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Basic nutritional investigation

Effects of an immune-enhancing diet in endotoxemic rats

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Abstract

Objective: This work compared the nutritional efficiency of a recently available enteral formula enriched with arginine, ω-3 fatty acids, and antioxidants and supplied nitrogen as peptides (Crucial, Nestlé Clinical Nutrition) with that of a standard polymeric formula (Sondalis HP, Nestlé Clinical Nutrition) in endotoxemic rats.

Methods: Male Wistar rats (209 ± 2 g) underwent catheter gastrostomy and received Sondalis HP until they recovered their preoperative weight. At that time (day 0), an endotoxemic shock was induced by an intraperitoneal injection of lipopolysaccharide (*Escherichia coli*, 8 mg/kg) and rats then received 290 kcal · kg^{-1} · d^{-1} and 3.29 g of nitrogen · kg^{-1} · d^{-1} in the form of Crucial (IED group, $n = 7$) or Sondalis HP (S group, $n = 6$) for 3 d. Another group underwent no treatment and was fed ad libitum (AL group). Rats were killed on day 3. Results are presented as mean ± standard error of the mean (analysis of variance and Newman-Keuls test).

Results: The endotoxemic shock induced a weight loss in group S on days 1 and 2 and a weight gain in group IED (−3.5 ± 1.3 g in group S versus +6.0 ± 2.2 g in group IED, $P < 0.05$). In the same way, atrophy of extensor digitorum longus muscle was observed in group S, whereas wasting was limited in group IED (102 ± 4 mg in group IED versus 90 ± 3 mg in group S versus 119 ± 3 mg in group AL, $P < 0.05$). Muscular atrophy was associated with muscular glutamine depletion and correlated with hyperphenylalaninemia ($R = 0.60$), with the latter being blunted in group IED (57 ± 1 μM/L in group AL versus 77 ± 4 μM/L in group S versus 66 ± 2 μM/L in group IED, $P < 0.05$). No difference was observed between the experimental groups of endotoxemic rats with respect to nitrogen balance, urinary excretion of 3-methyl histidine, or total tissue protein content.

Conclusion: Crucial counteracts injury-mediated weight loss, extensor digitorum longus muscle atrophy, and hyperphenylalaninemia in endotoxemic rats.

Keywords: Amino acids; Endotoxemia; Arginine; Protein metabolism; Enteral nutrition

Introduction

Metabolic responses to injuries such as trauma and sepsis involve an increased loss of lean body mass, which results mainly in a sustained increase in the rate of net protein breakdown in skeletal muscle [1]. Amino acids released from muscle are available for the synthesis of acute-phase proteins (C-reactive protein, α_1-acid glycoprotein, fibrinogen, etc.) [2–4] and for gluconeogenesis [5]. In addition to an altered nutritional status, these disturbances in amino acid metabolism may impair the immune system, as often observed in these situations, and that select nutrients (e.g., arginine, glutamine, cysteine, taurine, medium-chain triacylglycerols, or ω-3 fatty acids) may have pharmacologic effects and could restore immune function [6]. This provides a rationale for enriching diets with large amounts of such nutrients to form so-called immune-enhancing diets (IEDs).

Over the past decade, studies on IEDs have evaluated their effects on clinical outcomes. One meta-analysis [7]

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showed that the use of an IED in postsurgical patients decreased infectious complications and length of hospital stay, but no effect on mortality was observed in this population. However, this meta-analysis underlined that immunonutrition does not decrease the rate of infectious complications in critically ill patients and that, when considering only high-quality scored studies, there was an increased mortality rate in these populations when using this type of diet. Although the design of this meta-analysis may be questioned [8], the clinical results are conflicting and further studies are required to find the populations most likely to benefit from this nutritional therapy, as concluded by Montejo et al. [9] in the most recent meta-analysis available on the subject, and to clarify the effects of IEDs at the tissue level.

One recently available IED formula, Crucial (Nestlé Clinical Nutrition, Noisiel, France), is now available in the United States and in some European countries. This enteral formula is enriched with nutrients recognized for their immunologic properties: arginine, taurine, ω-3 fatty acids, and antioxidants (vitamins A, E, and C, zinc, selenium, and copper). It is a hypercaloric (1500 kcal/L) and hyperproteic (94 g/L) semielemental solution that supplies nitrogen as peptides and therefore should be suitable for the nutritional support of critically ill patients. To date, few studies are available on this IED: only one report by Jeevanandam et al. [10] has described the immunomodulatory properties of this IED, but there are no data available on its efficiency in restoring nutritional status. Also, for ethical reasons, it is difficult to obtain information at the tissue level in patients. Probably for technical reasons (i.e., difficulties in performing continuous enteral nutrition in rats using polymeric or semielemental based-diets), there are also very few data available in the literature for any IED. Thus this study evaluated nutritional efficiency at the tissue level of a recently available IED formula, Crucial, in endotoxemic rats. As previously demonstrated [11–13], an intraperitoneal injection of lipopolysaccharide (LPS) from *Escherichia coli* can be used as a reliable model of septic shock because it produces many of the metabolic disturbances found in patients in this situation.

Materials and methods

Surgical procedure for enteral nutrition

Thirty-one 6-wk-old male Wistar rats (Charles River, L'Arbresle, France) were housed individually in a controlled-temperature environment (21 ± 1°C), with a 12-h light, 12-h dark cycle, and maintained on rat chow (A04, UAR, Epinay/Orge, France) and water ad libitum for a 6-d acclimatization period. They were then housed in individual metabolic cages and after an overnight fast were randomized to an ad libitum group (AL; $n = 7$) or a group subjected to gastrostomy ($n = 24$). Rats in the AL group underwent no treatment and were fed ad libitum throughout the study. At this time, all rats had similar body weights (209 ± 2 g, mean ± standard error of the mean).

Rats undergoing surgery were anesthetized with isoflurane (2% in oxygen; Minerve, Esternay, France). Abdominal and neck areas were shaved, and the skin was carefully cleaned with polyvidone iodine (Betadine, Sarget Laboratory, Merignac, France). Animals underwent a left-side laparotomy, and the greater curvature of the stomach was isolated. A silicone catheter (Fisher Bioblock Scientific, Illkirch, France) was placed inside the stomach and secured with a purse-string suture. The catheter was tunneled subcutaneously to the neck and attached to a spring coil-swivel mechanism. A polyethylene tube (Fisher Bioblock Scientific) connected the cannula to a push syringe (model 1140-101, Harvard Apparatus, Les Ulis, France). Rats received a single intramuscular injection of analgesic approximately 20 min before the end of surgery (ketoprofen, 10 mg/kg) and were starved of food but were allowed to drink for the next 24 h.

The research protocol complied with the guidelines of our institution for animal care, and two of us have French government authorization to use animal models of stress (L.C., No. 75.461) and to perform surgery on rats (C.M., No. 75.522).

Nutritional program and endotoxemia

Rats received Sondalis HP (290 kcal · kg^{-1} · d^{-1}; Nestlé Clinical Nutrition) until they recovered their preoperative weight. At this time (day 0 [D0]), an endotoxemic shock was inflicted by intraperitoneally injecting 8 mg/kg of LPS (*E. coli*, 0127:B8 serotype; Sigma, Saint-Quentin-Fallavier, France), and rats were randomized to two groups: an experimental group received Crucial (IED group) and a control group receiving Sondalis HP (S group). The S diet was isovolumic and rendered isonitrogenous and isocaloric compared with Crucial by adding a casein hydrolysate (Sigma). Compositions of the diets are listed in Table 1. Enteral nutrition was infused at a constant rate over 24 h and provided 290 kcal · kg^{-1} · d^{-1} and 3.29 g · kg^{-1} · d^{-1} of nitrogen.

Animal care and death

Rats were weighed and urine was collected daily in a receptacle that contained a preservative (Amukin, Gifrer Barbezat, France). On D3, nutrition was stopped 2 h before rats were killed by decapitation (after anesthesia with isoflurane). Blood was collected in heparin-containing tubes, which were immediately centrifuged (10 min, 2500*g*, 4°C). Part of the plasma was deproteinated (with sulfosalicylic acid, 30 mg/mL) and samples were then stored at −80°C until analysis. Ten centimeters of proximal jejunum and proximal ileum was promptly removed and then washed with ice-cold 0.9% NaCl (w/w) through the lumen and