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Immune and metabolic responses in early and late sepsis during mild dietary zinc restriction

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ABSTRACT

Background: Mild dietary zinc (Zn) deficiency is widespread in human populations, but its influence on recovery after acute illness is poorly understood. In a mouse model of abdominal sepsis (cecal ligation puncture), systemic immune responses and liver metabolism were monitored in early (24 h) and late (5 d) phases, under control conditions and during mild dietary Zn restriction.

Methods: Mice were fed diets adequate or marginally deficient (ZM) in Zn (30 *versus* 10 mg zinc/kg diet) for 4 wk, before undergoing laparotomy alone (nonseptic control) or cecal ligation puncture (septic).

Results: Among nonseptic mice, the ZM state was not associated with differences in inflammation or metabolic responses. Among septic mice, mortality did not differ between the zinc adequate and ZM groups. In the early phase, the ZM state amplified increases in plasma interleukin (IL) 6, tumor necrosis factor alpha, and IL-10, while dampening the interferon gamma response. In the late phase, subtle but significant ZM-associated increases were observed in plasma IL-5 and interferon gamma levels and hepatic protein synthesis, the latter of which appeared to be mammalian target of rapamycin independent and was associated with increased hepatic tumor necrosis factor alpha messenger RNA content.

Conclusions: Without increasing mortality, the ZM state is associated with a more disordered acute systemic inflammatory response and persistence or enhancement of acute phase responses within the liver parenchyma.

Introduction

Clinical and experimental evidence indicates that the micronutrient zinc (Zn) plays an important role in the early response and recovery after acute systemic illness or major surgical procedures. In response to sepsis, trauma, and hemorrhagic shock, plasma Zn levels decline while Zn accumulates in metabolically and immunologically active

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organs such as the liver and spleen.[1-4] Turnover of Zn within the body is also increased, reflected in sequestration in injured tissues, and increased rates of disposal in the urine and stool.[5-9] Conversely, it is well recognized that severe Zn deficiency delays recovery and increases mortality after major surgery, peritonitis, or sepsis.[9-11] These considerations suggest that, in the setting of major systemic illness or surgical stress, the body's demand for Zn increases and the risk of adverse outcomes escalates if that demand cannot be satisfied.

Although severe, isolated Zn deficiencies are distinctly uncommon in developed countries, modest imbalances are not uncommon in the general adult population and more specifically in hospitalized patients. It has been estimated that 7.5% of the US population are at risk for inadequate Zn intake and a mild Zn deficiency that may not be recognized in the absence of systemic stress.[12] In addition, modest Zn deficiencies occur in ≈40% of individuals with advanced age, obesity, diabetes, or patients being considered for major surgical procedures.[13-17]

Understanding the role of Zn in recovery from acute illness or major surgery has been hindered by the absence of animal models that reflect the more modest Zn deficiency that is observed in human populations and medically complex patients. Such models are required to understand the conditions under which a modest Zn deficiency may impair initial responses to and recovery from acute illness or major surgical stress. Such models may also be useful in identifying novel biomarkers of Zn status, for which there are currently no well-validated methods for assessment.[18,19] It is notable that simple measurements of Zn in serum, tissues, and other body fluids do not provide reliable assessments of "Zn status," that is, the ability of the body's Zn stores to meet its demands under conditions of stress.[20]

We have previously reported the adaptation of such a model to determine how mild Zn deficiency may influence local and systemic inflammatory responses to sterile chemical or mechanical irritation of the peritoneum.[21] Our findings suggested that mild Zn restriction is capable of altering regional inflammatory responses to local tissue injury or manipulation. In studies reported here, we have used similar conditions to determine the influence of mild Zn deficiency on systemic immune responses and liver metabolism in response to a severe systemic illness. Using a well-recognized model of abdominal sepsis (cecal ligation puncture [CLP]), we monitored levels of pro-inflammation and anti-inflammation plasma cytokines 24 h (acute phase) and 5 d (chronic phase) after induction of illness. Also measured were markers of metabolism in the circulation and indices of inflammation and metabolism in the liver, which would be critical in the program of resolution and recovery. We hypothesized that a state of mild Zn deficiency, induced by marginal dietary Zn deprivation (ZM), would not necessarily increase susceptibility to organ failure and death, but would impede timely resolution of inflammation and metabolic disturbances induced by an acute illness such as abdominal sepsis.

Materials and methods

Animal care

Viral antibody-free male C57BL/6 mice (Taconic, Hudson, NY), aged 10-12 wk, (26.4 ± 2.3 g) were acclimated for 1 wk before the experiment and fed standard rodent chow (Teklad Globak 2019; Harlan Teklad, Boston, MA) and water *ad libitum*. Mice were housed in polycarbonate cages with corncob bedding and maintained in a controlled environment (22°C) with a 12:12-h light–dark cycle. All experiments were approved by the Institutional Animal Care and Use Committee at the Pennsylvania State University College of Medicine and adhered to National Institutes of Health guidelines.

Zn deprivation model

After acclimation, cages were changed, and mice were randomized to receive either a commercial diet (AIN-93M; MP Biomedical, Santa Ana, CA) containing either adequate (ZA; 30 mg Zn/kg diet) or marginally reduced (ZM; 10 mg Zn/kg diet) Zn concentrations. The Zn concentration of each diet in this study was verified by atomic absorption spectrophotometry (AAnalyst 400; PerkinElmer, Waltham, MA). Diets were fed *ad libitum* for 4 wk before the experiments. Feeding the ZM diet for this length of time to mice alters whole-body Zn balance by affecting fertility, testicular Zn concentration, sperm activity, and Zn concentration in milk of lactating female.[22-24] Although this specific ZM diet does not appear to alter plasma Zn levels,[22-25] it has been reported to lower hepatic Zn content in the nonstressed state.[22] Body weights and food intake were recorded weekly during the diet protocol.

Body composition

Nuclear magnetic resonance (^{1}H-NMR) spectroscopy (Minispec LF90; Bruker Corporation, Woodlands, TX) was used to quantify longitudinal changes in body composition during the protocol, as previously reported.[26,27] The minispec uses magnetic resonance imaging technology to determine free water, and then can differentiate tissue as fat or muscle. The nuclear magnetic resonance has higher accuracy in determining body composition in the mouse model than dual-energy x-ray absorptiometry scan.[28] Before and after 4 wk on the respective diet, conscious mice were analyzed noninvasively to quantify whole-body lean and fat mass. In addition, body composition was measured on day 5 after induction of sepsis in surviving animals in the chronic sepsis group (described in the following). The ratio of fat and lean mass normalized to body weight observed in our laboratory is similar to values published by other laboratories.[29]

Sepsis model

Mice were randomly assigned to one of four experimental groups (nonseptic ZA, nonseptic ZM, septic ZA, and septic ZM). Polymicrobial sepsis was produced by CLP. Mice were