02 (0.005 - 0.029)	0.23 (0.11 - 0.36)	0.69 (0.42 - 0.95)

Table 2: Total amount of Rhamnitol in mg in urine collected over a three hour period. Values expressed as mean (95% confidence intervals).

In the adult volunteer rhamnitol was present in the urine after the ingestion of the sugar solution. In urine collected 4 h after loading, 5.1% (76.8 mg) of the ingested rhamnose was recovered and 0.11% (1.7 mg) was found to be metabolized into rhamnitol. In the whole 24 h urine these figures were 6.7% (107 mg) and 1.2% (18.3 mg), respectively.

Discussion

Assessment of gut permeability using the DSPT was introduced in the late seventies[2]. The L/R ratio is considered to be a parameter for intestinal permeability. The strength of the test relies on the fact that both are assumed to be inert sugars not metabolized by the organism. In humans, the proportion of rhamnose not excreted in the urine is assumed to be fermented by colonic bacteria into short chain fatty acids (acetate, propionate and butyrate)[3]. Some bacteria metabolize rhamnose into rhamnulose but not to rhamnitol[4].

However we have consistently found rhamnitol in the urine of patients in our study. Traces of rhamnitol were generally found in urine of children who were screened for inborn errors of metabolism. It is likely that the conversion of rhamnose into rhamnitol by the human body follows a pathway similar to what has been proposed for another previously thought inert sugar (L-arabinose)[5]. A possible metabolite such as rhamnoate however was not present. The findings in the adult volunteer as well as the data presented in table 1 indicate that rhamnose is slowly metabolized. Therefore almost certainly the rhamnitol found in the urine of the patients after the second and third instillation is largely a metabolite of the previous dose(s).

Xylitol was also increased in the urine samples of the patients in this study (data not shown), indicating that xylose is also not an inert sugar. The DSPT is used as a research tool in human and animal studies. The addition of rhamnitol to rhamnose can reduce the L/R ratio strongly. Lactulose/Rhamnose ratios (L/R) changed significantly when rhamnitol was added to rhamnose in our previous study; group with CPB ($P < 0.02$) 0.57 (0.24 – 0.91) (without rhamnitol) and 0.49 (0.22 – 0.76) (with rhamnitol). Group without CPB ($P < 0.01$) 0.6 (0.36 – 0.85) (without rhamnitol) and 0.55 (0.32 – 0.78) (with rhamnitol) (normal L/R <0.05). This may be clinically irrelevant when the L/R ratios are as high as in our investigations. However it must be taken into consideration when L/R ratios are close to normal or when urine samples are collected for longer periods after administration.

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SECTION 2

Cardiac troponin T and paediatric cardiac surgery

CHAPTER 6

Effect of three different anaesthetic agents on the postoperative production of cardiac troponin T in paediatric cardiac surgery.

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Background: Paediatric cardiac surgery is associated with some degree of myocardial injury. Ischaemic preconditioning (IP) has been widely investigated in the adult population. Volatile agents have been shown to simulate IP providing extra protection to the myocardium during adult cardiopulmonary bypass while propofol seems to act through different mechanisms. IP has not been investigated in the paediatric population to the same extent. Cardiac troponin T (cTnT) is a reliable marker of myocardial injury in neonates and children. We have investigated the relationship between three anaesthetic agents, midazolam, propofol and sevoflurane, and postoperative production of cTnT.

Methods: Ninety patients undergoing repair of congenital heart defect with cardiopulmonary bypass were investigated in a prospective randomized study. cTnT was measured four times during the first 24 hours following admission to the paediatric intensive care unit. Other parameters measured included arterial blood gases, lactate, fluid balance, use of inotropic drugs, PaO_2/FiO_2 ratio and ventilator hours.

Results: cTnT was elevated in all three groups throughout the study period. The differences between the three groups were not statistically significant. Eight hours after admission to the intensive care unit cTnT concentrations tended to be higher in the midazolam group (mean (95% confidence intervals)); 2.7 (1.9 – 3.5) ng ml^{-1} . Patients receiving a propofol based anaesthesia had similar concentrations 2.6 (1.7 – 3.5) ng ml^{-1} while those receiving sevoflurane tended to have a lower cTnT production 1.7 (1.3 – 2.2) ng ml^{-1}.

Conclusions: Midazolam, propofol and sevoflurane seem to provide equal myocardial protection in paediatric cardiac surgery when using cTnT as a marker of myocardial damage.

During repair of a congenital heart defect the child is exposed to myocardial hypoxia. Exposing the adult myocardium to brief periods of ischaemia and reperfusion induces greater tolerance to a subsequent more prolonged ischaemic insult, a phenomenon known as ischaemic preconditioning (IP).

Animal experiments have shown that the effects of ischaemic preconditioning are mimicked by inhalational anaesthetics[1], morphine[2], and possibly other opioids. This is often referred to as anaesthetic preconditioning. The use of inhalational anaesthetics improves clinical and biochemical parameters after coronary artery bypass surgery.[3,4] The subject has been reviewed extensively.[5,6] There is little available information as to whether IP also occurs in paediatric patients. IP is absent in rats at birth and only develops in the second week of postnatal life.[7] Preconditioning can be induced in isolated perfused immature rabbit hearts.[8] It has been suggested that the normoxic paediatric heart is less likely than the adult heart to undergo injury with bypass or surgical ischaemia.[9] Recent studies, however, have suggested that the paediatric myocardium is more sensitive to hypoxia and cardioplegic arrest than the adult one.[10] On the other hand cyanotic patients are exposed to high concentrations of oxygen when bypass starts (similar to reperfusion injury).

The effect of halogenated agents and propofol on ischaemia and reperfusion injury has been widely investigated in adults. Midazolam has not been investigated to the same extent and information about its potential benefits or lack thereof is scarce. Midazolam, sevoflurane and propofol are three anaesthetic agents commonly used in paediatric practice.

Cardiac troponin T (cTnT) is a specific marker of myocardial infarction.[11] It is also a reliable marker of myocardial injury in the paediatric population.[12] There is no information in the literature about the relationship between postoperative production of cTnT in children and the anaesthetic agents, midazolam, propofol and sevoflurane. In this study we investigated whether midazolam, propofol or sevoflurane afford equal myocardial protection during paediatric cardiac surgery, as assessed by postoperative cTnT production.

Materials and Methods

After approval by the hospital ethics committee and parental consent 90 patients were prospectively investigated. cTnT was measured four times during the first 24 h following admission to the paediatric intensive care unit. This is standard practice in our institution.

Patients were randomized using standard randomization tables to receive either midazolam, propofol or sevoflurane as the main anaesthetic agent. We used a process of minimization to achieve similar number of patients for each surgical procedure. The first patient for each operation is allocated at random. For each subsequent patient we determined which treatment would lead to a better balance between the groups with respect to the type of operation. The patient is then randomized using a weighting system in favour of the treatment which will minimize the imbalance.[13] Measurement of cTnT was done by the hospital clinical chemistry laboratory, and the analysts were unaware of the conduct of this study.

Patients received premedication consisting of oral atropine (0.02 mg kg^{-1}) and midazolam (0.5 mg kg^{-1}) 30 min before induction of anaesthesia. Anaesthesia was induced with sevoflurane followed by a bolus of sufentanil (1 μg kg^{-1}) and pancuronium (0.2 mg kg^{-1}). For maintenance of anaesthesia, (30 patients in each group) patients received either a continuous infusion of midazolam (0.2 mg kg^{-1} h^{-1}), a continuous infusion of propofol (6 to 8 mg kg^{-1} h^{-1}), or an end tidal concentration of sevoflurane of 2 to 3% throughout the operation.

Each patient received a continuous infusion of sufentanil (2 μg kg^{-1} h^{-1}) throughout the operation. The lungs of the patients were mechanically ventilated with a mixture of oxygen and air. Mechanical ventilation was maintained until the start of cardiopulmonary bypass (CPB). After heparin administration (3 mg kg^{-1}) and aorta cannulation, CPB was instituted with a Dideco hollow fibre oxygenator with a blood flow between 200 and 300 ml kg^{-1} min^{-1}.

The prime volume 325 to 750 ml according to the patient's weight, contained lactate free Ringer's solution, albumin, mannitol, blood and heparin. Body temperature during bypass was maintained at 28 °C except for patients undergoing circulatory arrest who were cooled to 20 °C during the period of circulatory arrest. Patients underwent modified ultrafiltration at the end of the bypass.

Blood samples (0.5 ml) were taken immediately after admission, and 8, 15 and 24 h after admission to the paediatric intensive care unit. Samples were collected in a Gel-Microtainer tube and immediately analysed by the hospital clinical chemistry department using the Elecsys Modular E170 immunochemistry analyzer (Cardiac Troponin T, Roche Diagnostics, Mannheim).

Briefly, this immunoassay employs two monoclonal antibodies specifically directed against human cardiac troponin T. The antibodies recognize two epitopes located in the central part of the cardiac troponin T protein. The lower detection limit is 0.01 ng ml^{-1}. Ten patients admitted to the paediatric intensive care unit before surgery had cTnT levels measured as part of standard clinical practice.

Arterial oxygen tension (PaO_2), pH, base excess (BE), bicarbonate and lactate were measured immediately after admission to the intensive care unit and 24 h later (Chiron 865, Bayer, Mijdrecht, The Netherlands). Fluid balance in the 36 h following admission, and ventilator hours were also recorded.

The type of vasoactive drugs and their amount were recorded after admission to the intensive care unit and 24 h later. To quantify inotropic support, inotrope scores were calculated as the sum of all inotrope doses correcting for potency (dopamine, dobutamine = 1, milrinone = 15, epinephrine = 100).[14,15] Fluid intake (including crystalloids, colloids and blood products), output (urine, blood and serous fluid loss) and fluid balance were recorded over a 36 h period following admission to the intensive care unit.

Statistical analysis

In a retrospective analysis of fifteen patients the mean (SD) cTnT postoperatively was 1.92 (2.13) ng ml^{-1}. A power analysis based on these findings showed that we would need 90 patients to detect a difference in cTnT of 2 ng ml^{-1} with = 0.05 and a power of 95%.

Data were analysed with the statistical package SPSS v10, and are summarized as mean and 95% confidence intervals. Patient's characteristics (age, weight, surgery times and ventilator hours) and fluid balance were analyzed with ANOVA Bonferroni correction for post-hoc analysis. Because cTnT concentrations were not normally distributed, the data were first subjected to a natural logarithmic transformation before analysis by repeated measures ANOVA with the Greenhouse-Geisser correction. Differences (T8) from baseline values were analysed with ANOVA Bonferroni correction for pos-thoc analysis. Blood gas variables were also analyzed with repeated measures ANOVA. Categorical data were analysed using the Chi-squared test. Correlation coefficients between variables were calculated using Pearson test for normally distributed data and Spearman test for non normally distributed data. Values of $P < 0.05$ were considered statistically significant.

Results

The groups were comparable with respect to sex, age, weight, type of surgery, and cardiopulmonary bypass, aortic cross clamp and circulatory arrest times (Table 1). The number of cyanotic patients and those undergoing a ventriculotomy were well matched in each group. Table 2 shows the type of operations performed in each group. In the 10 patients from whom blood samples were obtained preoperatively (midazolam = 3, propofol = 4 and sevoflurane = 3), cTnT concentrations were less than 0.02 ng ml^{-1}. These patients were admitted to the intensive care unit preoperatively for logistical reasons the day before surgery. Table 3 shows cTnT concentrations throughout the study period in the three groups. Figure 1 shows the changes in cTnT in the three groups throughout the study period. Although the cTnT concentrations in the midazolam group tended to be higher at T8 than in the other two groups, the differences were not significant.

	Midazolam	Propofol	Sevoflurane
N	30	30	30
Age (months)	6 (0.9 – 11)	14 (2.9 – 26)	13 (5.4 – 21)
Sex (M/F)	17/13	20/10	15/15
Weight (kg)	5 (3.8 – 6.2)	7.5 (4.8 – 10)	6.8 (5.3 – 8.4)
Surgery time (min)	183 (159 – 208)	190 (166 – 215)	194 (172 – 216)
Bypass time (min)	114 (90 – 137)	122 (99 – 144)	120 (102 – 138)
Clamp time (min)	77 (56 – 98)	74 (53 – 96)	67 (50 – 85)
Arrest time (min)	9 (0 – 19)	9 (0 – 18)	7 (0 – 13)
Ventriculotomy (Y/N)	9/21	7/23	10/20
Cyanotic (Y/N)	14/16	17/13	16/14
Ventilator hours	87 (60 – 115)	106 (59 – 153)	84 (48 – 120)

Table 1: Patient characteristics. Values are expressed as mean (95% confidence intervals).

The differences of cTnT concentrations between T8 (maximum peak) and baseline for the three groups were (mean (95% Confidence Intervals)) 0.85 (1.2 – 1.49) ng ml^{-1} for midazolam, 0.7 (0.13 – 1.27) ng ml^{-1} for propofol and 0.05 (0 – 0.38) ng ml^{-1} for sevoflurane. Between groups variations were not statistically significant ($p < 0.49$) either.

There were no significant differences in PaO_2, pH, base excess, bicarbonate or lactate between the groups. The differences in fluid balance, PaO_2/FiO_2 ratios and ventilator hours in the 36 h following admission to the PICU also were not significantly different. There were no differences between the groups in the use of inotropic drugs on admission to the intensive care or 24 hours later. One patient in the sevoflurane group died 30 days after readmission to the intensive care unit due to irreversible cardiac failure.

	Midazolam	Propofol	Sevoflurane
VSD	8	8	6
ToF	3	3	6
TAPVC	2	1	2
Arterial switch	6	6	3
Norwood stage I	2	3	2
IAA	1	0	1
Pulmonary homograft	2	1	2
Fontan	1	2	2
AVSD	2	3	3
Aortic stenosis	2	1	0
Glenn	1	2	2
MVR	0	0	1
Total	30	30	30

Table 2: Surgical procedures. Ventricular septal defect (VSD), Tetralogy of Fallot (ToF), Total anomalous pulmonary venous connection (TAPVC), Interrupted aortic arch (IAA), Atrioventricular septal defect (AVSD), mitral valve replacement (MVR).

	T0	T8	T15	T24
Midazolam	1.9 (1.5 – 2.3)	2.7 (1.9 – 3.5)	2.4 (1.8 – 2.9)	1.9 (1.5 – 2.3)
Propofol	1.9 (1.4 – 2.4)	2.6 (1.7 – 3.5)	2.3 (1.5 – 3.1)	2.1 (1.3 – 2.8)
Sevoflurane	1.7 (1.3 – 2.1)	1.7 (1.3 – 2.2)	1.6 (1.2 – 1.9)	1.5 (1.2 – 1.8)

Table 3: Cardiac troponin T concentrations (ng ml^{-1}) in blood immediately after admission to PICU and at 8, 15 and 24 h after admission. Values are expressed as mean (95% confidence intervals).

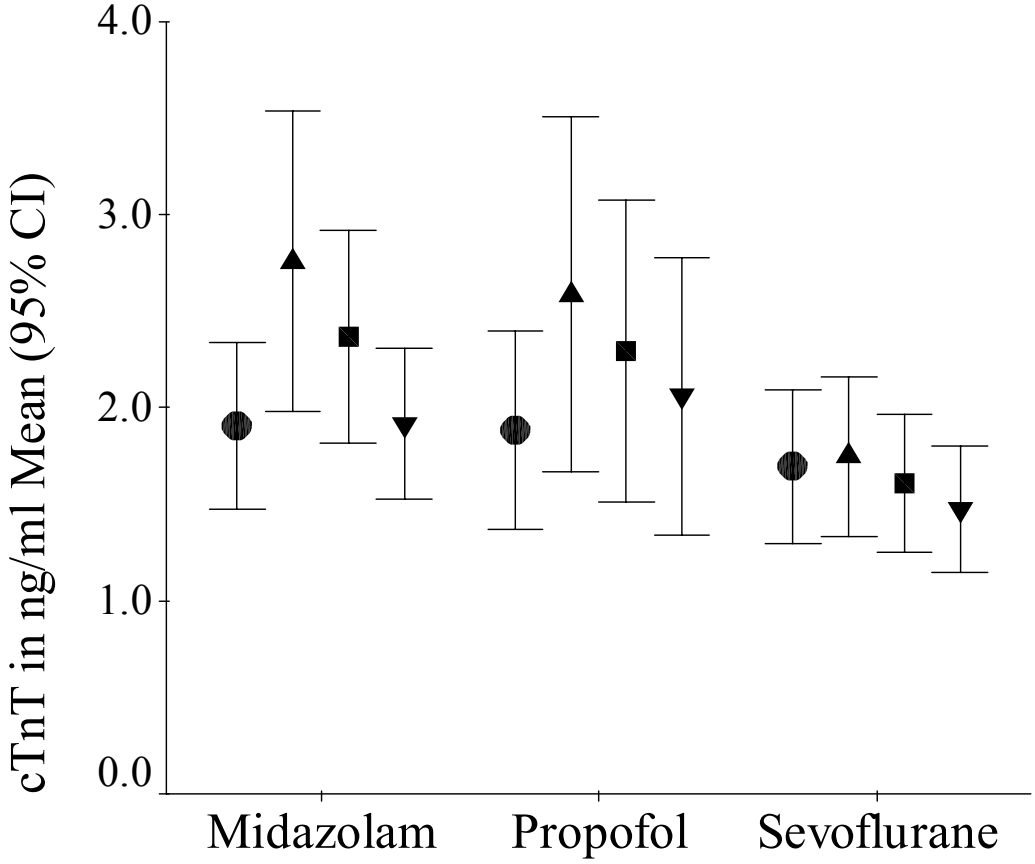

Fig 1: cTnT concentrations in the three groups at the four different time points. Values are expressed as mean (95% confidence intervals).T0 (●), T8 (▲), T15 (■) and T24 (▼).

Concentrations of cTnT peaked at T8 in all three groups. Univariate analysis of variance showed that cTnT concentrations at this time point were not related to sedation, cyanosis or the use of ventriculotomy. At T8 acyanotic patients had lower concentrations of cTnT than cyanotic patients in all groups although the differences were not significantly different. The values for acyanotic patients were 2.68 (1.8 – 3.5) ng ml^{-1} for midazolam, 2.28 (1.3 – 3.3) ng ml^{-1} for propofol and 1.67 (1.2 – 2.2) ng ml^{-1} for sevoflurane. The corresponding values for cyanotic patients were 2.84 (1.4 – 4.3) ng ml^{-1} for midazolam, 2.82 (1.3 – 4.3) ng ml^{-1} for propofol and 1.81 (1.1 – 2.5) ng ml^{-1} for sevoflurane.

There was a significant correlation between ventilator hours and cTnT concentrations at T8 in the sevoflurane group (r = 0.45) and midazolam group (r = 0.50), but not in the propofol group. There was no correlation between cTnT concentrations and the inotropic score on admission to the PICU.

Discussion

Our study shows that the postoperative production of cTnT in paediatric patients undergoing cardiac surgery is similar with midazolam, propofol or sevoflurane anaesthesia. To our knowledge this is the first time this has been reported. Peak concentrations of cTnT 2.7 (1.9 – 3.5) ng ml^{-1} (mean (95 % Confidence Intervals)) are similar to those reported in other studies. Immer and colleagues[12,16] reported mean cTnT concentrations of 4.06 ng ml^{-1} and 5.5 ng ml^{-1} in two consecutive studies with a patient population similar to ours. Unfortunately they failed to mention the type of anaesthetic technique used by them.

Hovels-Gurich and colleagues[17] reported a mean value of 5 ng ml^{-1} in neonates with transposition of the great arteries undergoing arterial switch operation. However all these patients underwent surgery with circulatory arrest. Seven patients in our study underwent a similar period of circulatory arrest, with a maximum cTnT concentration at T8 of 3.9 ng ml^{-1}.

IP has been little investigated in children. Classical IP in rats is not present at birth, and the enhanced recovery of contractile function develops only at the end of the first postnatal week.[7] Baker and colleagues found that preconditioning can be induced in isolated perfused normoxic immature rabbit hearts.[8] In the same study they found that isolated immature rabbit hearts that were chronically hypoxic from birth could not be preconditioned, even when the number of occlusion periods was increased. In an animal study,[18] pregnant rats were exposed chronically to intermittent periods of hypoxia. Their new born offsprings underwent periods of IP immediately after birth. Neither procedure in isolation increased tolerance to subsequent periods of hypoxia, while the combination increased cardiac tolerance.

A phenomenon similar to reperfusion injury happens at the start of CPB in cyanotic patients. Allen and colleagues[19] took biopsies of myocardial tissue in acyanotic and cyanotic patients before and 10 minutes after initiating bypass to measure antioxidant reserve capacity. In contrast to acyanotic patients, abrupt reoxygenation of cyanotic patients resulted in a significant depletion of endogenous tissue antioxidants. The effect of cyanosis on cTnT production was not our primary end point. Univariate analysis demonstrates that cyanosis did not have any influence in our results. cTnT concentrations were consistently higher in cyanotic patients in all three groups.

The differences however were not statistically significant. This is in contrast with two other studies[20,21] where unfortunately there is no description of the anaesthetic technique.Preliminary data indicate that cTnT values shortly after surgery for congenital heart disease are potentially useful prognostic indicators of postoperative recovery.[12,17] Once again those studies did not mention the type of anaesthetic agent used perioperatively.

We have demonstrated in our study that only ventilator hours correlates with cTnT production, and this was limited to the midazolam and sevoflurane group. Although statistically significant the correlations are weak.

Both cardiac troponin I (cTnI) and cTnT seem to evolve in a similar way after paediatric cardiac surgery.[16] Reported baseline values for cTnT remain below the standard cutoff point of 0.1 ng ml^{-1}. False pathological values of cTnT in patients with renal failure make cTnI theoretically a better choice. None of our patients developed renal failure during their admission to intensive care. However cTnI also has its limitations. Sasse and colleagues[22] showed that for up to nine months after birth in healthy infants, and for up to two years in infants with congenital heart disease, cTnI is not expressed solely in the myocardium. Slow twitch skeletal muscle troponin I is expressed in variable amounts in these infants.

Propofol decreases postischaemic myocardial mechanical dysfunction, infarct size and histological degeneration. It also suppresses the activity of neutrophils, and may therefore produce its beneficial effects by reducing free radicals, Ca^{2+} influx and neutrophil activity.[23] Other studies have failed to demonstrate a protective effect of propofol on myocardial function during ischaemia and reperfusion, and it does not appear to act as a preconditioning agent.[24]

Volatile anaesthetics improve recovery of contractile function of the stunned myocardium. Sevoflurane mimics ischaemic preconditioning, with an improvement of postischaemic contractility in isolated guinea pig hearts.[25] It also appears to reduce myocardial infarct size and to decrease the time threshold for ischaemic preconditioning in dogs, through activation of adenosine triphosphate-regulated potassium (K_{ATP}) channels.[26,27] The cardioprotective effect was independent of changes in coronary blood flow or a reduction in cardiac work. In addition to its preconditioning effects, sevoflurane also appears to exhibit cardioprotective effects against reperfusion injury. This effect has been attributed to its radical scavenging properties and the reduction of postischaemic adhesion of neutrophils.[28]

It has been suggested that sevoflurane may preserve myocardial function better than propofol in patients undergoing coronary artery bypass surgery.[29] Troponin I concentrations were significantly lower in the sevoflurane group than in the propofol group.[29] Compared to propofol, anaesthesia with either sevoflurane or desflurane resulted in a shorter length of stay in the ICU in adults after coronary artery surgery.[30] In our study the type of anaesthesia did not influence length of stay in the paediatric intensive care unit.

Benzodiazepines have not been investigated extensively in the adult population, and we could find no references in the literature to their myocardial effects in paediatric cardiac surgical patients. In isolated perfused guinea pig hearts subjected to ischaemia midazolam reduced neutrophil adhesion to non-ischaemic control levels.[31] Adhesion of polymorphonuclear neutrophils (PMN) to the coronary endothelium is a crucial step in the development of ischemic myocardial injury. In this study ketamine and thiopental behaved in a similar way. Midazolam may interfere with Ca^{2+} influx and free radical production but the data is contradictory in this respect.[32,33]

If IP occurs in children, sevoflurane seems to lack the IP-like effect demonstrated in the adult population.[30] Propofol and sevoflurane may provide protection to the adult myocardium by different mechanisms. However, they both appear equally effective in our study.

In conclusion we have demonstrated that midazolam, propofol and sevoflurane produce similar concentrations of cTnT postoperatively in paediatric cardiac surgery. Contrary to what has been published in adult patients undergoing coronary bypass surgery, sevoflurane did not reduce significantly cTnT production when compared to propofol.

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CHAPTER 7

Effect of dexamethasone on postoperative cardiac troponin T production in paediatric cardiac surgery

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Objectives: Paediatric cardiac surgery is associated with a temporary rise in cardiac troponin T (cTnT) during the postoperative period. We have tested the hypothesis that dexamethasone given before cardiopulmonary bypass starts may have myocardial protective effects as assessed by the postoperative production of cTnT.

Design: Prospective randomized interventional study.

Setting: Paediatric intensive care unit in a university hospital.

Interventions: Patients were randomly allocated to act as controls or receive a single dose of dexamethasone (1 mg kg^{-1}) during induction of anaesthesia.
Measurements and results: cTnT was measured four times postoperatively; immediately after admission to the paediatric intensive care unit (PICU) and at 8, 15 and 24 h thereafter. Both groups had similar cTnT concentrations on PICU admission; 2 (1.56 – 2.51) ng ml^{-1} (mean (95% Confidence Intervals)) in the group without dexamethasone and 1.85 (1.55 – 2.15) ng ml^{-1} in the group with dexamethasone. Concentrations of cTnT 8 h after admission to the PICU were 3.08 (2.46 – 3.69) ng ml^{-1} in patients not receiving dexamethasone and 1.99 (1.53 – 2.45) ng ml^{-1} in patients receiving dexamethasone. Overall differences between the two groups were significant ($P < 0.035$). After subgroup statistical analysis differences between the two groups remained significant only at 8 h ($P < 0.005$). At 15 h and 24 h after admission to the PICU differences were no longer significant.

Conclusions: The use of dexamethasone (1 mg kg^{-1}) before cardiopulmonary bypass starts is associated with a short lived significant reduction in postoperative cTnT production. The clinical significance of this effect is unclear.

The use of cardiopulmonary bypass (CPB) can induce a systemic inflammatory response syndrome in the postoperative period. This phenomenon is probably more exaggerated in the paediatric population as a greater proportion of the patient's blood is exposed to the surface of the CPB circuit.[1] The proinflammatory response associated with the use of CPB in children has been reviewed extensively elsewhere.[2] Within the paediatric population, neonates may react differently to CPB exposure, with higher proinflammatory cytokine production.[3]

Corticosteroids have been used extensively in adult cardiac surgery. Patients given methylprednisolone before CPB started had higher cardiac indices than those given placebo.[4] More recent studies, however, have shown that the use of methylprednisolone offered no clinical advantage in adults undergoing cardiac surgery with CPB.[5] Experience with steroids in paediatric cardiac surgery, on the other hand, is limited. The assumption has always been that the paediatric myocardium is more resistant to hypoxia than the adult one. This may not be the case.[6]

Steroids given before CPB starts reduce significantly the postoperative production of proinflammatory cytokines in children[7] and may provide myocardial protection during cardiac surgery. The use of troponins as surrogate markers to assess the potential myocardial protective effect of steroids is not a new concept.[8,9] However in those studies the investigators measured cardiac troponin I (cTnI). Sasse and colleagues[10] showed that up to nine months after birth in healthy infants, and for up to two years in infants with congenital heart disease, cTnI is not expressed solely in the myocardium, but is also expressed in variable amounts from slow twitch skeletal muscle.

Cardiac troponin T (cTnT) is a specific marker of myocardial infarction.[11] It is also a reliable marker of myocardial injury in children. Cardiac troponin T (cTnT) concentrations rise postoperatively in paediatric patients undergoing cardiac surgery with CPB;[12] up to three times the average cTnT that could be found in adult patients undergoing coronary artery bypass surgery.[13]

We have tested the hypothesis that dexamethasone given before CPB starts may have myocardial protective effects as assessed by the postoperative production of cTnT, the inotropic support and ventilator hours necessary after surgery. Subgroup analysis in cyanotic and neonatal patients was also evaluated for the same hypothesis. This study has been presented in part at a recent international meeting.[14]

Materials and Methods

After approval by the hospital ethics committee and parental consent 140 patients were prospectively investigated. This study was conducted between October 2003 and December 2004. Approximately 300 paediatric patients per year undergo cardiac surgical procedures in our institution. Patients operated without CPB were not recruited. cTnT was measured four times during the first 24 h following admission to the paediatric intensive care unit (PICU). This is standard practice in our institution. Patients were randomized using standard randomization tables to receive either dexamethasone (1 mg kg^{-1}) during induction of anaesthesia or act as controls. The use of placebo is not allowed by our hospital ethics committee. We used a process of minimization to achieve similar number of patients for each surgical procedure. The first patient for each operation was allocated at random. For each subsequent patient we determined which treatment would lead to a better balance between the groups with respect to the type of operation. The patient was then randomized using a weighting system in favour of the treatment which would minimize the imbalance.[15] cTnT concentrations were measured by the hospital clinical chemistry laboratory, and the analysts were unaware of the conduct of this study.

The anaesthetic technique was similar for both groups. Patients received a premedication consisting on oral atropine (0.02 mg kg^{-1}) and midazolam (0.5 mg kg^{-1}) 30 min before induction of anaesthesia. Anaesthesia was induced with sevoflurane followed by a bolus of sufentanil (1 μg kg^{-1}) and pancuronium (0.2 mg kg^{-1}). Maintenance of anaesthesia consisted on a combined continuous infusion of either midazolam (0.2 mg kg^{-1} h^{-1}) or propofol (6 mg kg^{-1} h^{-1}), and sufentanil (2 μg kg^{-1} h^{-1}). The lungs of the patients were ventilated with oxygen/air (F_IO_2 = 0.5). Ventilation was discontinued during CPB. After heparin administration (3 mg kg^{-1} or 300 IU kg^{-1}) and aorta cannulation, CPB was instituted with a Dideco hollow fibre oxygenator with a blood flow between 200 and 300 ml kg^{-1} min^{-1}. The prime volume, 325 to 750 ml according to the patient's weight, contained lactate-free Ringer's solution, albumin, mannitol, blood and heparin. Body temperature during bypass was maintained at 28 °C except for patients undergoing circulatory arrest, who were cooled to 20 °C during the period of circulatory arrest. Patients underwent modified ultrafiltration at the end of the bypass. The amount of fluid ultrafiltered was 60 (49 – 63) ml kg^{-1} (mean 95% Confidence Intervals).

In the PICU, patients were sedated with a combination of midazolam (0.1 -0.2 mg kg^{-1} h^{-1}) and morphine (10-20 μg kg^{-1} h^{-1}). Our practice is not to use diuretics in the first 24 h after admission to the PICU.

Blood samples (0.5 ml) were taken for measurement of cTnT concentrations immediately after admission to the paediatric intensive care unit (PICU), and 8, 15 and 24 h after admission. Samples were collected in a Gel-Microtainer tube and immediately analysed using the Elecsys Modular E170 immunochemistry analyzer (Cardiac Troponin T, Roche Diagnostics, Mannheim, Germany). Briefly, this immunoassay employs two monoclonal antibodies specifically directed against human cardiac troponin T. The antibodies recognize two epitopes located in the central part of the cardiac troponin T protein. The lower detection limit is 0.01 ng ml^{-1}. Ten patients admitted to the PICU before surgery had preoperative cTnT concentrations measured as part of standard clinical practice.

Arterial oxygen tension (PaO_2), pH, base excess (BE), bicarbonate and lactate were recorded immediately after admission to the intensive care unit and 24 h later (Chiron 865, Bayer, Mijdrecht, The Netherlands). Ventilator hours were also recorded.

The type and amount of vasoactive drugs were recorded after admission to the PICU and 24 h later. To quantify inotropic support, inotrope scores were calculated as the sum of all inotrope doses correcting for potency (dopamine, dobutamine = 1, milrinone = 15, epinephrine = 100). Fluid intake (including crystalloids, colloids and blood products), output (urine, blood and serous fluid loss) and fluid balance were recorded over a 36 h period following admission to the PICU.

Statistical analysis

In a retrospective analysis of fifteen paediatric patients who had undergone cardiac surgery the mean (SD) cTnT concentration postoperatively was 1.92 (2.13) ng ml^{-1}. A power analysis based on these findings showed that we would need 90 patients to detect a difference in cTnT of 2 ng ml^{-1} with = 0.05 and a power of 95%. In the same retrospective analysis the mean (SD) ventilator hours was 64 (37) hours and more than 50% of the patients needed two or more inotropic drugs postoperatively.

A power analysis based on these findings showed that we would need 136 patients to detect a reduction of 18 h in ventilation time and 130 patients to detect a 50% reduction on inotropic support. These two variables with = 0.05 and a power of 80%.

Data were analysed with the statistical package SPSS v10, and are summarized as mean and 95% confidence intervals. Patient characteristics (age, weight, surgery times and ventilator hours), fluid balance and blood gas variables were analyzed with unpaired t test for normally distributed data and Mann-Whitney test for non-normally distributed data. Because cTnT concentrations were not normally distributed, the data were first subjected to a natural logarithmic transformation before analysis by repeated measures ANOVA with the Greenhouse-Geisser correction. Categorical data were analyzed using the Chi-squared test. Correlation coefficients between variables were calculated using Pearson test for normally distributed data and Spearman test for non normally distributed data. Values of $P < 0.05$ were considered statistically significant. Data are presented as mean (95% Confidence Intervals).

Results

The groups were comparable with respect to age, sex, weight, surgery times, aortic cross-clamp, bypass and arrest times (Table 1). The number of cyanotic patients and those who underwent a ventriculotomy were similar in both groups. There were no statistically significant differences between the groups with regards to ventilator hours, PaO_2/FiO_2 ratios, and lactate. PaO_2/FiO_2 ratios did not change significantly when values from acyanotic and cyanotic patients were analyzed separately. Fluid intake did not differ between the groups throughout the study period, although the positive balance in the patients receiving dexamethasone was significantly less during the first postoperative day. During day two there were no differences between the two groups in terms of fluid intake, output and total fluid balance. Table 2 shows the type of operations performed in each group. Table 3 shows the type of operations performed in neonates while table 4 shows the type of operations performed in cyanotic patients. In the 10 patients from whom blood samples were obtained preoperatively, cTnT concentrations were less than 0.02 ng ml^{-1}.

Differences between the two groups were statistically significant ($p < 0.035$). Both groups had comparable cTnT concentrations on PICU admission (T0); 2 (1.56 – 2.51) ng ml^{-1} in the group without dexamethasone and 1.8 (1.54 – 2.14) ng ml^{-1} in the group given dexamethasone.

	No dexamethasone	Dexamethasone
Number of patients	70	70
Age (months)	12.8 (6.4 – 19.3)	12.2 (6.7 – 17.8)
Sex (M/F)	33/37	43/27
Weight (kg)	7.3 (5.6 – 8.9)	6.4 (5.3 – 7.5)
Surgery time (min)	198 (184 – 212)	207 (188 – 226)
Bypass time (min)	124 (110 – 137)	127 (112 – 142)
Aortic clamp time (min)	78 (64 – 91)	72 (60 – 84)
Arrest time (min)	7.5 (2.1 – 12.8)	6.5 (2.7 – 10.3)
Ventriculotomy (Y/N)	20/50	16/54
Cyanotic (Y/N)	35/35	40/30
Ventilator hours	97 (74 – 119)	99 (76 – 122)
Inoscore day one	10 (8 – 13)	9 (6 – 12)
Inoscore day two	11 (8 – 14)	10 (8 – 13)
Fluid balance day one		
Intake (ml kg^{-1})	69 (62 – 77)	62 (55 – 69)
Output (ml kg^{-1})	34 (28 – 40)	37 (32 – 42)
Balance (ml kg^{-1})	35 (28 – 43)	25 (17 – 32)*
Fluid balance day two		
Intake (ml kg^{-1})	100 (86 – 114)	99 (88 – 110)
Output (ml kg^{-1})	73 (60 – 85)	80 (60 – 100)
Balance (ml kg^{-1})	27 (16 – 37)	18 (-2.5 – 39)
Lactate day one	1.98 (1.44 – 2.53)	1.59 (1.28 – 1.91)
Lactate day two	1.80 (1.58 – 2.02)	1.57 (1.40 – 1.74)
PaO_2/FiO_2 day one	41.8 (35.8 – 47.7)	36.6 (30.4 – 42.8)
PaO_2/FiO_2 day two	32.9 (27.9 – 38)	33.3 (27.8 – 38.7)

Table 1: Patient characteristics. Values expressed as mean (95% Confidence Intervals).* Denotes statistical significance.

However, subgroup analysis demonstrated that only at 8 h after admission, the cTnT concentrations were significantly higher ($p < 0.005$) in the non-dexamethasone group (3.1 (2.5 – 3.7) ng ml^{-1}) than in the dexamethasone group (1.9 (1.5 – 2.4) ng ml^{-1}). There were no significant differences in the cTnT concentrations between the groups at the other times. cTnT concentrations at T15 were 2.65 (2.12 – 3.19) ng ml^{-1} in the non-dexamethasone group and 1.95 (1.46 – 2.43) ng ml^{-1} in the dexamethasone group.

	No dexamethasone	Dexamethasone
AS	3	1
Atrial septation	1	
AVSD	7	6
Fontan	6	2
Glenn	5	10
Homograft	2	2
IAA	1	1
MAPCA	1	1
MVA	1	2
MVR	1	
Norwood	4	5
PS	1	1
Rastelli	1	1
Switch	10	10
TAPVC	2	3
ToF	9	9
Truncus	2	1
TVA		1
TVR	1	
VSD	12	14
Total	70	70

Table 2: Type of operations. Aortic stenosis (AS), Atrioventricular septal defect (AVSD), Interrupted aortic arch correction (IAA), Major aortopulmonary collateral arteries (MAPCA), Mitral valve anuloplasty (MVA), Mitral valve replacement (MVR), Pulmonary stenosis (PS), Total anomalous pulmonary venous connection (TAPVC), Tetralogy of Fallot (ToF),Tricuspid valve anuloplasty (TVA), Tricuspid valve replacement (TVR), Ventricular septal defect (VSD).

At T24 were 2.27 (1.77 – 2.76) ng ml^{-1} in the non-dexamethasone group and 1.81 (1.33 – 2.28) ng ml^{-1} in the dexamethasone group. Figure 1 is a graphical representation of the changes in cTnT concentrations in the two groups. Figure 2 and 3 show changes in cTnT concentrations in cyanotic and neonatal patients respectively. Correlation between cTnT concentrations at T8 and ventilator hours was significant although weak. Correlation coefficient was r = 0.40 in patients not receiving dexamethasone and r = 0.49 in those receiving dexamethasone.

	No dexamethasone	Dexamethasone
Homograft		1
IAA	1	1
MAPCA	1	
MVR	1	
Norwood	4	5
Switch	8	8
TAPVC	1	1
Truncus	1	1
VSD	2	1
Total	19	18

Table 3: Type of operation in neonatal patients. Interrupted aortic arch correction (IAA), Major aortopulmonary collateral arteries (MAPCA), Mitral valve replacement (MVR), Total anomalous pulmonary venous connection (TAPVC).

	No dexamethasone	Dexamethasone
Fontan	6	2
Glenn	5	10
Homograft		1
MAPCA	1	1
Norwood	4	5
Rastelli	1	1
Switch	10	10
TAPVC		2
ToF	8	7
TVA		1
Total	35	40

Table 4: Type of operation in cyanotic patients. Major aortopulmonary collateral arteries (MAPCA). Total anomalous pulmonary venous connection (TAPVC). Tetralogy of Fallot (ToF). Tricuspid valve anuloplasty (TVA).

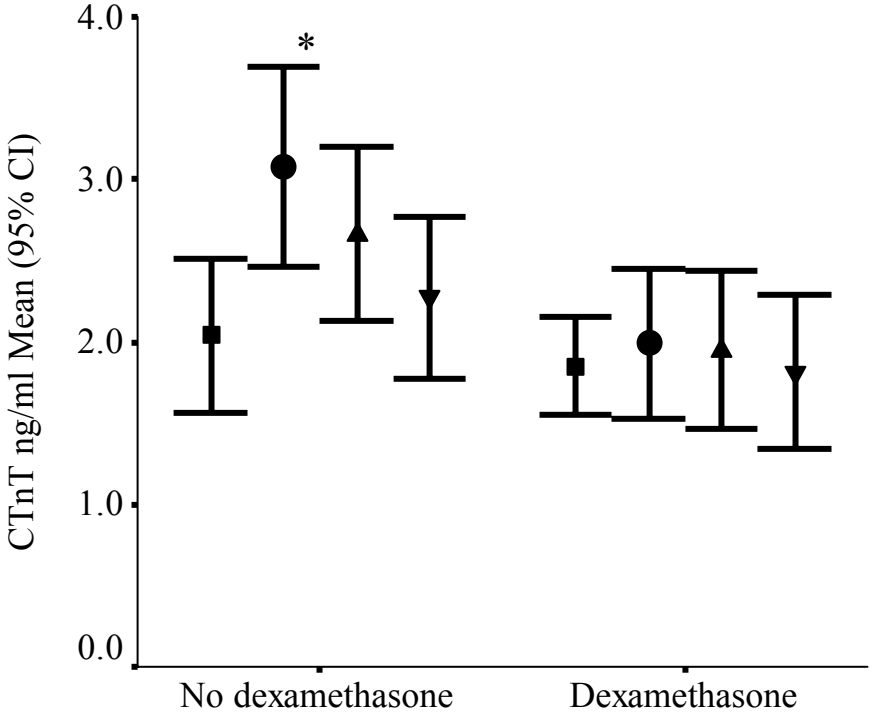

Fig 1: Changes in cTnT concentrations during the first 24 h after operation. (■) T0, (●) T8, (▲) T15 and (▼) T24.Values expressed as mean (95% Confidence Intervals). * Denotes statistical significance at that time point between the two groups.

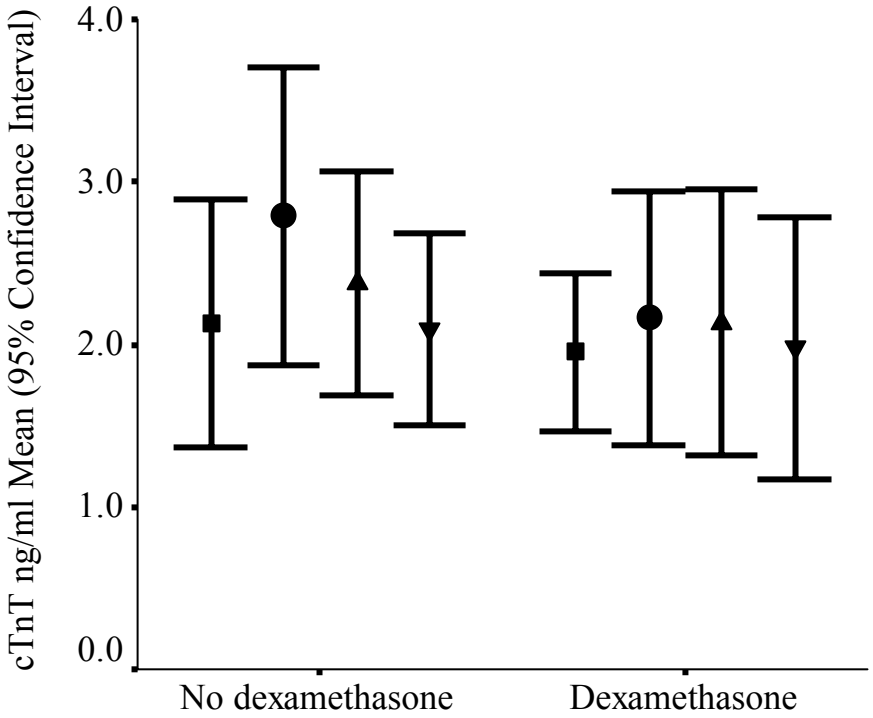

Fig 2: Changes in cTnT concentrations during the first 24 h after operation in cyanotic patients. (■) T0, (●) T8, (▲) T15 and (▼) T24. Differences are not significant.

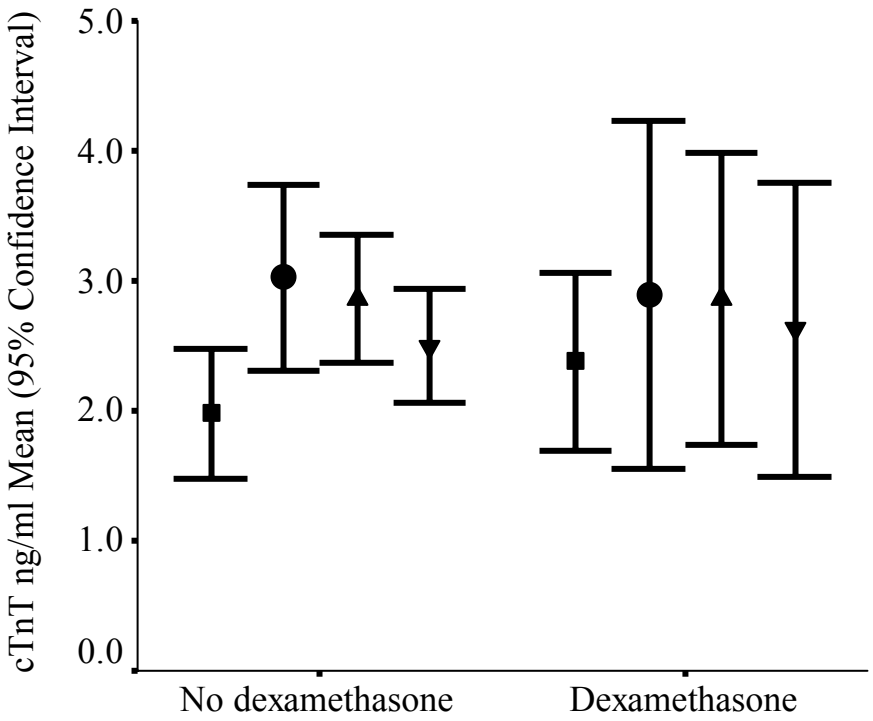

Fig 3: Changes in cTnT concentrations during the first 24 h after operation in neonatal patients. (■) T0, (●) T8, (▲) T15 and (▼) T24. Differences are not significant.

When both groups were analyzed together the correlation coefficient was r = 0.45. Correlations between cTnT concentrations (T8) and inotropic scores at admission and 24 h later were also weak in both groups; no dexamethasone group r = 0.38 (admission inotropic scores) and r = 0.51 (inotropic scores 24 h). In the dexamethasone group correlations were r = 0.29 (admission inotropic scores) and r = 0.55 (inotropic scores 24 h).

Discussion

The systemic inflammatory response syndrome following CPB has been implicated in myocardial injury leading to low cardiac output and increased inotropic requirements in the postoperative period.[16] One of the strategies aimed at protecting the myocardium is the use of steroids before CPB starts. We have shown in the present study that while dexamethasone reduces the postoperative production of cTnT in paediatric cardiac surgery, its effects are short lived. Fifteen hours after the end of the surgical procedure, there are no statistically significant differences between the dexamethasone and the control group.

The concentrations of cTnT found in our study are in line with what it has been reported in other studies. Immer and colleagues[12,17] reported mean cTnT concentrations of 4.06 ng ml^{-1} and 5.5 ng ml^{-1} in two consecutive studies with a patient population similar to ours. A mean concentration of 5 ng ml^{-1} was reported in neonates with transposition of the great arteries undergoing arterial switch operation with circulatory arrest.[18]

Checchia and colleagues[8] investigated the effect of dexamethasone (1 mg kg^{-1}) on the postoperative production of cTnI in 28 paediatric patients undergoing cardiac surgery with CPB. They found a statistically significant reduction in cTnI concentrations 24 h after surgery in patients who received dexamethasone compared to those given a placebo. In our study we did not find any difference on the cTnT concentrations 24 h after the surgical procedure between the two groups. Other investigators have found that cTnT peaked at 4 hours after CPB,[18] 30 minutes after CPB[20] and 2 hours after declamping.[21] It is not clear why cTnT concentrations peaked 8 h after admission to the PICU in our patients.

A beneficial effect of steroids on cTnI degradation has been demonstrated.[9] However this study was performed in animals subjected to a 2 h period of deep hypothermic circulatory arrest (DHCA) and methylprednisolone was given twice, 6 h before and immediately before CPB started.

Imura and colleagues[19] showed an age-dependent and hypoxia-related difference in myocardial injury during CPB. Other investigators[20] have also noted that cyanotic patients have higher cTnT concentrations postoperatively than the acyanotic counterparts. Reperfusion injury may explain this phenomenon. When CPB starts cyanotic patients are suddenly exposed to normoxic concentrations of oxygen. According to our results, cyanotic patients do not shown any improvement in the postoperative production of cTnT when dexamethasone is used.

The neonate myocardium has a distinct systemic inflammatory response to CPB, with higher production of proinflammatory cytokines when compared to older patients.[3] We could not find any improvement in cTnT production in neonates treated with dexamethasone. Bronicki and colleagues[7] found a significant reduction on postoperative fluid requirements in the paediatric cardiac surgical patients (n = 15) who received dexamethasone compared to the placebo group (n = 14). The timing and amount of dexamethasone was similar to our study design.

Fluid balance in the first postoperative day is significantly less in the dexamethasone group. However we consider this difference not clinically relevant. Fluid balance in the dexamethasone group on day one and the no dexamethasone group on day two are similar.

The use of steroids in adult cardiac surgery remains controversial.[5] To our knowledge there are no prospective or retrospective cohort studies that clearly show the influence of steroids on the clinical postoperative course. Nevertheless the use of steroids in paediatric cardiac surgery has become accepted practice in many institutions. Lindberg and colleagues[22] consider that omitting the use of dexamethasone in children weighing less than 10 kg scheduled for cardiac surgery is unethical.

Dexamethasone reduces C-reactive protein production without any effect on the release of protein S100B and Von Willebrand factor.[22] The concentration of proinflammatory cytokines decreases when steroids are used before CPB starts.[7,24] The reduction is even more pronounced if steroids are given before and during CPB.[23] Oxygen delivery and cardiac output improve faster when steroids were used in an animal model.[25] Steroids may not exert their effects through anti-inflammatory properties but by up-regulation of calpastatin,[9] a protein that prevents the degradation of cTnI at the intracellular level. Even the timing of steroid administration seems to be relevant.[26]

However when clinical end points have been used to test the benefits of steroids the results are not so impressive.[27] Our study was powered to detect a 50% reduction on inotropic support or an 18 h reduction in ventilator hours. For these targets we had to include 68 patients in each group. Our results however show that the use of dexamethasone was not associated with any significant improvement in the postoperative use of inotropic drugs, fluid requirements, lactate production, ventilator hours or PaO_2/FiO_2 ratios. Some concerns have been raised regarding the use of cTnT in patients with chronic renal failure.[28] None of the patients included in the study had either acute or chronic renal failure during the study period. Some studies have suggested that cTnT concentrations may be used as prognostic indicators of postoperative recovery.[17,18] In our study ventilator hours and inotropic scores correlated with cTnT production. However, although statistically significant the correlations were weak.

The present study has a number of limitations. We did not investigate cardiac function parameters (shortening fraction, ejection fraction) and its relationship with cTnT elevations in the postoperative period. Atriotomy[29] and ventriculotomy[30] influence cTnI production independent of myocardial damage related to other factors. The number of patients undergoing atriotomy was similar in both groups ($p < 1$) and therefore unlikely to influence the results.
More patients required a ventriculotomy in the control group ($n = 20$) than in the dexamethasone group ($n = 16$). While the difference is not significant ($p < 0.56$) it could have affected the results.

Ventilator hours in our study appear unacceptably high at first glance. However, some of the patients included in this study are known to have prolonged postoperative course (i.e. HLHS, TAPVC, Truncus arteriousus, etc.) while in the majority of patients (i.e. VSD, AVSD, ToF, TGA, valve surgery, Glenn, Fontan, etc.) extubation within 24 to 48 h is standard of care. The use of the mean as statistical instrument to describe the population may have interfered with these results. Power analysis for ventilator hours and inotropic support were based on average retrospective values from our intensive care unit.

In conclusion, this study has demonstrated that in paediatric patients undergoing cardiac surgery the use of dexamethasone given before CPB starts is associated with a significant albeit short lived reduction in postoperative production of cTnT. Subgroup analysis showed that dexamethasone did not improve postoperative production of cTnT neither in cyanotic nor in neonatal patients.

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SUMMARY

Cardiopulmonary bypass (CPB) induces a systemic inflammatory response syndrome (SIRS) in patients following cardiac surgery that can lead to major organ injury and postoperative morbidity. Initiation of CPB sets in motion an extremely complex and multifaceted response involving complement activation along with activation of platelets, neutrophils, monocytes, and macrophages. This in turn initiates the coagulation, fibrinolytic, and kallikrein cascades, increasing blood concentrations of various endotoxins and cytokines and increasing endothelial cell permeability.

The basic physiological insults caused by CPB have been associated with major postoperative morbidity, including neurological, pulmonary adrenal dysfunction, and/or haematological abnormalities. Additional clinical manifestations associated with the SIRS include increased metabolism (fever), fluid retention, myocardial oedema, and detrimental haemodynamic alterations.

The use of steroids to minimize or prevent the consequences of SIRS in the postoperative period has been extensively investigated in adults. Clinical investigations in the paediatric population are scarce. Our aim was to investigate how dexamethasone could influence the associated side effects of CPB in two organs, the small intestine and the heart. To that effect we chose two surrogate markers, gut permeability and cardiac troponin T production.

Intestinal mucosal ischaemia, although transient, can occur in infants and children during and after CPB. Gut permeability had not been previously investigated in children undergoing cardiac surgery. In *chapter two* we describe, in an observational study, the natural course of gut permeability in patients undergoing cardiac surgery with and without CPB. Gut permeability has been investigated in healthy children and neonates not undergoing surgical or medical interventions during the study period. Patients with congenital cardiac diseases have preoperative gut permeability values up to seven times what we could expect in healthy children of similar age.

In patients operated without CPB gut permeability was reduced in the postoperative period returning to near normal values 24 hours after surgery. On the other hand, in patients undergoing surgery with CPB gut permeability deteriorated even further in the postoperative period.

In *chapter three* we report the results of a study designed to test the hypothesis that dexamethasone has beneficial effects on intestinal permeability during the postoperative period. Dexamethasone given before CPB starts reduced intestinal permeability within 24 h after surgery. The differences are highly significant when compared to control patients not given dexamethasone. In the investigation reported in *chapter four* we studied the changes in intestinal permeability in patients undergoing stage I of the Norwood procedure.

Neonates with hypoplastic left heart syndrome (HLHS) undergo surgical repair in three stages. These patients suffer from an imbalanced circulation potentially exposing the intestine to chronic ischaemia. The surgical repair requires a period of circulatory arrest. It comes as no surprise, therefore, that HLHS patients are at high risk of developing necrotizing enterocolitis in the postoperative period, with devastating consequences. We found that HLHS patients have abnormal intestinal permeability before and after surgery.

Rhamnose is one of the four sugars used to test intestinal permeability. For the last thirty years it has been assumed that rhamnose is an inert sugar not metabolized by the human body. We have found this not to be the case, and the results are presented in *chapter five.*

The type of anaesthetic agent used during adult coronary bypass surgery may influence considerably the postoperative production of cardiac troponin T (cTnT), a protein that reflects the extent of myocardial damage after a period of hypoxia. In particular halogenated ethers may exert its effect through a process called anaesthetic preconditioning, a phenomenon similar to ischaemic preconditioning.

Anaesthetic preconditioning has not been investigated in paediatric cardiac surgery to the same extent as in adult cardiac surgery. In *chapter six* we present a study of the effects of three different anaesthetic agents, propofol, midazolam and sevoflurane, on the postoperative production of cTnT in paediatric cardiac surgical patients. Contrary to what happens in adult patients we could not find significant differences in the postoperative production of cTnT when midazolam, propofol or sevoflurane were used as anaesthetic agents.

In *chapter seven*, we report on a study designed to test the hypothesis that dexamethasone given before CPB starts may have myocardial protective effects as assessed by the postoperative production of cTnT. Subgroup analysis in cyanotic and neonatal patients was also evaluated for the same hypothesis.

We found that dexamethasone did reduce postoperative cTnT concentrations. However, the reduction was short lived and was not accompanied by improvements in any of the other clinical parameters measured.

SAMENVATTING

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CURRICULUM VITAE

Ignacio Malagon was born in Madrid, Spain on 3 December 1962. He attended medical school at the Universidad Complutense Madrid and graduated in 1986. In 1988 he obtained a Master in Philosophy on biochemistry at the same university. His anaesthetic training started in 1990 in Great Britain, and included a senior house officer rotation at Leicester Royal Infirmary and registrar positions in Birmingham and St Bartholomew's Hospital in London. In December 1994 he successfully completed the final exam for the fellowship at the Royal College of Anaesthetists of United Kingdom (FRCA). The subsequent training included a post as fellow in adult intensive care at St George's Hospital in London and a senior registrar rotation at Guy's and St. Thomas' Hospital in London. To round up his training he completed a year paediatric intensive care fellowship program at the Vancouver Children's Hospital in British Columbia, Canada.

In 1999 he was appointed consultant cardiac anaesthetist with a special interest in paediatric cardiac surgery at the Leiden University Medical Centre, where he has been working ever since.

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