Gut permeability and myocardial damage in paediatric cardiac surgery

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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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