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Pretreatment with arginine preserves intestinal barrier integrity and reduces bacterial translocation in mice

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Abstract

Objective: To evaluate the effects of arginine on intestinal barrier integrity and bacterial translocation (BT) in mice undergoing intestinal obstruction.

Methods: Mice were divided into 3 groups, treated for 7 d before surgical intervention with isocaloric and isoprotein diets. The ARG group received a diet containing 2% arginine, the IO (intestinal obstruction) and Sham groups, standard chow diet. On the eighth day of treatment, all animals received diethylenetriamine pentaacetic acid (DTPA) solution labeled with ^{99m}Technetium (^{99m}Tc-DTPA) by gavage for intestinal permeability analysis. After 90 min, the animals were anesthetized and the terminal ileum ligated. The Sham group only underwent laparotomy. After 4, 8, and 18 h, blood was collected for radioactivity determination. Samples of ileum were collected 18 h after surgery for histological analysis. In another set of animals, BT was evaluated. After 7 d of treatment, all animals received 10^8 CFU/mL of ^{99m}Tc-*E.coli* by gavage; 90 min later they were submitted to the surgical procedure described above. BT was determined by the uptake of ^{99m}Tc-*E.coli* in blood, mesenteric lymph nodes, liver, spleen, and lungs, assessed 18 h after the surgery.

Results: The intestinal permeability and BT were higher in the IO group when compared with the Sham group ($P < 0.05$). Arginine supplementation reduced intestinal permeability and BT to physiologic levels. Histological analysis showed mucosal ileum preservation in animals treated with arginine.

Conclusion: Arginine was able to preserve barrier integrity, thus reducing BT. © 2010 Elsevier Inc. All rights reserved.

Keywords: Immunonutrition; Arginine; Bacterial translocation; Intestinal barrier

Introduction

The gastrointestinal tract has a multitude of functions in addition to digestion. One important function is the ability to serve as a barrier against living organisms and antigens within the lumen, the so-called intestinal barrier function. The breakdown of this barrier may result in the crossing of viable bacteria and their products to mesenteric lymph nodes and more distant sites, a process known as bacterial translocation (BT) [1].

Increased intestinal permeability, BT across the intestinal mucosa, and decreased mucosal integrity are associated with critical illness. Loss of the gut mucosal barrier and subsequent translocation of bacteria from the gut have been implicated in the development of hypermetabolism, distant organ injury, and sepsis [2].

The benefits of immune-modulating specific nutrients and formulas have been shown to up-regulate host immune responses and to modulate inflammatory responses, nitrogen balance, and protein synthesis after injury [3]. Although

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most of the current industrialized formulas contain a combination of arginine, n-3 fatty acids, glutamine, antioxidants, and nucleic acids, arginine *per se* has gained specific attention [4].

Arginine is a conditionally essential amino acid that plays an important role in the transport, storage, and excretion of nitrogen and in the disposal of ammonia via the urea cycle. In catabolic states such as trauma and sepsis, arginine may become essential because of alterations in its metabolism [5]. Besides being a major nitrogen carrier and a component of proteins, it is a precursor for the synthesis of molecules with enormous biological importance including urea, ornithine, polyamines, nitric oxide, creatine, agmatine, and many others [6].

Arginine supplementation is effective in improving intestinal barrier function and vascular development [7]. Pretreatment with arginine enhances survival and intestinal mucosal barrier function after intestinal mesenteric ischemia [8]. Arginine-enriched diets protect gut mucosa from radiation-induced enteritis, as indicated by accelerated healing ability, as well as prevent BT [9].

In this context, the purpose of the current study was to investigate the effects of oral arginine on intestinal barrier integrity and, consequently, BT after an extremely aggressive model of intestinal obstruction in mice.

Materials and methods

Animals and treatment

Adult Swiss male mice weighing between 25 g and 30 g were used in this study. For each experiment (determination of intestinal permeability and BT), animals were randomized into three groups: (1) ARG group, which received arginine-supplemented chow (2% of total diet kilocalories) and underwent intestinal obstruction; (2) IO group, fed with a standard chow diet, and also underwent intestinal obstruction; and (3) Sham group, fed a standard chow diet and did not undergo intestinal obstruction.

The animals were fed for 7 d before surgical intervention with isocaloric and isoprotein diets, and were monitored daily for diet intake and weight gain. All animals had free access to water throughout the experiment.

The supplemented diet was prepared using as reference the diet composition described by Quirino et al. [10], using the same ingredients, with modifications in the quantities. This procedure was made with the purpose to prepare a chow with the same composition of macronutrients present in the standard chow.

This study was approved by the Ethics Committee for Animal Experimentation at the Federal University of Minas Gerais (CETEA/UFMG) and complies with the guide for the care and use of laboratory animals recommended by the Institute of Laboratory Animal Resources.

Surgical procedure

Mice were anesthetized intraperitoneally with xylazine (8 mg/kg) and ketamine (60 mg/kg) solutions. The abdomen was opened through a midline incision and the terminal ileum was isolated and ligated. The abdominal wound was closed in two layers. Animals in the Sham group underwent a simulated operation only [10,11].

Intestinal permeability

To evaluate intestinal permeability, 45 mice were divided into groups of 15 animals and were treated as described above. After 7 days, all mice received 0.1 mL of diethylenetriamine pentaacetic acid (DTPA) solution labeled with 18.5 MBq of ^{99m}Tc by gavage. After 90 min, all mice underwent intestinal obstruction as previously described. Later, at 4, 8, and 18 h, 5 animals per period were anesthetized once again and 500 μL of blood was collected and placed in appropriate tubes for radioactivity determination. Data were expressed as % dose, using the following equation:

$$\%\ \text{of injected dose in blood} = \frac{\text{cpm in the blood}}{\text{cpm of the administrated dose}} \times 100$$

where cpm is counts per minute.

Ileum histology

Samples of small bowel were taken for histological analysis 18 h after surgery. A 1-cm ring of distal ileum, adjacent to the intestinal obstruction, was resected, fixed in a 4% buffered formalin solution, dehydrated, cleared, embedded in paraffin, cut into sections 4 to 5 μm thick, stained by hematoxylin and eosin (H&E), and coded and analyzed by optical microscopy by a single pathologist who was unaware of the experimental conditions of each group.

Radiolabeling of E. coli

A sample of *E. coli* ATCC-10536 culture grown overnight in trypticasein agar was transferred to 10 mL of sterile saline solution. The bacterial concentration was adjusted to 31% of transmittance in a spectrophotometer at 580 nm, which corresponds to approximately 10^8 colony-forming units/mL. An aliquot of bacterial suspension (2 mL) was incubated in tubes containing 1 mL of stannous chloride solution (580 μM, pH 7.0) at 37 °C for 10 min. After incubation, 37.0 to 55.5 MBq of technetium-99m (^{99m}Tc) obtained by elution from the sterile ^{99}Mo/^{99m}Tc generator (IPEN/Brazil) was added and the preparation was kept at 37 °C for another 10 min. The tubes were then centrifuged at 3000 × *g* for 25 min. This procedure was repeated three times. After the last centrifugation, the radioactivity of the supernatant and precipitate were measured in a dose calibrator (CRC®-25R Dose Calibrator, Capintec, Ramsey, NJ, USA) and the percent ^{99m}Tc incorporated into the bacterial cells was determined using the following equation [12]:

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