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Plasma Postheparin Diamine Oxidase Activity after Major Abdominal Surgery

Makoto Usami¹, Seiji Haji², Kazuya Sakata², Hiroshi Kasahara², Masahiro Yamamoto², Ikuo Imamura³, and Yoshiki Tabuchi¹

Plasma diamine oxidase (DAO) activity after heparin injection, a circulatory marker for the intestinal mucosal integrity, was evaluated shortly after major abdominal surgery. DAO activity was measured by high-performance liquid chromatography method after 50 units/kg of heparin injection after esophagectomy in 8 patients, total gastrectomy in 16 patients, and pancreatectomy in 7 patients. Blood samples were collected before and at 2, 10, and 30 days after operation. Postheparin DAO activity at the 2nd day after operation decreased to 60% of the value before operation (p<0.05), then recovered gradually until the 30th day. The activity decreased in cases after esophagectomy and pancreatectomy (p<0.01). It is suggested that the intestinal mucosal integrity decreases in the early course after the major gastroenterological surgery.

Key Words
Plasma diamine oxidase, Gastrointestinal surgery.

INTRODUCTION

Damage to the intestinal mucosa promotes translocation of luminal bacteria across the bowel wall to regional lymph nodes and systemic organs.¹ This process termed “bacterial translocation” has been identified as a possible mechanism for the development of sepsis in various experimental conditions and in varying disease status under the surgical trauma.²,³ However, the study of changes of the intestinal mucosa has always been limited by the need for mucosal biopsies to examine the histology and the enzymes characteristic of an intact mucosa, and to follow the mucosal integrity during perioperative period is more difficult. Therefore, introduction of the plasma marker that can both be easily measurable and serve suitable information is important to evaluate mucosal integrity after surgery.

Diamine oxidase (DAO) is an enzyme found in high concentrations in the intestinal mucosa and plasma DAO is at or below assay detection limits in rats and humans.⁴⁻⁸ Anti-cancer drug administration,⁹ massive intestinal resection,⁴ ischemia and reperfusion of the intestine,¹⁰ and mucosal atrophy under total parenteral nutrition¹¹ have been reported to decrease in mucosal and/or plasma DAO activity in animal studies. Also, decreased their activity in
patients with inflammatory bowel diseases, including Crohn's disease,\textsuperscript{5,12} celiac disease,\textsuperscript{5}\textsuperscript{3} and ulcerative colitis,\textsuperscript{5} has been reported. Recently, plasma DAO levels have been evaluated after the intravenous heparin injection in rats and humans.\textsuperscript{4,5,12} Heparin releases DAO from binding sites of the gut mucosa and its clearance is rapid.\textsuperscript{6} Rokkas shows reproducibility of postheparin plasma DAO activity measurement and significant linear relationship between postheparin plasma DAO activity and jejunal mucosal DAO activity.\textsuperscript{5}\textsuperscript{3} From these studies, postheparin plasma DAO activity is considered as a suitable plasma marker of intestinal mucosal integrity in humans.\textsuperscript{5,12}\textsuperscript{3}

The aim of the present study was to evaluate postheparin plasma DAO activity with measurement modification in cases after major gastrointestinal surgery as a marker of intestinal mucosal integrity. In a pilot study, feasibility and bleeding diathesis of this measurement was evaluated in small number of normal subjects.

**MATERIALS AND METHODS**

**Pilot Study**

Doses of 3000-15000 U of heparin have been used to release DAO into the plasma.\textsuperscript{5,6,12} Although this dose effectively does so, it would be safer and ethically more adaptable to use a lower heparin dose in patients after major surgery. Therefore, in the pilot study in three normal subjects (3 men, mean age 34.7 ± 7.2 years; range 30 - 43 years old), we administered 5000 and 10000 U heparin intravenously and evaluated bleeding diathesis. Venous sampling was carried out before and at 15, 30, 60, and 120 minutes after heparin injection. Plasma DAO activity was measured by radio labeled putrescine decrease catabolized by DAO.\textsuperscript{13}\textsuperscript{3} One putrescine unit of DAO activity equaled 1 nmol of putrescine metabolized per hour at 37 °C and pH 7.2. Postheparin DAO activity is expressed as the calculated area under the time concentration curves (AUC).\textsuperscript{5}\textsuperscript{3}

<table>
<thead>
<tr>
<th>Heparin dose</th>
<th>5 points AUC (0, 15, 30, 60, 120)</th>
<th>4 points AUC (0, 15, 30, 60)</th>
<th>3 points AUC (0, 15, 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC* value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(putrescine U · min/ml)</td>
<td>5000 U</td>
<td>310 ± 156**</td>
<td>179 ± 117</td>
</tr>
<tr>
<td></td>
<td>10000 U</td>
<td>1054 ± 346</td>
<td>485 ± 220</td>
</tr>
<tr>
<td>Ratio of AUC value</td>
<td>5000 U</td>
<td>1.00</td>
<td>0.550 ± 0.083</td>
</tr>
<tr>
<td></td>
<td>10000 U</td>
<td>1.00</td>
<td>0.445 ± 0.088</td>
</tr>
</tbody>
</table>

* AUC; area under the time-concentration curve, **; mean ± SD
Plasma DAO activity after abdominal surgery

[Graph showing plasma DAO activity after heparin injection in normal subjects and after injection of different doses of heparin]

Figure 1. Plasma DAO activity after heparin injection in normal subjects. ○—○ after 5000 U and ●—● after 10000 U heparin injection.

Heparin plasma DAO activity values calculated from different sampling points were compared to decide suitable sampling time and points. Blood coagulation activity was evaluated by prothrombine time and activated partial thromboplastine time measurements by a routine clinical method at 60 and 120 minutes after heparin injection.

Clinical Study

Thirty-one patients after major operations, including 8 esophagectomies, 16 total gastrectomies and 7 pancreatectomies, were entered in the evaluation (Table 2). Blood samples for postheparin plasma DAO activity measurement were collected before and at 2, 10 and 30 days after the operation after an overnight fast. Fifty U/kg of bolus heparin was injected following the result of pilot study and venous sampling was carried out before and at 15, 30, 45 and 60 minutes after heparin injection. The samples were centrifuged at 3000 × g and the resultant plasma stored at −20 °C until DAO activity was measured within 4 weeks of performing the study. Plasma DAO activity was measured by high-performance liquid chromatography (HPLC) method. To a plasma sample, histamine (as substrate) was added to the final concentration of 10 μmol with potassium phosphate buffer, pH = 7.0, 50 mM (final concentration). After 1000 minutes incubation at 37 °C, reaction was terminated by adding perchloric acid to the final concentration of a 3%. A blank value was obtained by adding aminoguanidine (DAO inhibitor) to the final concentration of 1μM. Histamine destroyed by DAO was calculated by determination of histamine remained in the test tube compared to a blank tube. The HPLC method developed by Yamatodani et al.14) was employed in histamine determination. One histamine unit of DAO activity equaled 1 pmol of histamine metabolized per minute.

Postheparin DAO activity is expressed as the calculated AUC. Correlation among the basal DAO activity (the level before heparin injection), DAO activity at 30 minutes after
Table 2. Patient profile

<table>
<thead>
<tr>
<th></th>
<th>Esophagectomy</th>
<th>Total gastrectomy</th>
<th>Pancreatectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Age (year)</td>
<td>65.5 ± 7.1</td>
<td>66.7 ± 9.9</td>
<td>60.4 ± 7.1</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>7/1</td>
<td>14/2</td>
<td>5/2</td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>551 ± 114</td>
<td>355 ± 92*</td>
<td>524 ± 76</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>755 ± 238</td>
<td>958 ± 969</td>
<td>2056 ± 2036</td>
</tr>
</tbody>
</table>

Mean ± SD, *, p<0.01 vs. others

heparin injection, and postheparin DAO activity calculated from AUC were analyzed at different points after surgery. The data at postoperative day (POD) 2 was compared in different operations and in patients with or without early operative complication.

Statistical analysis

All data were expressed as mean ± standard deviation (SD), and Student's t test was employed to compare mean value from two groups and Dunnett test for the three groups. Chi square test was employed to compare incidence. The statistically significance was assured when p value was less than 0.05.

RESULTS

Postheparin Plasma Diamine Oxidase Activity Profile and Bleeding Diathesis in Normal Subjects

Intravenous administered heparin produced an increase in plasma DAO activity (Figure 1). Peak times were in between 15 and 30 minutes after 5000 U heparin injection and in between 30 and 60 minutes after 10000 U heparin injection. Their peak levels after 10000 U heparin injection, 4.5 - 16.2 putrescine U/ml, were more than two folds of that after 5000 U heparin injection, 1.3 - 7.2 putrescine U/ml. Plasma DAO activity decreased gradually after peak and it was still greater than the pretreatment baseline level at study end. Comparison of AUC data calculated from different samples in the same normal subjects, consisting of 5 points (0, 15, 30, 60, 120 minutes samples), 4 points (0, 15, 30, 60 minutes samples), and 3 points (0, 15, 60 minutes samples), are shown in Table 1. AUC data decreased in 4 points and 3 points, but correlation coefficients of each AUC data were over 0.91.

APTT levels in all samples at 30 and 60 minutes after 10000 U heparin injection and in one sample at 30 minutes after 5000 U heparin were over 150 seconds. PT levels at 60 minutes after 10000 U heparin injection were over 70 seconds. Nasal
bleeding was observed 3 hours after 5000 U heparin injection in one normal individual.

Postheparin Plasma Diamine Oxidase Activity Profile in Patients after Major Abdominal Surgery

Patients' characteristics are shown in Table 2. Operation time was shorter in total gastrectomy cases (p < 0.01). Age, sex, blood loss and the incidence of postoperative complications were the same in the three disease groups. Early postoperative complication until POD 2 includes 2 adult respiratory distress syndrome and 1 anastomotic leakage.

Mean plasma DAO activity after heparin injection in different time points after surgery is shown in Figure 2. DAO profile in patients after 50 U/kg heparin injection indicated similar pattern to that in normal subjects after 5000 U heparin injection. Time-concentration curves were lower at POD 2, recovered gradually at POD 10, then higher at POD 30 than that before surgery. Mean peak times before operation and at POD 2, 10, and 30 were 33.9, 32.5, 30.8, and 40.0 minutes, respectively without statistical differences. Plasma DAO activity before heparin injection (basal), 30 minutes after injection, and calculated values by AUC are shown in Figure 3. DAO activity before surgery in basal level was 0.3 ± 0.3 histamine U/ml and mean level at 30 minutes after heparin injection was 2.2 ± 1.3 histamine U/ml, however, the level calculated by AUC was around 98.0 ± 57.0 histamine U·min/ml, that is double-digit bigger value than the basal level. No statistically significant difference was observed in basal and 30 minutes DAO activity. DAO activity calculated from AUC decreased at POD 2 with statistical significance (p < 0.05). The value at POD 2 was 60.5 ± 24.2 histamine U·min/ml.

Basal and postheparin DAO activity shows statistically significant correlation in all samples (r = 0.395, p < 0.001, Figure 4). Correlation among basal, 30 minutes level and the value calculated from AUC in different sampling points after surgery was shown in Table 3. Postheparin DAO activity 30 minutes after heparin injection and that calculated from AUC shows high correlation over 0.8 in POD 2 and 30 (p < 0.01). Basal level did not show any correlation to the 30 minutes level and data from AUC.

The incidence of decreased DAO activity less than 80% was 7/8 (87.5%) in esophagectomy, and 5/6 (83.3%) in pancreatectomy vs. 2/12 (16.7%) in total gastrectomy (p < 0.01). Mean postheparin plasma DAO activity at POD 2 in cases with postoperative complication was 41.1 ± 17.1 histamine U·min/ml and less than that in the cases without complication 81.7 ± 43.0 histamine U·min/ml (p < 0.01).

DISCUSSION

The results of this study provide one of the first observations on postheparin plasma DAO activity changes after major abdominal surgery. Heparin dosage and sampling points for application of postheparin plasma DAO activity measurement in cases shortly after surgery were selected as follows; Side effect due to heparin
administration should be neglected in clinical setting. Also, Thompson’s the least heparin dosage reported for DAO release, 3000 U/body\textsuperscript{12}, is not suitable for every patient after surgery, for example, a common case with remarkable weight-loss due to gastroenterological cancer in the elderly. Finally, 50 unit/kg heparin was used for clinical study, because bleeding diathesis was observed in a normal subject even after 5000 U heparin injection in the pilot study. As a next step, sampling points after heparin injection and calculation method were evaluated. Thompson reported 30 minutes level alone as a postheparin plasma activity,\textsuperscript{12} but our results indicate that one point level was inadequate to use due to differences of peak times in each subject. Calculation of AUC was selected for clinical study following Rokkas.\textsuperscript{5)}

Four sampling points, consisting of 0, 15, 30, 45 minutes, were selected, because results of correlation were acceptably high. And, also fewer sampling is required for a clinical study.

No side effect of heparin usage was observed in patients after surgery. The heparin dosage is acceptable for DAO release from the gut mucosa in the results of comparable plasma DAO profile in a pilot study and a clinical study. One hundred and twenty minutes DAO data after 5000 U heparin injection by Rokkas shows slight decrease after 45 minutes and sampling even until 120 minutes is not sufficient to show the whole changes of DAO activity after 5000 U heparin injection.\textsuperscript{5)} Correlation data among different DAO measurements in the clinical study indicate the importance of the calculation of AUC after heparin injection.

As indicated by lack of correlation between basal and data after heparin injection, heparin stimulation is important to measure their decreased levels in various diseases, as a marker of decreased mucosal integrity.\textsuperscript{5,12} Our results indicate that plasma postheparin DAO activity decreases shortly after gastrointestinal surgery and recovers gradually.
The change is transient and reversible. Patients after esophagectomy or with postoperative complication show lower levels of plasma postheparin DAO activity.

As for the mechanisms, decreased plasma postheparin DAO activity is reported as a sensitive marker for quantitating length of acute intestinal mucosal injury in the rat.\textsuperscript{5,6} However, operation methods employed in this study do not require major intestinal resection. The short period to change DAO activity suggests several mechanisms. First, it could be due to loss of functioning ileal mucosa with fasting. Short time of DAO catabolism after fasting in animal experiment and mucosal hypoplasia is reported.\textsuperscript{5} Recovery at the 30th day in this study supports the increase of plasma postheparin DAO activity under sufficient oral intake.\textsuperscript{5,11} Second, it could be due to the results of active inflammation. Surgical stress introduces humoral mediator.

Table 3. Correlation coefficients among basal DAO activity, DAO activity 30 minutes after heparin injection, DAO activity calculated from area under the time-concentration curve (AUC) after surgery.

<table>
<thead>
<tr>
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<th>basal-30 min</th>
<th>basal-AUC</th>
<th>30 min-AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>0.215</td>
<td>0.216</td>
<td>0.556 *</td>
</tr>
<tr>
<td>POD 2</td>
<td>0.030</td>
<td>0.088</td>
<td>0.864 **</td>
</tr>
<tr>
<td>POD 10</td>
<td>-0.177</td>
<td>-0.207</td>
<td>0.634 *</td>
</tr>
<tr>
<td>POD 30</td>
<td>0.000</td>
<td>0.202</td>
<td>0.880 **</td>
</tr>
</tbody>
</table>

\*p<0.05, \textsuperscript{**}p<0.01

Figure 3. Plasma postheparin DAO activity in patients after surgery. (histamine U/ml for basal and 30 minutes values and histamine U/min/ml for AUC value, mean ± SD, *; p<0.05)

Figure 4. Correlation between basal and postheparin plasma DAO activity. (histamine U/ml for basal value and histamine U/min/ml for AUC value, Y=56.39X+69.45, r=0.395, p<0.001).
including inflammatory cytokines, secretion and various metabolic derangement. Decreased intestinal integrity cause certain changes in inflammatory response and inflammatory changes may cause changes in intestinal integrity on the contrary. Third, the intestinal microcirculation could have been compromised, resulting in either impaired delivery of heparin to the intestinal mucosa or a direct reduction in the number of endothelial binding sites for DAO. Redoan reports that even gentle bowel manipulation causes bacterial translocation as great as that associated with ligation of the superior mesenteric artery using rats.

Decreased mucosal integrity suggested by decreased postheparin plasma DAO activity introduces mucosal enzyme change and possible increase in mucosal permeability combined with bacterial translocation. Further investigation is required to make it clear the intestinal mucosal integrity, cytokine release and the severity of stress reaction in clinical study following the gut hypothesis using the measurement of plasma postheparin DAO activity established in this study. Also, the relationship among plasma postheparin DAO activity and factors affecting intestinal mucosal integrity, including duration of preoperative fasting, perioperative cancer chemotherapy, recovery after surgery, and effectiveness of early enteral feeding should be analyzed.

REFERENCES