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Tyrosine: A Possible Marker of Severe Intestinal Injury During Ischemia

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Background. **Long periods of ischemia can cause organ injury and dysfunction. The protein degradation occurring in the muscular layer and in the mucosa of the intestinal wall during ischemia may release amino acids into the intestinal lumen or into the circulation. The small intestine, like skeletal muscle, cannot synthesize or degrade tyrosine. Thus, the tyrosine concentration released from the gut mucosa reflects the balance between protein synthesis and degradation. We aimed to determine whether tyrosine can be used as a marker of intestinal injury during ischemia.**

Methods. **In 19 anesthetized rabbits, an ultrasonic flow probe was placed around the superior mesenteric artery to estimate blood flow. A segment from the ileum was isolated using two multilumen catheters with inflated balloons to create a closed segment for perfusion. Animals were allocated into three groups: a sham group without intervention (group I); a group submitted to superior mesenteric artery ligation only (group II); and a group submitted to 1 h of SMA clamping followed by 1 h of reperfusion (group III). Concentrations of lactate and tyrosine (fluorometry) were determined in the serum and the gut luminal perfusate.**

Results. **Gut luminal perfusate tyrosine concentrations increased significantly in group II (from 10 ± 8 to 93 ± 63 mM/mL at 2 h) and were significantly higher than in group I (26 ± 24 mM/mL) and group III (11 ± 13 mM/mL) ($P < 0.05$ for all).**

Conclusion. **Tyrosine is released from cells into the lumen during severe intestinal ischemia. Regional measurements of tyrosine levels may be a useful indicator of severe intestinal villus compromise.**

Key Words: **intestinal ischemia; ischemia marker; reperfusion; tyrosine; lactate; rabbits.**

INTRODUCTION

Shock with low cardiac output is the most common cause of intestinal ischemia in critically ill patients [1]. Reperfusion injury may create further damage after blood flow is restored. Injury to the gastrointestinal tract may result in changes in permeability, allowing translocation of bacteria and toxins into the bloodstream, potentially contributing to mortality in critically ill patients [2].

In clinical practice, there are no reliable serum markers to detect gut ischemia, and regional measures may be preferable. Recently, luminal concentrations of lactate were obtained from patients through rectal equilibrium dialysis [3, 4]. Local injury may affect protein metabolism, but in contrast to skeletal muscle, little is known about the mechanism by which catabolic factors produce loss of protein in the small intestine *in vivo* during ischemic injury. It has been shown that decreased protein synthesis cannot explain the bulk of protein wasting [5–7]. Protein losses in the small intestine may compromise digestive and absorptive functions, barrier integrity, and immune competence [8, 9].

There are no published methods for the measurement of intestinal protein metabolism either *in vivo* or *in vitro*. To indirectly assess protein catabolism during intestinal ischemia, we measured tyrosine levels in serum and gut luminal perfusate. Since the small intestine, like skeletal muscle, cannot synthesize or degrade tyrosine, the tyrosine concentration released from the gut mucosa rep-

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resents the balance between protein synthesis and degradation in the tissue [10]. We hypothesized that an increase in the concentration of tyrosine in the gut perfusate would, therefore, indicate a shift in the balance toward net degradation. We measured tyrosine concentrations in the serum and regionally in the mucosa and muscular layer of the ileum wall and in the intestinal lumen in an animal model of intestinal ischemia.

METHODS

Animal Model

The study included 19 male and female New Zealand white rabbits (2100 to 3200 kg body weight). Animals were handled according to the rules of the local Animal Care Committee after institutional approval for animal investigation. Chew and water *ad libitum* were allowed before the experiments. Induction doses of ketamine (20 mg/kg) and xylazine (4 mg/kg) were given intramuscularly for sedation and anesthesia, followed by a continuous infusion of ketamine (15-35 mg/kg/h) starting 3 h after induction.

After induction of anesthesia, a bolus of 6 mL/kg of saline was given to avoid hypotension. A tracheotomy was then performed. Mechanical ventilation was started (Inter 5; Intermed, São Paulo, Brazil) with a FiO_2 of 40–60%, a tidal volume of 5 mL/kg, and a respiratory rate of 40 breaths/min. Ventilatory conditions were further adjusted to maintain a $PaO_2 > 80$ mmHg and a $PaCO_2$ between 35 and 45 mmHg. Ringer's lactate was infused at a rate of 12 mL/kg/h throughout the experiment.

Surgical Procedure

A 22-G polyethylene catheter was inserted into the right carotid artery and connected to a pressure transducer to enable continuous monitoring of arterial pressure. Another catheter (Surflo I.V. catheter, 20 G × 2″) was placed into the jugular vein for venous access. A midline laparotomy was performed and an ultrasonic flow probe (Transonic System Inc., Ithaca, NY) was placed around the superior mesenteric artery to continuously estimate blood flow (QSMA). A bowel segment was delineated in the mid ileum. An antimesenteric enterotomy was performed and 8-Fr Foley inflow and 10-Fr Foley outflow catheters were placed to delimit a 5-cm segment. The larger outflow catheter size was used to facilitate perfusate withdrawal. The catheter balloons were gently inflated with water to allow total recovery of the gut luminal perfusate (GLP) solution without causing any gut damage. In a second gut segment, a tonometric catheter (TRIP Tonometry Catheter, Datex, Finland) was placed through a minimal antimesenteric wall incision and secured with a purse-string suture. The superior mesenteric artery (SMA) and identified collaterals were ligated in animals in group II. In group III, the SMA was occluded with a clamp for 1 h and then released. The abdomen was closed using Backhaus clamps. Body temperature was maintained between 37 and 38°C with a heating lamp.

Experimental Protocol

All animals received 20 mL/kg of a 6% hydroxyethyl starch solution (molecular weight 200,000; D/0.5, Haes-steril), as well as a 20-mL bolus at the end of the surgical instrumentation, with the remainder infused over 5 h. A 45- to 60-min stabilization period was allowed after the end of the surgical procedure. Baseline measurements were then obtained and the animals randomized into the three following groups: (a) group I (SHAM, $n = 6$); (b) group II (4-h ischemia, $n = 8$); and (c) group III (1-h ischemia + 3-h reperfusion; $n = 5$). Blood samples and hemodynamic measurements were obtained at baseline and every hour up to 4 h. In groups II and III the SMA was ligated/clamped just after the baseline measurements and sampling.

Gut Luminal Perfusate

The gut segment was carefully rinsed with warmed (37°C) 0.9% saline, before a perfusate solution (phosphate buffer solution) was infused at a rate of 8 mL/h. The rate of infusion of saline was chosen based on previous pilot studies where we observed that this rate was sufficient to fill the total length of the gut segment evaluated in a 1-h period. The lumen of the proximal catheter was used for continuous infusion and the lumen of the distal catheter was used to recover the perfusate infused every hour. GLP samples were obtained at baseline and every hour for 4 h after LPS administration. GLP samples were stored at −70°C.

Analytical Methods

After the stabilization period, mean arterial pressure (MAP), heart rate (HR); rectal temperature, right atrial pressure, superior mesenteric artery (QSMA), and ventilator parameters were measured every hour for 4 h. Arterial blood samples were obtained hourly for blood gas analysis. Serum and GLP concentrations of lactate (EML 105 Radiometer, Copenhagen, Denmark) were measured every hour. For tonometry measurements, 1 mL of 0.9% saline was placed in the silicone balloon of the tonometer and allowed to equilibrate for 60 min. The first 0.7 mL aspirated was discarded and the remaining 0.3 mL was immediately analyzed using a blood gas analyzer (ABL 500 radiometer, Copenhagen, Denmark). Ileal PCO_2 was corrected for incomplete equilibration during the 30-min sampling periods by multiplying the PCO_2 by 1.26. The ileal mucosal-arterial PCO_2 gap was calculated. Samples of arterial blood and GLP were collected from the rabbits and further centrifuged to perform tyrosine analysis (Fluorometer). Tyrosine was analyzed by the method of Waalkes and Udenfriend, in which the red blood cells are separated and a fraction of the perfusate sample is deproteinized using trichloroacetic acid 10% (1:4 vol/vol) [11].

Histological Grade of Gut Injury

At the end of the experiment each animal received a lethal injection of potassium. The ileum was immediately removed and a segment was harvested for optical microscopy and fixed in 10% formaldehyde-saline for further histological processing using hematoxylin and eosin. Mucosal histology was graded as previously described [12] using the following scale: grade 0, normal mucosa; grade 1, subepithelial space formation; grade 2, extension of the subepithelial space with moderate lifting of the epithelial layer from the lamina propria; grade 3, massive epithelial lifting down the sides of the villi; grade 4, denuded villi with lamina propria and dilated capillaries exposed; and grade 5, digestion and disintegration of the lamina propria, hemorrhage, and ulceration.

Data Analysis

The results are presented as mean ± SD. Significance was tested by the analysis of variance for repeated measurements. Bonferroni adjustment was used for multiple comparisons. The degree of histological damage was evaluated using a Mann–Whitney rank sum test. Linear regression was used to test the relation between gut luminal and serum concentrations of lactate and tyrosine. For these analyses, a P value <0.05 was considered as statistically significant.

RESULTS

MAP, QSMA, serum, and GLP lactate concentrations are shown in Table 1. A significant decrease in MAP occurred in group II (from 57 ± 32 mm Hg to 44 ± 13 at 2 h and to 50 ± 6 mm Hg at 3 h, $P < 0.05$ for both) in comparison to group III. QSMA values were only significantly altered after ligation of the vessel. Serum lactate concentrations did not differ among groups. Luminal gut lactate concentrations signifi-