Bacterial translocation secondary to small intestinal mucosal ischemia during cardiopulmonary bypass. Measurement by diamine oxidase and peptidoglycan

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Object: To demonstrate that small intestinal mucosal ischemia occurs during cardiopulmonary bypass by measuring serum diamine oxidase activity, an index of small intestinal mucosal ischemia, in perioperative patients undergoing cardiovascular surgery with and without cardiopulmonary bypass. Methods: Twelve successive patients who underwent coronary artery bypass grafting with cardiopulmonary bypass (Group I) were compared to 10 patients who underwent off-pump coronary artery bypass grafting (Group II). Serum diamine oxidase activity, blood lactate concentration, and serum peptidoglycan concentration were measured perioperatively. Results: Serum diamine oxidase activity rose after the start of cardiopulmonary bypass and continued to rise throughout cardiopulmonary bypass in Group I, while activity was unchanged in Group II. The serum lactate concentration mirrored the change in the diamine oxidase activity in both groups. The peptidoglycan concentration in Group I rose after the start of cardiopulmonary bypass and returned to near normal concentrations after surgery. Conclusions: The parallel rise in diamine oxidase activity and the serum lactate concentration in Group I implies that ischemic injury to the mucosa of the small intestine occurs during cardiopulmonary bypass, and the rise in the serum peptidoglycan concentration indicates that bacteremia did occur. Thus, cardiopulmonary bypass causes hypoperfusion of small intestinal mucosa and consequently bacterial translocation.

Keywords: Cardiopulmonary bypass; Ischemia of small intestinal mucosa; Diamine oxidase; Peptidoglycan; Bacterial translocation

1. Introduction

Although cardiopulmonary bypass (CPB) is essential for some procedures in cardiovascular surgery, it causes peripheral hypoperfusion because flow is non-pulsatile. Furthermore bacterial translocation is thought to occur during CPB because the serum endotoxin concentration rises [1–3]. However it is difficult to measure small intestinal mucosa directly, unlike organs that are more accessible, such as the stomach or the rectum. Therefore, it has not been established conclusively whether the small intestinal mucosa is ischemic during CPB or not. Diamine oxidase (DAO) activity is particularly high in the upper portion of the small intestinal villi [4–6], and serum DAO activity has been reported to be elevated in intestinal ischemia [7,8]. Therefore measurement of serum DAO activity can be used as an index of small intestinal mucosal ischemia.

Since the small intestine is colonized with both Gram-positive and Gram-negative bacteria, measuring the serum endotoxin concentration will not detect all types of bacteremia [9,10]. Peptidoglycan (PG) is a common component of both Gram-positive and Gram-negative bacteria, and serum concentrations are measurable during bacteremia [11,12]. This study looked for direct evidence of small intestinal mucosal ischemia and bacteremia during CPB by measuring serum DAO activity and the serum PG concentration.
2. Materials and methods

2.1. Patients

Twenty-two patients who underwent elective coronary artery bypass grafting (CABG) in our department from February 2000 to April 2002 were enrolled in this study. Patients were classified into two groups: Group I, 12 patients who underwent CABG with CPB, and Group II, 10 patients who underwent CABG without CPB (Table 1). All patients in Group II had stenosis of the cervical or intracranial arteries, a cerebrovascular accident, or required only a single graft for coronary insufficiency. All subjects enrolled in this research study gave informed consent for participation. The study protocol was approved by the Institutional Committee on Human Research.

2.2. Anesthesia

Standard anesthetic agents and neuromuscular blockade (fentanyl, midazolam, pancuronium, and propofol) and monitoring techniques (electrocardiography, central venous pressure, and arterial blood pressure) were used in all patients.

2.3. Operative procedure in Group I

After median sternotomy, all patients were heparinized to achieve an activated clotting time of greater than 400 s. After inserting an arterial straight cannula via the ascending aorta and a dual-stage venous cannula via the right atrial appendage, patients were placed on CPB. Perfusion was driven by a non-pulsatile roller pump (Stockert SIII; Stockert-Shiley, Irvine, CA), and a membrane oxygenator (HMO-1040; Jostra Bentley Corporation, Irvine, CA) and an arterial line filter (LH-40AH; JMS Co. Ltd, Hiroshima, Japan) were used. CPB flow was initiated at a perfusion index of 2.4 l/min per m² and then decreased to keep the SvO₂ at about 75%. Blood pressure was maintained between 40 and 60 mmHg using chlorpropramide as a systemic vasodilator. Mild hypothermia (rectal temperature, 32 °C) was instituted immediately after the start of CPB. The circuit was primed with 1000 ml of Ringer’s lactate (Lactec; Otsuka Pharmaceutical Co. Ltd, Tokyo, Japan), 300 ml of D-mannitol (Mannigen; Nihon Pharmaceutical Co. Ltd, Tokyo, Japan), 50 ml of sodium bicarbonate (Meylon; Otsuka Pharmaceutical Co. Ltd, Osaka, Japan), and 5000 IU of sodium heparin. Methylprednisolone (SoluMedrol; Pfizer Inc., Tokyo, Japan) 1 mg/kg and 300 000 U of urokinase (Miracle; Mochida Pharmaceutical Co. Ltd, Tokyo, Japan) were infused into the reservoir of the circuit during CPB. Myocardial protection consisting of antegrade induction of cardioplegia was performed initially using, crystalloid 10 ml/kg, and after the second time, blood cardioplegia 5 ml/kg was infused over 30 min. Rewarming was started 5 min before concluding the procedure. Induction of terminal warm blood cardioplegia (1000 ml) was followed by aortic unclamping. Retrograde cardioplegia was infused continuously during aortic clamping. About 5 min before weaning from CPB, dopamine (5 μg/kg per min), nitroglycerin (0.3 μg/kg per min), and diltiazem (0.6 μg/kg per min) were administered routinely as a continuous infusion. Thirty minutes after aortic unclamping, when patient’s rectal temperature was at least 34 °C, separation from CPB was initiated, and heparin was neutralized with protamine sulfate.

2.4. Operative procedure in Group II

After median sternotomy, all patients were heparinized to achieve an activated clotting time of > 250 s. Trendelenburg position and deep pericardial suture technique (Lima suture) were employed throughout the creation of the distal anastomoses. The distal anastomoses were completed with the use of a mechanical stabilizer (Octopus 3; Medtronic Ltd, Warford, UK) and an intracoronary shunt tube (Clearview, Medtronic Ltd). During CABG, dopamine (5 μg/kg per min), nitroglycerin (0.3 μg/kg per min), and diltiazem (0.6 μg/kg per min) were administered routinely as a continuous infusion. After grafting was complete, heparin was neutralized with protamine sulfate.

2.5. Measurement of lactate, DAO, and PG

Blood samples were obtained aseptically peripherally; preoperative, 1 h after the start of CPB in Group I or 1 h after start of CABG in Group II, 2 h after the start CPB in Group I, after termination of CPB in Group I or after grafting was complete in Group II, immediately postoperatively, 4 h postoperatively, 12 h postoperatively, and 24 h postoperatively. Samples were centrifuged at 3000 × g
for 10 min at 4 °C and stored at −70 °C until measurement of serum DAO activity and PG concentration.

Serum lactate concentration was made into the index of peripheral circulation insufficiency in this study. The lactate concentration was measured immediately byABL 725 (Radiometer Medical A/S; Copenhagen, Denmark).

Serum DAO activity was measured by the method of Takagi et al. [13]. In brief, DAO catalyzes the substrate cadaverine, and DA-67 (Wako Pure Chemical Industries Co. Ltd, Osaka, Japan) is quantitatively oxidized by peroxidase in proportion to the amount of hydrogen peroxide produced, resulting in the production of methylene blue with an absorption maximum at 668 nm. Serum DAO activity in healthy subjects is 5.0 ± 1.5 U/l (mean ± SD).

To detect bacteremia, the serum PG concentration was measured using the SLP-HS Single Reagent Set (Wako Pure Chemical Industries Co. Ltd) and Toxinometer 2000 (Wako Pure Chemical Industries Co. Ltd) as a computerized instrument. This reactive principle is as follows [12]. A freeze-dried preparation derived from the body fluids of the silkworm is added to calcium chloride, and 3,4-dihydroxy-phenylalanine (L-DOPA) is used as a substrate. Silkworm larva plasma (SLP) contains all factors of the pro-phenol-oxidase cascade. PG can initiate this cascade, leading to activation of the enzyme phenol-oxidase, which converts L-DOPA into melanin. As a result, the color changes to black. This color change and activation time are measured and the PG concentration is calculated by the Toxinometer 2000.

2.6. Statistical analysis

All data are expressed as the mean ± SD. Data were small scale, and therefore nonparametric statistical methods were used (StatView 5.0 for Macintosh, SAS Institute Inc., Cary, NC). At each point, the Mann–Whitney U-test for continuous variables or Fisher’s exact probability test for categorical variables was applied to compare groups of patients. The Bonferroni–Dunn test was used for inspection of differences between preoperative values and different time points within each group.

3. Results

3.1. Operative and postoperative results

All patients completed the study protocol, and no patient was excluded. Although there were some differences in intraoperative management between groups, no patient developed postoperative complications or died. However one patient with a cerebral infraction in Group II had a tracheostomy created to improve pulmonary toilet (Table 2).

3.2. Serum lactate concentration

The serum lactate concentration rose after the start of CPB in Group I, reached a maximum of 61.8 ± 19.3 mg/dl at the termination of CPB, and returned to baseline by 12 h after surgery. The serum lactate concentration did not rise intraoperatively in Group II (Fig. 1).

3.3. Serum DAO activity

Preoperative serum DAO activity in the two groups was similar. DAO activity did not rise in Group II, but raised progressively during CPB in Group I (Fig. 2). The maximum value (34.7 ± 22.2 U/l) was recorded after the termination of CPB, and activity returned to near normal by the end of surgery.

3.4. Serum PG concentration

Serum PG was undetectable preoperatively in both groups. PG concentration in Group I raised significantly at the termination of CPB but it decreased gradually after then (Table 3). On the other hand, the PG concentration in Group II did not rise significantly.

### Table 2

Clinical results of patients who underwent CABG with (Group I) or without (Group II) CPB

<table>
<thead>
<tr>
<th></th>
<th>Group I (n = 12)</th>
<th>Group II (n = 10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grafts (No)</td>
<td>3.2 ± 0.7</td>
<td>2.3 ± 1.1</td>
<td>0.065</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>168 ± 43</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean PI or CI (l/min per m²)</td>
<td>2.3 ± 0.1</td>
<td>2.3 ± 0.7</td>
<td>0.87</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>52.4 ± 5.6</td>
<td>72.9 ± 7.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.01</td>
<td>7.39 ± 0.04</td>
<td>0.49</td>
</tr>
<tr>
<td>SvO₂ (%)</td>
<td>75.6 ± 2.1</td>
<td>74.3 ± 5.6</td>
<td>0.80</td>
</tr>
<tr>
<td>Transfusion (U)</td>
<td>1.7 ± 2.5</td>
<td>2.3 ± 2.7</td>
<td>0.55</td>
</tr>
<tr>
<td>Minimum RT (degrees)</td>
<td>32.6 ± 1.4</td>
<td>36.0 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Minimum Hb (g/dl)</td>
<td>5.6 ± 0.71</td>
<td>8.1 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total heparin (IU/kg)</td>
<td>357 ± 50</td>
<td>119 ± 29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Initial ACT (s)</td>
<td>443.2 ± 53.1</td>
<td>349.0 ± 80.5</td>
<td>0.0019</td>
</tr>
<tr>
<td>Postoperative RI (12 h)</td>
<td>1.15 ± 1.00</td>
<td>1.12 ± 0.80</td>
<td>0.74</td>
</tr>
<tr>
<td>Postoperative CCr</td>
<td>157.5 ± 78.8</td>
<td>148.8 ± 61.5</td>
<td>&gt;0.9999</td>
</tr>
<tr>
<td>(24 h, ml/min per 1.48 m²)</td>
<td>0.219 ± 0.047</td>
<td>0.208 ± 0.043</td>
<td>0.62</td>
</tr>
<tr>
<td>Postoperative K-ICG (24 h)</td>
<td>18.2 ± 7.4</td>
<td>18.8 ± 6.1</td>
<td>0.97</td>
</tr>
<tr>
<td>Hospital deaths</td>
<td>none</td>
<td>none</td>
<td>1.000</td>
</tr>
</tbody>
</table>

PI, perfusion index in Group I; CI, cardiac index in Group II; BP, blood pressure; RT, rectal temperature; Hb, hemoglobin concentration; ACT, activated clotting time; RI, respiratory index; RI, alveolar-arterial oxygen index/arterial oxygen pressure (PaO₂), which simplifies to (713 − PaO₂)/PaO₂, which simplifies to (713 − PaO₂)/PaO₂, which simplifies to (713 − PaO₂)/PaO₂, which simplifies to (713 − PaO₂)/PaO₂; CCr, creatinine clearance; K-ICG, elimination rate constant of indocyanine green; CRP, serum C-reactive protein concentration.
Fig. 2. Sequential changes in serum diamine oxidase (DAO) activity during coronary artery bypass grafting (CABG) with and without cardiopulmonary bypass (CPB). The Bonferroni–Dunn test was used to compare differences between preoperative values and different time points (*P < 0.05). The Mann–Whitney U-test was applied to compare groups of patients at each point (\#P < 0.05). Serum DAO activity rose at the start of CPB in Group I. But in Group II, serum lactate was essentially unchanged intraoperatively. OP, operation.

4. Discussion

The surface area of the intestinal mucosa is large owing to the extensive network of villi. Since they are supplied by end-arteries, it is likely that the tip of villi is particularly sensitive to ischemia. In animal models of CPB, blood flow to the jejunal and ileal mucosa during CPB is about one third of pre-CPB blood flow [14,15]. However, these changes have not been documented clinically.

DAO reduces the concentration of the polyamines that are necessary for cellular proliferation. DAO is localized to the small intestine, kidney, and placenta, with rapid cellular metabolic turnover [13]. In humans, DAO activity is particularly high in the upper portion of small intestinal villi [4–6]. Therefore serum DAO activity has been used as an index of small intestinal mucosal mass and integrity [16–18]. It was found that serum DAO activity increases markedly when the small intestine is strangulated, and therefore it was hypothesized that serum DAO activity would also rise in response to ischemia of the small intestinal mucosa [7,8]. We previously measured the change in intraoperative serum DAO activity in successive patients who underwent aortic arch replacement by the open distal anastomosis method [19]. Serum DAO activity increased after restoration of blood flow to the lower half of the body. The high serum lactate concentration seen in Group I might reflect anaerobic metabolism caused by peripheral ischemia during CPB or inactivation of pyruvate dehydrogenase in an endotoxic state [20]. Although direct detection of small intestinal mucosal ischemia implies actually measuring blood flow, it is not yet possible to do at this stage. Then considering the parallel changes in the serum lactate concentration and serum DAO activity in Group I, ischemia involving small intestinal mucosa most likely occurs during CPB, and a rise in the serum DAO activity seems to be an index of small intestinal mucosal ischemia.

A confounding factor is that serum DAO activity rises when heparin is present; DAO activity rises almost immediately after injection of heparin and peaks within 30–60 min, decreasing gradually after that [18,21]. Our other data showed that DAO activity (n = 5) increased from 6.5 ± 2.2 U/l preoperatively to 7.1 ± 3.5 U/l (P = 0.92) at 15 min after injection of heparin (331.2 ± 15.9 IU/kg), a dose equivalent to that received by patients in Group I. Furthermore the present study showed that DAO activity rose to over 20 U/l after the start of CPB in Group I and continued to rise throughout CPB, while remaining unchanged in Group II. Considering the blood dilution during CPB, it is thought that there is still more product of serum DAO. Thus, it can be inferred that increased DAO activity during CPB primarily reflects ischemia of the villi in the upper portion of small intestine, although some effect due to heparin also might be present.

Blood culture and measurement of the serum endotoxin concentration have been used to detect bacterial translocation [1–3]. Since the sensitivity of blood bacterial culture is very low in clinical specimens, and endotoxin originates only from Gram-negative bacteria, neither method is comprehensive or reliable. In contrast, PG is a component of about 70 and 20% of Gram-positive and Gram-negative bacterial cell walls and the sensitivity and specificity of this test for identifying bacteremia are 86.2 and 90.6% [12]. These data justify the use of the PG concentration as an index of bacteremia. In Group II, a little PG had been detected perioperatively but there was no significant
increase. Considering the rise in the serum PG concentration in Group I, we conclude that bacterial translocation is common during CPB.

No surgical complications arose in any patient in either group. However, considering the second attack theory [22], additional stress, a ‘second attack’, facilitates bacterial translocation during CPB, which is the first attack, and predisposes the patient to multiple organ failure. Therefore, to minimize the risk of complications, splanchnic vessels should be kept dilated and perfused during CPB to minimize the risk of bacterial translocation due to ischemic injury. Furthermore, one report has found that administration of glutamine protects against ischemic injury [23]. Another strategy is to reduce the bacterial burden in the small intestine by administering a ‘bowel prep’ preoperatively, as is done in digestive surgery. Lower bacterial counts may decrease the likelihood of bacterial translocation during CPB.

References


