Noninvasive Detection of Experimental Intestinal Ischemia

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INTRODUCTION

Acute intestinal ischemia is associated with a mortality of more than 80% [2, 3, 14, 18]. Periods of ischemia greater than 6 to 12 hr often commit the surgeon to an extensive resection of gangrenous bowel from which few of these critically ill patients can recover [14]. Delay in diagnosis is recognized as the major factor contributing to the high morbidity associated with this condition [3]. Clearly, early detection is essential for the successful treatment of acute intestinal ischemia.

No methods are currently available for the rapid, noninvasive screening of patients at high risk for intestinal ischemia before substantial infarction has occurred. Findings by physical examination are not diagnostic, and when present, often indicate that the diagnosis has been made too late [14, 18]. Other means of evaluation, including visceral arteriography [3], serum chemistries [4, 6, 10], radiologic appearance [16], dye dilution [7], intestinal radionuclide imaging [1, 11, 12, 13], and intestinal D-xylose absorption [15], have not proved to be accurate and specific in the early detection of intestinal ischemia.

Recent investigations into the metabolic requirements of the small intestine have demonstrated that glutamine is extracted from the mesenteric bloodstream as a primary metabolic substrate for cells of the small intestinal mucosa [8, 9, 17]. We have investigated the possibility of using the expiration of CO₂ produced from the oxidation of glutamine as a noninvasive metabolic indicator for the early detection of intestinal ischemia in an experimental model.

MATERIALS AND METHODS

Twenty fasted rats were anesthetized using 0.1 ml of Innovar-Vet (0.04 mg fentanyl and 2.0 mg droperidol) administered intramuscularly and hydrated intravenously using D10W (5 ml per kilo) administered via a femoral vein catheter. An arterial catheter was placed at the base of the tail for arterial pressure monitoring and pH determinations. Rats were then placed on a warming blanket to maintain core temperature at 37°C. Ten rats underwent a sham operation with laparotomy, evisceration, and manipulation of the small intestine. In another ten rats, acute, total, small intestinal ischemia was produced by ligation of the superior mesenteric artery at its origin and ligation of the small intestine with its adjacent mesentery at the ligament of Treitz and at the ileocecal valve. In both groups the bowel was then returned to the peritoneal cavity and the abdomen closed to prevent insensible fluid loss. Fifteen minutes later all rats were given an intravenous bolus of 0.5 μCi of L-[U-14C]glutamine (0.0019 μmole of glutamine). A plastic hood was placed over the rat's head and a pressure-controlled vacuum system bubbled expired air through a solution of 3 ml methylbenzeth-
ontium (hyamine) hydroxide and 27 ml 100% ACS methanol to trap expired CO₂. Expired $^{14}$CO₂, the metabolic product of L-[U-$^{14}$C]glutamine oxidation, was collected in this manner for 2 hr at 10-min intervals. Following the 2-hr collection period the abdomen was reopened and the intestine examined for gross evidence of gut viability. Aliquots were taken from every 10-min sample of the collecting solution and the $^{14}$CO₂ radioactivity measured by scintillation counting. Cumulative results were expressed as microcuries $^{14}$CO₂ expired and as percent of L-[U-$^{14}$C]glutamine oxidized to $^{14}$CO₂ by the formula

$$\frac{\mu\text{Ci}^{14}\text{CO}_2 \text{ expired}}{0.5 \mu\text{Ci glutamine infused}} \times 100 = \% \text{ glutamine oxidation to CO}_2.$$ 

The data were subjected to one-way analysis of variance (MINITAB, Pennsylvania State Univ.) and were considered statistically significant if $P < 0.05$.

### RESULTS

Rats in both groups were comparable in body weight (Table 1). There was no difference in systolic blood pressure between the two groups over the 2-hr time of observation. In Table 1 are shown the systolic blood pressure and arterial pH for the two groups at time zero and after 60 min. There was no difference in arterial pH between the two groups over the observation period. The mild metabolic acidosis in both groups was associated with the fasting state and was not corrected with bicarbonate. Gross examination of the small bowel at the beginning and end of the experiment showed no changes in the group undergoing sham operation. In the group with superior mesenteric artery ligation, there was typical mottling and discoloration throughout the small bowel suggesting ischemia, but no areas of gross infarction.

Glutamine oxidation, both as microcuries of $^{14}$CO₂ expired and percent L-[U-$^{14}$C]glutamine expired as $^{14}$CO₂, was significantly decreased in rats with acute intestinal ischemia at all time periods (Table 2). Even after 10 min of collection (25 min from the time of operation), the $^{14}$CO₂ expired in the sham operation group was higher than that in the group with acute intestinal ischemia (0.019 ± 0.007 vs 0.010 ± 0.005, $P < 0.01$). Table 2 shows cumulative glutamine metabolism for the two groups at representative times over the 2-hr observation period.

### DISCUSSION

Windmueller and Spaeth [17] reported that isolated, vascularly perfused segments of rat intestine extracted large amounts of glutamine from the bloodstream but no other amino acids. Fifty-seven percent of the carbon from L-[U-$^{14}$C]glutamine appeared with little delay in the intestinal venous blood as $^{14}$CO₂. Perfusate glutamine was the source of 32% of the CO₂ produced by the small bowel in their preparation. Results of their work suggest that glutamine derived from blood is an important respiratory substrate for cells of the
small intestinal mucosa, which is the primary site of systemic glutamine metabolism in a variety of animals models. Hanson and Parsons [8] reported that deprivation of food and metabolic acidosis had little effect upon the metabolism of glutamine per unit length of jejunum. In starved rats the metabolism of glutamine in jejunal segments was 112% of glucose metabolism suggesting that glutamine may be an even more important metabolic fuel in the intestine of rats deprived of food. Glutamine metabolism in their model was dependent on the concentration of glutamine in the vascular perfusate and was not affected by the absence of glucose. Heitmann and Bergman [9] reported, in a sheep model, that renal glutamine demand increased during conditions of acidosis and fasting. This was compensated for by a decreased net hepatic glutamine removal and an increased muscle glutamine release, respectively. It was demonstrated that, under conditions of acidosis and fasting, glutamine metabolism by other body tissues must be altered to compensate for renal changes. Although, in our model, acidosis was mild and the rats were glucose loaded, there was significant systemic, possibly renal, glutamine metabolism. In spite of this, glutamine metabolism by the vascularly intact rats was still significantly greater than in rats with acute intestinal ischemia at all time periods.

Boley et al. [3] emphasized the need for early recognition of the patient with acute intestinal ischemia and the value of visceral arteriography prior to operative intervention in the successful treatment of these patients. Arteriography by itself demonstrates arterial anatomy and pathology, but does not address the question of intestinal viability. Intraoperative assessment of intestinal viability by gross appearance is notoriously unreliable. There have been many attempts to improve diagnostic accuracy in identifying patients with intestinal ischemia. Radiologic appearance is not specific until after intestinal infarction occurs and is thus an unsuitable test of early intestinal ischemia [16]. Serum chemistries including phosphate level [10], alkaline phosphatase isozymes [4, 6], xanthine oxidase [6], transaminases [4, 6], and other indicators such as hemoconcentration, leukocytosis, and metabolic acidosis all occur with acute intestinal ischemia but are too nonspecific or occur too late in the disease process to be valuable. Other tests, such as d-xylose intestinal absorption [15] or determinations of splanchnic blood flow by dye dilution [7], depend upon the provocative enteric administration of agents and are thus better suited to the investigation of chronic intestinal ischemia rather than the acute situation. Radionuclide imaging of ischemic intestine by 99mTc-labeled microspheres [11, 12, 13, 18] or leukocytes [1] has had limited success because significant intestinal infarction is required for

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**TABLE 2**

<table>
<thead>
<tr>
<th>Glutamine Oxidation</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham operation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>µCi ¹⁴CO₂ expired</td>
<td>0.019 ± 0.007*</td>
<td>0.067 ± 0.014</td>
<td>0.118 ± 0.023</td>
<td>0.194 ± 0.032</td>
</tr>
<tr>
<td>% Glutamine oxidation as expired CO₂</td>
<td>4%</td>
<td>13%</td>
<td>24%</td>
<td>39%</td>
</tr>
<tr>
<td><strong>Intestinal ischemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>µCi ¹⁴CO₂ expired</td>
<td>0.010 ± 0.005</td>
<td>0.042 ± 0.015</td>
<td>0.089 ± 0.025</td>
<td>0.157 ± 0.030</td>
</tr>
<tr>
<td>% Glutamine oxidation as expired CO₂</td>
<td>2%</td>
<td>9%</td>
<td>18%</td>
<td>31%</td>
</tr>
<tr>
<td><em>P &lt; 0.01</em></td>
<td><em>P &lt; 0.01</em></td>
<td><em>P &lt; 0.05</em></td>
<td><em>P &lt; 0.05</em></td>
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</tr>
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*Mean ± SD.*
successful imaging, there is often only limited uptake of labeled particles by ischemic areas, and there is significant tracer uptake in adjacent tissue areas with normal vascular supply [5]. Another imaging technique using the intestinal absorption and washout of $^{33}$Xe injected into the peritoneum [5] overcomes some of these objections by directly placing the radioactive marker in the proximity to the ischemic tissue where it is trapped while being washed out of adjacent areas with intact arterial flow. Since $^{33}$Xe is a gas and is rapidly exhaled, this technique allows positive imaging of areas of ischemia [5].

It is yet to be determined if glutamine oxidation studied in this way is sensitive enough to detect regional or limited acute intestinal ischemia. In our model, there was considerable background metabolism of glutamine. Manipulation of other metabolic parameters (e.g., arterial pH, glucose, or amino acid concentrations) may improve the specificity of this technique. Glutamine oxidation may have clinical relevance to other syndromes with altered intestinal metabolism, such as neonatal necrotizing enterocolitis, inflammatory bowel disease, short gut syndrome, and strangulation–obstruction of the intestines. This technique has the potential to be modified for clinical situations by the use of stable $^{13}$C-glutamine with breath $^{13}$CO$_2$ quantitated by mass spectrometry. These investigations explore the possibility of developing a bedside test of intestinal metabolism that will allow earlier diagnosis of ischemia in high-risk patients with improved survival.

REFERENCES


