Effects of dopexamine on blood flow in multiple splanchnic sites measured by laser Doppler velocimetry in rabbits undergoing cardiopulmonary bypass

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Decreased gut perfusion has been reported during cardiopulmonary bypass (CPB). Studies of treatments to avoid splanchnic ischaemia during CPB have given conflicting results. We studied 12 rabbits during mild hypothermic non-pulsatile CPB. Tissue blood flow in three different splanchnic areas (gastric, jejunum and ileum) was measured by laser Doppler velocimetry (LDV) before CPB (T0), after steady state (T1), after administration of dopexamine 2 µg kg⁻¹ min⁻¹ (T2) and 4 µg kg⁻¹ min⁻¹ (T3), and after return to baseline (T4). Splanchnic blood flow decreased during CPB. Dopexamine increased significantly jejunum LDV (100% at T1 to mean 271 (SD 210)% at T2) and ileum LDV (100% at T1 to 187 (112)% at T2). Gastric LDV was not altered by infusion of dopexamine during CPB. This could partly explain the conflicting results on the value of gastric tonometry as an index of splanchnic injury.

Keywords: heart, cardiopulmonary bypass; heart, dopexamine; gastrointestinal tract, blood flow, mucosal perfusion; rabbit

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Splanchnic blood flow is altered during low flow states (e.g. cardiogenic shock) and possibly during cardiopulmonary bypass (CPB). The incidence of reduced gut perfusion has been reported to be as high as 60%1 during this type of surgery. Even if the majority of these patients do not suffer directly from splanchnic sequelae, this reduction could act as a trigger for bacterial translocation, inflammatory mediator release2 3 and multi-organ dysfunction4

Unfortunately, human studies are difficult to perform because splanchnic blood flow is difficult to measure clinically. Some measurements are available only for gastric flow assessment (intramucosal pH, laser Doppler), others are invasive (hepatic vein cannulation) or difficult to perform during a short perioperative period (rhamnose permeability) and others are not accessible in human clinical practice (ileum blood flow). For these reasons, there is still debate on the regulation of splanchnic blood flow during CPB,5–8 monitoring of gastric flow9 and the effects of drugs on splanchnic regional blood flow.9–13 These changes differ from splanchnic injury induced by sepsis.

A CPB model with small animals (rabbits) has been developed by some teams14–16 using neonatal oxygenators and has proved useful in such studies. We used dopexamine, a DA1 receptor and β2 adrenoreceptor agonist with a weak activity on β1 receptors.17 18 DA1 agonists are potent mesenteric vasodilators.19

We have studied the effects of CPB on splanchnic blood flow assessed directly by laser Doppler in three different splanchnic areas (gastric, jejunum and ileum) and tested the potential of dopexamine to prevent CPB-induced decreased mesenteric blood flow.

Materials and methods

Twelve New Zealand white rabbits of both sexes (weight 3.0–3.5 kg) were given xylazine 1 mg/kg body weight i.m. as premedication and then anaesthetized with ketamine 75 mg/kg body weight i.m. (Imalgene, Rhone-Mérieux, Lyon F. France). Adequate depth of anaesthesia was ensured before any surgical procedures by the absence of pedal and palpebral reflexes. The marginal ear vein was cannulated (20-gauge cannula) for administration of fluids (hetastarch 3 ml kg⁻¹ h⁻¹) and drugs. The central ear artery was also cannulated (20-gauge cannula) and connected to a pressure
transcutaneous for measurement of arterial pressure during surgery. All catheters were flushed with heparinized saline 10 u. ml⁻¹ to prevent clotting during the experiment. A tracheotomy was performed (tracheal tube size 3.5 mm od) and the lungs ventilated mechanically using a neonatal apparatus (Dräger Cicero, Germany) with 100% oxygen. Tidal volume was set at 15 ml/kg body weight and ventilatory frequency 35 bpm. Ventilation was adjusted to maintain Pco₂ and pH in the physiological range. End-tidal gas concentrations were measured by integrated capnography. Body temperature was recorded using the thermistor of a 7-French gauge pulmonary artery catheter (Baxter, San Diego, CA, USA) inserted orally into the oesophagus to maintain temperature at 38.5–39.5°C before CPB. Limb lead II of the electrocardiogram was monitored continuously using subcutaneous needle electrodes. Continuous anaesthesia was maintained with ketamine 25 mg kg⁻¹ h⁻¹ and midazolam 0.125 mg kg⁻¹ h⁻¹ during surgery and measurements. The left carotid artery was cannulated with a 20-gauge cannula and connected to a fluid filled pressure transducer (Medex medical SARL Nantes, France). This catheter was used to obtain blood samples during the experiment and to measure non-pulsatile arterial pressure during CPB.

A laparotomy was performed by a midline incision and haemostasis obtained by diathermy. Systemic heparinization was performed (300 u./kg body weight). The femoral arteries were then ligated for the CPB time and the distal abdominal aorta was retrogradely cannulated with an 8-French gauge arterial perfusion cannula (DLP, Medtronic, Grand Rapids MI, USA) approximately 10 mm above the femoral bifurcation. The aortic cannula was flushed with methylene blue at the end of the preparation to check the integrity and normality of the mesenteric arteries. This technique is similar to that described previously and is controlled easily in terms of metabolic effects, as the surgical time is less than 1 h. After checking the pulsatility of flow in the arterial cannula, sternotomy was performed. The right atrium was cannulated with a 16-French gauge venous perfusion cannula (Stockert, Munich, Germany) via a purse string suture. Both aortic and venous cannulae were connected to the cardiopulmonary bypass circuit.

Cardiopulmonary bypass

We used a small size neonatal oxygenator (Microsafe, Polystan, Ballerup, Denmark) with a venous reservoir and heat exchanger, and connected with 7 mm tubing. Temperature was maintained at 38°C by a thermostatic water pump connected to the heat exchanger. Blood-gas concentrations were checked regularly and 0.42% sodium bicarbonate (10-ml bolus w:v) was injected to maintain base excess greater than –5 mmol litre⁻¹ and pH greater than 7.30 to avoid acidosis from femoral occlusion. Circuit priming consisted of 6.5% Hetastarch 300 ml, 0.42% sodium bicarbonate 20 ml, calcium chloride 250 mg, cefazoline 1 g, heparin 1000 u. and 100 ml of rabbit blood. This priming fluid has been found to achieve a rabbit haemoglobin concentration of 6–10 g dl⁻¹. Bypass was initiated at 100 ml kg⁻¹ min⁻¹ using a calibrated roller pump (Hospal BSM, Lyon, France). Oxygenation was ensured by continuous infusion of oxygen in the oxygenator (100 ml kg⁻¹ min⁻¹) and was adjusted to maintain arterial blood-gas tensions (Pao₂, Paco₂, pH, base excess) in the physiological range and particularly Paco₂ at 4.7–6.0 kPa. Pulmonary ventilation was disconnected. The left ventricle was vented by a 16-gauge cannula inserted in the left ventricle to drain the blood in the venous circuit of the bypass. Some pleural or mediastinal blood was scavenged and reinfused to the venous line.

Drugs

Dopexamine (IPSEN, Paris, France) was dissolved in 0.9% NaCl (w/v). The dissolved dopexamine was infused using a continuous infusion pump in the arterial line of the cardiopulmonary bypass close to the animal to avoid biomaterial adsorption.

Procedure

Baseline measurements (T0) were made before CPB. After steady state, CPB was instituted and a second set of measurements was performed (T1). Dopexamine was infused at 2 µg kg⁻¹ min⁻¹ (T2) and 4 µg kg⁻¹ min⁻¹ (T3) over 15 min for each period. At the end of infusion, a 15-min recovery period was allowed; a final measurement set was then performed (T4).

Each set of measurements included direct intestinal laser Doppler flowmetry (Perimed, Jarfalla, Sweden) in three different areas: gastric, ileum and jejunum. After calibration with a Perimed motility standard kit, a laser Doppler probe (Perimed P 424) was gently placed directly on the intestinal wall with a flexible design holder. This probe was connected to a 632 nm helium–neon laser and calibrated to obtain a penetration depth of 1 mm. This assumed a global mucosa and muscularis measurement.

Jejunum biopsies were performed at the four different times (before CPB, before and after infusion of dopexamine and at the end of CPB) with a flexible myocardial biotome gently introduced into the jejunum by a short puncture. Samples were fixed by Trump solution and examined under electron microscopy by an independent pathologist blinded to the study to evaluate the degree of mucosal ischaemic injury.

Data collection and analysis

Laser Doppler flow data were collected using a multichannel monitor and two probes, during steady state periods of 10 min. Samples were computerized and stored by specially designed software (Perisoft, Jarfalla, Sweden). Curves were analysed and mean values calculated for each period.

The laser Doppler flow data were expressed in perfusion units and normalized to values measured at the onset of CPB (T1).
Table 1 Mean arterial pressure (MAP) (mm Hg), temperature (Temp.) (°C) and absolute value of splanchnic laser Doppler velocimetry at steady state (LDV) (perfusion unit) (mean (SD)). *P<0.05 compared with T1; †P<0.05 compared with T0.

<table>
<thead>
<tr>
<th></th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP</td>
<td>70 (17)</td>
<td>73 (20)</td>
<td>58 (17)*</td>
<td>55 (20)*</td>
<td>49 (18)*</td>
</tr>
<tr>
<td>Temp.</td>
<td>37.8 (0.9)</td>
<td>37.9 (1.0)</td>
<td>37.8 (1.1)</td>
<td>37.9 (0.5)</td>
<td>37.6 (0.9)</td>
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<tr>
<td>Gastric LDV</td>
<td>507 (158)</td>
<td>182 (77)†</td>
<td>206 (110)</td>
<td>209 (144)</td>
<td>102 (90)*</td>
</tr>
<tr>
<td>Jejunum LDV</td>
<td>356 (154)</td>
<td>126 (114)†</td>
<td>213 (118)*</td>
<td>182 (104)</td>
<td>81 (50)</td>
</tr>
<tr>
<td>Ileum LDV</td>
<td>314 (118)</td>
<td>97 (65)†</td>
<td>149 (75)*</td>
<td>160 (116)*</td>
<td>94 (73)</td>
</tr>
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Statistical analysis

Initial sample size was calculated from previous results validating the model (not published) in order to obtain alpha and beta risks of more than 50% and a power of more than 50%, which are acceptable for this type of model.

Values are expressed as mean (SD). Statistical analysis was performed using Systat version 7.0 for Windows, 1997 SPSS INC statistical software. Comparisons were made by one-way ANOVA for repeated measurements followed by post hoc adjusted by Bonferroni correction. For jejunum data analysis, the within-subject variability for regional blood flow was significant for Fisher and Greenhouse–Geisser epsilon tests, and between-time analysis was not allowed. Then a paired t test adjusted by Bonferroni correction which was considered to be more robust, was performed. P<0.05 was considered significant.

Results

We performed 11 complete experiments. One case was discarded because of surgical bleeding problems at the beginning of CPB.

Data are summarized in Table 1. Central oesophageal temperature was maintained constant during CPB. It remained mildly hypothermic between 37.6 and 37.9°C (normal temperature is approximately 39°C). Mean arterial pressure was unchanged after onset of CPB (70 (17) to 73 (20) mm Hg); however, it decreased significantly after infusion of dopexamine (58 (17) at T2 and 55 (20) mm Hg at T3). Commencement of CPB decreased regional splanchnic blood flow in the three areas measured (Table 1).

Normalized values of splanchnic blood flow during CPB are represented in Figure 1. Infusion of dopexamine did not significantly change gastric tissue blood flow. However, ileum blood flow was increased by the two infusion rates of dopexamine (100% at T1, 187 (112)% with dopexamine 2 µg kg⁻¹ min⁻¹ and 209 (109)% with dopexamine 4 µg kg⁻¹ min⁻¹). Jejunum regional blood flow was enhanced by dopexamine 2 µg kg⁻¹ min⁻¹ (100% at T1, 271 (210)% at T2) with no significant difference at T3. Return to baseline after stopping infusion of dopexamine occurred in the jejunum (125 (119)% and ileum (119 (64)%), however, gastric blood flow was decreased at T4 (84 (32)%) compared with T1.

Severe ischaemic damage was not detected by surface electron microscopy examination of biopsy sample. Jejunal biopsies showed a stretched microvillus and junctional area.

Fig 1 Effect of dopexamine on splanchnic laser Doppler velocimetry (% of normalized value) before CPB (T0), after steady state (T1), after administration of dopexamine 2 µg kg⁻¹ min⁻¹ (T2) and 4 µg kg⁻¹ min⁻¹ (T3), and after return to baseline (T4). Gastric blood flow was not altered by infusion of dopexamine. Jejunum blood flow increased with dopexamine 2 µg kg⁻¹ min⁻¹. Ileum blood flow increased with both dopexamine infusion rates. Data are normalized to the first measurement after onset of CPB (T1). PU=Laser perfusion unit. *P<0.05 compared with T1.
Mucus was scarce even at the beginning of the preparation and more pronounced at the end.

**Discussion**

The main findings of this study were impairment of splanchnic blood flow during CPB and improvement in CPB-induced low flow rates in the jejunum and ileum after infusion of dopexamine. The low dose (2 µg kg\(^{-1}\) min\(^{-1}\)) dopexamine infusion rate was as efficient as the higher dose (4 µg kg\(^{-1}\) min\(^{-1}\)). Gastric blood flow was insensitive to dopexamine in this model.

Patients undergoing cardiac surgery with CPB are at risk of developing systemic inflammatory response syndrome after operation.\(^3\)\(^,\)\(^4\) Lack of ideal biocompatibility of the device circuitry has been identified as a trigger factor for this syndrome. Another trigger factor is impairment of splanchnic flow induced by CPB. In an experimental model, Tao and colleagues\(^2\) have shown that CPB increased superior mesenteric blood flow, whereas ileal mucosal blood flow assessed by laser Doppler decreased, associated with gut-derived metabolic acidosis. These authors related this CPB-induced mucosal ischaemia to mucosal hypoperfusion by vasoconstriction and to an increase in gut oxygen consumption. A marked difference from sepsis syndrome is that the trigger phenomena is a direct regional reduction in blood flow. During CPB, arterial oxygen saturation is always 100%, without any lactate production.

Preventing intestinal damage represents an important challenge because ischaemic intestines have been shown to allow bacterial translocation and endotoxaemia, which is involved in the development of the CPB inflammatory syndrome.\(^2\)\(^,\) Our study aimed to test if a vasodilating agent with splanchnic effects could prevent decreases in intestinal blood flow during CPB. Dopexamine is a β2 adreno-receptor agonist with moderate activity on α-receptor; it is free of α adrenoceptor activity and inhibits norepinephrine (noradrenaline) reuptake.\(^1\) Dopexamine vasodilates rat mesenteric artery by increasing intracellular cAMP.\(^2\) Clinical studies have shown that dopexamine can be used successfully before\(^15\) or after operation\(^2\) to treat low cardiac output syndrome without side effects. Dopexamine 2 µg kg\(^{-1}\) min\(^{-1}\) increased heart rate and left ventricular stroke index, decreased systemic vascular resistance but did not alter arterial pressure.\(^1\)\(^,\)\(^8\) Compared with dopamine, preoperative infusion of dopexamine can reduce gastrointestinal tract permeability in patients undergoing CPB.\(^2\)\(^,\)\(^3\) Acute phase responses after cardiac surgery are decreased when patients are pretreated with dopexamine. Berendes and colleagues\(^2\) have shown that perioperative infusion of dopexamine 2 µg kg\(^{-1}\) min\(^{-1}\) decreased the postoperative interleukin-6 (IL-6) peak, and attenuated the post-CPB systemic inflammatory syndrome.

In our study, the significant decrease in mean arterial pressure, despite constant blood flow maintained by the mechanical device, was explained by the vasodilatory property of dopexamine. The increase in laser Doppler flow in the jejunum and ileum returned to previous values after the end of infusion of dopexamine, but gastric blood flow did not return to baseline. A continuous decrease in gastric blood flow during CPB has also been described by Tao and colleagues\(^2\) in a pig model of CPB.

Laser Doppler flowmeter measurements were validated in various physiological situations\(^2\) such as an in vitro intestinal segment preparation,\(^2\) hepatic perfusion,\(^2\) rectal mucosal perfusion in neonates\(^5\)\(^,\)\(^9\) and gastric mucosal blood flow measurements during CPB\(^3\)\(^,\)\(^7\)\(^,\)\(^31\); these measurements were reproducible.\(^32\) One of the reasons for signal variability is change in red blood cell count. This did not occur in our model because i.v. and extravascular blood was heparinized and collected for reinfusion in the oxygenator.

We have shown contrasting results concerning the evolution of gastric and intestinal perfusion: in contrast with intestinal blood flow, gastric blood flow was unaltered by infusion of dopexamine. This observation could be related to existing differences in arterial inflow between the gastric region and small intestine. In the latter case, opposite directions of arterial and venous flow in the villus create a special at-risk network.\(^3\) Dopaminergic receptors have been described in gastric and jejunum mucosa which could act differently from β2 receptors.\(^3\)\(^4\) This could explain conflicting results of clinical studies in which gastric measurements were the main criteria. Such negative results have been published in dopexamine,\(^9\)\(^,\)\(^2\)\(^,\)\(^3\)\(^,\)\(^5\)\(^,\)\(^6\) enoximone\(^5\) and dobutamine\(^2\)\(^,\)\(^3\)\(^7\)\(^,\)\(^3\)\(^8\)\(^,\)\(^3\)\(^9\)\(^,\)\(^4\)\(^0\) studies. The smaller response to treatment in gastric mucosa during CPB can be explained by anatomical considerations.

Gastric intramucosal pH is not a validated index during mild hypothermic CPB: blood-gas analysis needs to be corrected by increasing sample temperature to 37°C. Moreover, pH, the most popular method of monitoring splanchnic dysfunction in human clinical practice, is not correlated with other oxygen transport markers such as oxygen delivery, blood lactate or pH.\(^3\) Gastric blood flow assessment is also probably not a good index to allow limitation of systemic inflammatory response since bacteria and macrophages are not numerous in the stomach.

**Limitation of the study**

In this study using small animals, Doppler signals are approximately 1 mm deep from the surface, including global mucosal and muscularis tissue flow measurements. A comprehensive approach to flow redistribution between mucosa and muscularis, as described in the endotoxaemic shock model,\(^3\)\(^8\) was not possible in our model. In situ studies with a needle probe to differentiate the two intestine layers are not feasible because of bleeding during CPB.

Ketamine was used to maintain anaesthesia and this does not affect intestinal motility.\(^3\) Animals were maintained free to eat and drink until the beginning of the experiment to prevent stress and intestinal mucosal injury.

Vasodilator drugs act only on the first step of ischaemia–
reperfusion injury. When prevention of ischaemia is not sufficient, leukocytes, free radicals and cytokines contribute to reperfusion-induced damage. 1 The intensity of the post-CPB inflammation syndrome is dependent on surgical duration, and deterioration of the preparation with time cannot be excluded. As this study was performed in a rabbit model, caution must be exercised in extrapolating these results to humans.

In summary, a marked decrease in splanchnic blood flow measured by Doppler laser velocimetry was detected during CPB in rabbits. Simultaneous measurements in different splanchnic areas indicated that prophylactic use of dopexamine protected jejunum and ileum blood flow but not gastric blood flow. Our data can explain the conflicting results in the literature concerning the effects of drugs on gastric ischaemia.

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