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Quantitative measure of intestinal permeability using blue food coloring

Stephanie A.K. Angarita, MD,[a] *Sergio Duarte, PhD,*[b] *Tara A. Russell, MD,*[a] *Piotr Ruchala, PhD,*[c] *Irmina A. Elliott, MD,*[a] *Julian P. Whitelegge, PhD,*[c] *and Ali Zarrinpar, MD, PhD*[b,*,1]

[a] *Department of Surgery, David Geffen School of Medicine, University of California, Los Angeles, California*
[b] *Dumont-UCLA Transplant Center, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, California*
[c] *Semel Institue for Neuroscience and Human Behavior, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, California*

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ABSTRACT

Background: Loss of intestinal barrier integrity plays a fundamental role in the pathogenesis of various gastrointestinal diseases and is implicated in the onset of sepsis and multiple organ failure. An array of methods to assess different aspects of intestinal barrier function suffers from lack of sensitivity, prolonged periods of specimen collection, or high expense. We have developed a technique to measure the concentration of the food dye FD&C Blue #1 from blood and sought to assess its utility in measuring intestinal barrier function in humans.

Materials and methods: Four healthy volunteers and 10 critically ill subjects in the intensive care unit were recruited in accordance with an institutional review board approved protocol. Subjects were given 0.5 mg/kg Blue #1 enterally as an aqueous solution of diluted food coloring. Five blood specimens were drawn per subject: 0 h (before dose), 1, 2, 4, and 8 h. After plasma isolation, organic extracts were analyzed by high-performance liquid chromatography/mass spectrometry detecting the presence of unmodified dye.

Results: We found no baseline detectable absorption in healthy volunteers. After including the subjects in the intensive care unit, we compared dye absorption in the six subjects who met criteria for septic shock with the eight who did not. Septic patients demonstrated significantly greater absorption of Blue #1 after 2 h.

Conclusions: We have developed a novel, easy-to-use method to measure intestinal barrier integrity using a food grade dye detectable by mass spectrometry analysis of patient blood following oral administration.

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* *Corresponding author*. Department of Surgery, University of Florida, College of Medicine, 1600 SW Archer Road, Gainesville, FL 32610-0118. Tel.: +1 352 265 0606; fax: +1 352 265 0678.
E-mail address: ali.zarrinpar@surgery.ufl.edu (A. Zarrinpar).
[1] Current address: Department of Surgery, University of Florida, Gainesville, FL 32610.
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Introduction

The gastrointestinal tract is an extensive surface charged with a multitude of functions including the establishment of a selective barrier between the internal and external environments.[1] This consists of a physical barrier created by a mucus layer, a lining of enterocytes of epithelial origin, and various tight junctions, as well as an immunologic barrier maintained by immune-sensing cells and Paneth cells that release lysozymes and defensins. Mounting evidence implicates the disruption of intestinal barrier integrity in the development of numerous ailments such as inflammatory bowel disease and celiac disease.[2,3] Furthermore, loss of intestinal barrier function is frequently associated with the onset of sepsis and multiple organ failure, especially when combined with intestinal hypoperfusion in trauma or major surgery.[4,5] Abnormal blood flow, such as portal hypertension and cirrhosis, is also linked to reduced intestinal barrier function.[6,7] Disruption of the intestinal barrier facilitates translocation of toxins, bacteria, and other pathogenic entities into the circulation, thereby eliciting an inflammatory response that may result in sepsis and multiple organ failure.[8-12]

Over the past 20 years, an array of methods has been developed to assess different components of intestinal barrier function.[1,2] These include analyzing epithelial cell damage by quantitating fatty acid–binding protein[13-18] or glutathione S-transferase in blood or urine,[19-22] measuring paracellular barrier integrity loss by detecting claudin-3 in the urine,[23-26] and assessing functional barrier compromise using differential sugar absorption tests,[2,3,27-29] the detection of polyethylene glycols,[2,30-34] and chromium-labeled ethylenediaminetetraacetate in the urine.[35] The detection of plasma endotoxin[36,37] and D-lactate levels[38] have also been proposed as indirect methods of assessing intestinal barrier function. However, these tests are impractical due to prolonged incubation periods, lack of sensitivity and specificity, difficulty obtaining large amounts of sample, such as 24-h urine studies, and high cost.[1,2,39-41]

A recent study evaluating the intestinal permeability of fruit flies demonstrated that FD&C Blue No. 1 (Blue #1) can be used effectively for this purpose.[42] With Blue #1, the authors demonstrated an association between loss of intestinal integrity and altered metabolic and immune signaling. Importantly, their findings showed that loss of intestinal integrity was the harbinger of death. Animal studies indicated that Blue #1, which is a water-soluble triphenylmethane dye, is minimally absorbed from the gastrointestinal tract and has rapid biliary and renal excretion with low metabolic transformation.[43-46] Remarkably, long-term studies have revealed no associated genotoxicity, physiologic, reproductive, or carcinogenic effects.[47,48] Approved for use in foods, drugs, and cosmetics by the U.S. Food and Drug Administration since 1969, Blue #1 has a recommended maximum daily intake of 12 mg/kg body weight.[45,49,50] Based on observed safety and efficacy of Blue #1 in various studies, we have developed a technique to measure the concentration of food dyes, including Blue #1, in blood to assess their utility in quantifying intestinal barrier function in humans.

Materials and methods

The study was approved by the institutional review board of the University of California, Los Angeles. All patients or their legal representatives gave written informed consent.

Study design

This study was a prospective single-center study in 14 adult subjects (10 in the intensive care unit [ICU] and four healthy volunteers). Phase 1 of the study recruited healthy volunteers and was designed to establish the lower limit of detection and to measure the baseline intestinal absorption in normal subjects. Phase 2 was performed in 10 critically ill patients recruited in the ICU. Clinically septic subjects were defined by the Surviving Sepsis Campaign (including presence, probable or documented, of infection together with systemic manifestations such as fever, tachycardia, leukocytosis, hypotension, organ dysfunction, or decreased tissue perfusion).[51] Inclusion criteria included English-speaking adult subjects over the age of 18, who were able to tolerate enteral intake and being up at $>45^{\circ}$ for 1 h after Blue #1 administration. Exclusion criteria included having a strict nothing by mouth (NPO) status, documented history of hypersensitivity to Blue #1, or for Phase 1, having any acute systemic illness.

Subjects were administered 0.5 mg/kg Blue #1 orally or per nasogastric tube as an aqueous solution of diluted food coloring (1.0 mg/mL). Five blood specimens were drawn per subject (5 mL/draw) at 0 h (before dose), 1 h, 2 h, 4 h, and 8 h after dye ingestion.

Detection of dye

Blue #1 presence was assessed in plasma samples using a modified liquid chromatography/tandem mass spectrometry method. In brief, plasma samples were mixed with three volumes of the mixture of isopropanol and acetonitrile (1:3, vol:vol) containing 0.1% of trifluoroacetic acid. After 15 min of incubation at room temperature, samples were spun down using table top centrifuge (16,000 rpm for 5 min) and supernatants transferred to fresh Eppendorf tubes and dried using Speed-Vac. Dried residue was dissolved in 50% N-methyl-2-pyrrolidone in water containing p-nitroanilide of N-benzyl-L-arginine (Bzl-Arg-pNA) as an internal standard. All samples were analyzed using the Agilent 6460 Triple Quadrupole LC-MS System (Agilent Technologies, Santa Clara, CA), equipped with a Cortecs C18+ 2.1 × 50 mm column (Waters Corp, Milford, MA). Mobile phases consisted of solvent A (20 mM $HCOONH_4$, 0.1 % formic acid in water) and solvent B (2 mM $HCOONH_4$, 0.1 % formic acid in mixture of methanol/acetonitrile/water [68:30:2; vol:vol:vol]). The dye was detected in the positive ionization mode using selected reaction monitoring transition (m/z 749.1→170.9) applying the linear gradient of solvent B from 0% to 100% over 10 min (flow rate: 0.2 mL/min).

Statistics and data analysis

All enrolled participants were included in the final analysis. Data on subjects were summarized and compared among the

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