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Sex differences in response to immunonutrition in sepsis

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Abstract

Background: Cell-mediated immune response is superior in females compared to males. Whether sex differences influenced mortality in sepsis while on immune-enhancing nutrients was investigated.

Materials and Methods: Seventy-two non-orchitectomized male and non-oophorectomized female rats (n = 36/group) were randomized into four groups. Rats were fed either an immune-enhancing diet or control diet for five days. Sepsis was induced with caecal ligation and puncture (CLP). An additional group of orchitectomized and oophorectomized rats (n = 36/group) were divided into four groups; fed either an immune-enhancing or control diet of one month, then following castration. Sepsis was induced with CLP. The influence of immune-enhancing nutrients on the effect of the rat's sex on mortality rates and serum cytokines were compared.

Results: Non-orchitectomized male rats had a decreased mortality (88.9% vs. 16.7%) on immune-enhancing diet. Low mortality among non-oophorectomized female rats persisted, on immune-enhancing diet (27.8% vs. 11.1%). Orchitectomized rats demonstrated reduced mortality (88.8% vs. 50%) on immune-enhancing diet. Oophorectomized rats showed a similar trend (55.6% vs. 44.4%). Orchitectomy increased mortality in spite of immune-enhancing diet (50% vs. 16.7%). Oophorectomy increased mortality on immune-enhancing diet (44.4% vs. 11.1%). Circulating IL-1β was higher in non-oophorectomized female rats on control diet compared to immune-enhancing diet. Non-orchitectomized male and non-oophorectomized female rats had similar increases in IL-10 on immune-enhancing diet.

Conclusions: Mortality rates on immunonutrition were less in male than female rats following sepsis. Orchitectomy did not confer an advantage for septic rats. Sex hormone was more important than immunonutrition in septic female rats.

Keywords: Gender difference; Immunonutrition; Sepsis; Cecal ligation and puncture; Sex hormone; Estrogen; Androgen; Orchidectomy; Oophorectomy

Introduction

Despite identical treatment after surgery, some patients succumb to overwhelming sepsis, whereas others do not. Biological variation, including genetic background, age, and gender may influence these outcomes. Gender differences have been reported after trauma and sepsis, with female patients have a significantly better prognosis, perhaps related to increased levels of anti-inflammatory mediators [1–6]. Experimental studies have demonstrated an improved cell-mediated immune response in females compared with males. In female mice, after cecal ligation and puncture (CLP), an improved cell-mediated immune response resulted in a significantly improved ability to tolerate sepsis compared with males [7,8].

Several experimental, clinical, and epidemiologic studies have demonstrated gender differences in susceptibility to septic challenge. Sex steroids may contribute to these observations regarding sexual dimorphism [9,10]. Androgens have been found to react immunosuppressively [11–15]. In the complex interaction between the immune and endocrine systems, sexual dimorphism may be influenced by the effects of sex hormones and a different ratio of proinflamma-

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tory and anti-inflammatory mediators in women compared with men [5]. The enhanced immune response in females result from an absence of immunosuppressive androgenic hormones in females or the immunostimulating properties of female sex steroids [16]. Modulation of the hormonal response to immunonutrition has been demonstrated to be effective in septic complications in animal studies.

Recent investigations have demonstrated that sepsis with multiple organ failure is related to immunologic disorders, and the excessive release of proinflammatory reacting mediators has been found to pivotal in the pathophysiology of sepsis with multiple organ failure. Based on previous animal experimental and clinical studies, a clear association between sexual dimorphism and various immune functions has been proposed. However, data are not yet available on the animal and clinical relevance of the sex-specific phenomena for immunomodulation changes after feeding septic animals or patients with an immune-enhancing diet. Therefore, this study assessed and compared the potential association between sex and the incidence of sepsis and immunologic response after immunonutrition.

Materials and methods

Animals study and grouping

Seventy-two Sprague-Dawley rats (36 male and 36 female) weighing 200–250 g were studied. The animals were fed in a cage for at least 5 d before the experiment, and diseased rats with body weight loss were excluded. All animals were fasted overnight with free access to water before the study.

The vaginal smears of female rats were examined to determine the stage of their estrous cycles. The day of estrous was characterized by large clumps of cornified cells. For the low levels of circulating female hormones in the beginning and reaching high levels at the end of diet feeding, the female rats were proven and determined to be in the estrous phase by vaginal smear examination [17]. All male and female rats were then divided as follows. Non-orchidectomized male rats (n = 36) were divided into an immune-enhancing group (n = 18) that received an immune-enhancing diet (Nu-immune, Nutritec-Enjory Nutrition Center, Taipei, Taiwan) for 5 d and had sepsis induced by CLP and a control group (n = 18) that received a control diet for 5 d and had sepsis induced by CLP. Non-oophorectomized female rats (n = 36) were divided into an immune-enhancing group (n = 18) that received an immune-enhancing diet (Nu-immune) for 5 d and had sepsis induced by CLP and a control group (n = 18) that received a control diet for 5 d and had sepsis induced by CLP.

Another 72 Sprague-Dawley rats (36 male and 36 female) weighing 150–200 g were studied. The animals were acclimated for at least 5 d before the experiment, and the diseased rats with body weight loss were excluded. All animals were fasted overnight with free access to water before orchidectomy or oophorectomy. The rats underwent general anesthesia with ether before surgery, with male rats receiving bilateral orchidectomy and female rats receiving oophorectomy after laparotomy. All rats were returned to their cages after castration and allowed a chow diet for 1 mo. The rats were then divided as follows. Orchidectomized male rats (n = 36) were divided into an immune-enhancing group (n = 18) that received an immune-enhancing diet (Nu-immune) for 5 d and had sepsis induced by CLP and a control group (n = 18) that received a control diet for 5 d and had sepsis induced by CLP. Oophorectomized female rats (n = 36) were divided into an immune-enhancing group (n = 18) that received an immune-enhancing diet (Nu-immune) for 5 d and had sepsis induced by CLP and a control group (n = 18) that received a control diet for 5 d and had sepsis induced by CLP.

The rats received 5 d of an immune-enhancing diet (Nu-Immune), which contained 11.1 g of glutamine and 11.6 g of arginine and a high ratio of ω-3 fatty acids in each 1000-kcal diet, or a non–immune-enhancing control diet. The contents of both diets are listed in Table 1. Sepsis was then induced in the rats using CLP.

Cytokine (tumor necrosis factor-α, interleukin [IL]-1β, IL-6, and IL-10) responses were measured using the sera of non-orchidectomized male and non-oophorectomized female rats at 0, 2, 4, 8, 12, 16, and 24 h after surgery. Comparisons were made within and between genders.

Induction of sepsis with CLP procedure

Sepsis was induced by CLP procedure as described by Wichmann et al. [18]. The cecum was ligated with a 3-0 silk ligature and two punctures were made with an 18-gauge needle. The cecum was returned to the peritoneal cavity and the abdomen was closed in two layers. All rats were resuscitated with normal saline (4 mL/100 g of body weight) immediately after and 7 h after surgery. The rats were fasted but with free access to water.

Assays of cytokines

Commercially available enzyme-linked immunosorbent assay kits were used to determine tumor necrosis factor-α, IL-1β, and IL-6 in supernatants of whole blood cultures or serum specimens based on instructions of the manufacturer. The assay sensitivity was 5 pg/mL. IL-10 was measured using a specific enzyme-linked immunosorbent assay that uses the rat anti-human IL-10 monoclonal antibody and a rabbit anti-human IL-10 polyclonal antibody.

Statistical analysis

The results were presented as mean ± SEM. Chi-square analysis was used to compare the mortality of rats. One-way analysis of variance and one-way analysis of variance on rank were used to determine the significance of differences