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Effect of Glycine on Serum IL-1 and IL-6 of NEC Rats Induced by Endotoxin and Hypoxia

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Abstract: **Objective** To explore the effect of glycine on serum levels of IL-1 and IL-6 in rats with necrotizing enterocolitis (NEC) induced by endotoxin and hypoxia. **Methods** Forty SD rats were randomly assigned into the glycine-treated group and the normal saline (NS) control group. In the glycine-treated group, glycine (1 g/kg) was injected intravenously and lipopolysaccharide (LPS) of 2 mg/kg was administrated five minutes later. The control group rats were treated with the same volume of NS as a substitute for glycine. In both groups, 90 minutes after injection of LPS, FiO_2 given was reduced from 21% to 5% and ventilation continued for 180 min or until the death of rats. At the end of the experiment, the blood samples and sections of the intestine were obtained immediately. Serum levels of IL-1 and IL-6 were measured using ELISA. The histopathological changes of the small intestine were studied. **Results** The survival time of the glycine-treated group was significantly longer than that of the control group [(159.25 ± 22.78) min vs (138.75 ± 19.05) min] ($P < 0.01$). The injury of the small intestine in the glycine-treated group was markedly alleviated ($P < 0.01$). The levels of IL-1and IL-6 in the glycine-treated group were significantly lower than those in the control group [(149.1 ± 76.1) ng/L vs (472.1 ± 505.6) ng/L, (204.8 ± 163.5) ng/L vs (585.8 ± 574.5) ng/L, respectively] ($P < 0.01$). **Conclusions** Glycine could reduce the levels of IL-1 and IL-6 and alleviate injuries of the intestine in rats with NEC induced by LPS and hypoxia. **[Chin J Contemp Pediatr, 2003, 5(2): 100-103]**

Key words: Glycine; Endotoxin; Hypoxia; Necrotizing enterocolitis; IL-1; IL-6

甘氨酸对坏死性肠炎鼠血清 IL-1 和 IL-6 水平的影响

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[摘 要] 目的 探讨甘氨酸对内毒素和缺氧诱导的坏死性肠炎(NEC)鼠血清炎性因子 IL-1 与 IL-6 的作用。**方法** 40只SD大鼠随机分为甘氨酸+LPS组和NS+LPS对照组。甘氨酸组大鼠静脉给予甘氨酸 1 g/kg，5 min后给予内毒素 2 mg/kg，NS对照组用等量的生理盐水代替甘氨酸，内毒素剂量同前。所有大鼠注射 LPS 90 min后氧吸入浓度从21%降至5%，继续机械通气至鼠死亡或存活 180 min，实验结束时采血样和小肠标本。用双抗夹心 ELISA 法测定血清 IL-1 与 IL-6 的含量，肠组织做病理检查并进行 NEC 分度。**结果** 甘氨酸组的存活时间 (159.25±22.78) min 长于 NS 对照组(138.75±19.05) min，差异有显著性($P < 0.01$)。甘氨酸组小肠病理损伤程度明显明显低对照组($P < 0.01$)。甘氨酸组血清 IL-1 的含量为(149.1±76.1) ng/L，显著低于对照组(472.1±505.6) ng/L($P < 0.01$)；血清 IL-6 的含量为(204.8±163.5) ng/L，亦显著低于对照组(585.8±574.5) ng/L ($P < 0.01$)。**结论** 甘氨酸可降低内毒素和缺氧诱导的坏死性肠炎(NEC)鼠血清 IL-1 和 IL-6 含量水平，减轻肠病理损伤。 **[中国当代儿科杂志，2003，5(2)：100-103]**

[关 键 词] 甘氨酸；内毒素；低氧；坏死性肠炎；白介素-1；白介素-6

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Necrotizing enterocolitis (NEC) is a very common gastrointestinal emergency of the newborn and has a high mortality[1]. NSE can be induced in rats by endotoxin and hypoxia[2]. Interleukin-1 (IL-1) and interleukin-6 (IL-6) were important mediators of NEC[3]. Recently glycine was found to have effects of prolonging the survival time and alleviating the pathological injury of the intestine in NEC rats induced by lipopolysaccharide and hypoxia[4]. So the serum levels of IL-1 and IL-6 were measured in this study to explore the protection mechanism of glycine.

Materials and methods

Main reagents

Glycine was obtained from the Institute of Shanghai Biological Products. Typhoid endotoxin was obtained from Sigma USA. The ELISA kits of IL-1 and IL-6 were the products of Shanghai Langka Trade Company.

Animals and grouping

Forty young male Sprague-Dawley rats (provided by Zhongshan University) weighing 150 - 200 g were anesthetized with pentobarbital (50 mg/kg, intraperitoneal injection) and the endotracheal was intubated to prevent upper airway obstruction. Assisted ventilations were applied (Stephan ABV ventilator). A catheter (26 G) was inserted into penial veins for drug administation. The rats were randomly assigned into the glycine-treated group and normal saline (NS) control group. In the glycine-treated group, glycine (1 g/kg) was injected, followed by typhoid endotox (2 mg/kg) 5 minutes later. In the control group, glycine was replaced by the same amount of NS. FiO_2 given was reduced from 21% to 5% in both groups 90 minutes after the administration of lipopolysaccharide (LPS). Mechanical ventilation continued for 180 minutes after injection of LPS or till the death of rats. The survival time of each rat was recorded. Thoraxe was opened and 2 ml blood was collected by heart puncture. The small intestine was removed for pathological study.

Preparation and assays of section of the intestine

The intestine was fixed by 10% formaldehyde. 10 - 12 pieces of the intestine were achieved every two centimetres from the near end in each case. The specimens were prepared for routine histologic testing by dehydration, embedding, slicing and hematoxylin-eosin staining. Then they were determined by a light microscope. Inflammatory necrosis of mucosa and submucosa was observed. The histopathology of the necrotic leisions was categorized according to the standard of Caplan[5]: Grade Ⅰ, focal mild injury confined to villous tips; Grade Ⅱ, partial loss of villi; Grade Ⅲ, necrosis extending to submucosa; Grade Ⅳ, transmural necrosis.

Preparation and asssays of blood samples

The serum was extracted after the blood sample was stored in a 4℃ environment for 6 - 8 hours and then was stored in another one with a temperature of -70℃. The levels of IL-1 and IL-6 were measured by the double antibody sandwich ELISA technique.

Statistical analysis

All the data were analyzed by t or rank sum tests. All calculations and tests were performed using the software package of SPSS 8.0.

Results

Effect of glycine on the survival time of NEC rats

In the control group, only 1 rat was still alive when the experiment was over, and the mean survival time was (138.75 ± 19.05) min. However, 9 rats were still alive in the glycine-treated group, and the mean survival time was (159.25 ± 22.78) min. There was a significant difference in the survival time between the two groups ($P < 0.01$) (See Table 1).

Table 1 Comparisons of serum levels of IL-1 and IL-6, and survival time in the two groups ($n = 20$, $\bar{x} \pm s$)

Group	Survival time (min)	Serum IL-1 (ng/L)	Serum IL-6 (ng/L)
Control	138.75 ± 19.05	472.1 ± 505.6	585.8 ± 574.5
Glycine-treated	159.25 ± 22.78[a]	149.1 ± 76.1[a]	204.8 ± 163.5[a]

Note: a vs the control group $P < 0.01$

Effect of glycine on the reduction of intestinal pathologic injuries of NEC rats

Most glycine-treated rats had Grade Ⅰ or Ⅱ pathologic injury, and only 15 percent had Grade Ⅲ injury. However, in the control group, the extent of injury in all the rats was greater than Grade Ⅱ, and 2 cases (10 percent) had Grade Ⅳ injury. The mean rank of the two groups were 24.95 and 16.05 respectively. The difference was significant ($P < 0.01$) (See Table 2).

Table 2 Frequence of histopathologic grade in the two groups [Number(%)]

Group	Grade Ⅰ	Grade Ⅱ	Grade Ⅲ	Grade Ⅳ
Control	0(0)	12(60)	6(30)	2(10)
Glycine-treated	6(30)	11(55)	3(15)	0(0)

$Z=2.699$, $P<0.01$

Influence of glycine on serum IL-1 and IL-6 levels of NEC rats

The serum IL-1 level in the glycine-treated group [(149.1 ± 76.1) ng/L] was apparently lower than that in the control group [(472.1 ± 505.6) ng/L]. The serum IL-6 level in the glycine-treated group was (204.8 ± 163.5) ng/L, which was obviously lower than that in the control group [(585.8 ± 574.5) ng/L] (See Table 1).

Discussion

The etiological factors of NEC in the newborn include: immaturity of the intestinal function and immunity, injury induced by hypoxia and ischemia, enteric feeding and infections. Caplan found that either endotoxin or hypoxia could induce NEC, but the incidence was low and the lesion was mild. When both were used, almost all rats developed NEC, up to 75% of which showed severe histopathological lesion[2]. LPS and hypoxia were chosen to induce NEC in rats. The different NEC histopathologic lesions of the intestine could be observed in both the glycine-treated group and the control group, indicating the reliability of the NEC model. Further analysis showed that the enteric histopathologic lesion in the glycine-treated group was less severe than that in the control group and the survival time of the glycine-treated group was evidentl longer. All this suggested that glycine could protect NEC lesions induced by endotoxin and hypoxia. Although the mechanism of NEC was not very clear, many studies demonstrated that inflammatory cytokines were the significant mediator. IL-1, once called lymphocyte activator, is mainly produced by mononuclear phagocyte, playing a role in the immune adjustment and inflammatory medium. When the body suffers from Gram-negative bacteria toxemia – endotoxin toxemia, high concentration of IL-1 goes into circulation and acts on every system by endocrine, which results in fever, activation of the thrombosystem, inhibition of the hematopoietic function, and circulatory failure as well as DIC[6]. Clinical studies found that the serum level of IL-1 in NEC increased significantly. Dai Fukumura considered IL-1 as an important mediator in endotoxin-induced rat NEC, with histopathologic changes by decreasing the flow velocity of the erythrocyte, increasing the activation and adhesion of the leucocyte, thereby changing the microcirculation[7]. Comparing the RNA of cytokines in the small intestine of normal newborns with that of NEC newborns, Viscardi found the expression of IL-1 β-mRNA in NEC newborns increased significantly, not only in the inflammation cells but also in the small intestinal mucosal epithelial cells. So IL-1 was considered to play a special role in the initiation of NEC[3]. The study of Lu showed that glycine could restrain the production of IL-1 and TNF of mononuclear phagocyte induced by endotoxin[8]. IL-6, once called differentiational factor of B cell and T killer cell, can not only strengthen the immunity and hematopoietic function but also participate in the inflammatory reaction. It can induce and magnify the inflammatory reaction by combining with TNF and IL-1[6]. Morecroft found the serum level of IL-6 in infants diagnosed as NEC which required surgery was 10 times higher than that of sepsis newborns with a positive blood culture result. It was proposed that in NEC, a high serum IL-6 level might be an indication of surgical operation[9]. Estimating the level of serum IL-6 in 39 preterm infants with the gestational age between 25 to 34 weeks, who had the sign and symptoms of sepsis,

Romagnoli found that the level of IL-6 in NEC patients was significantly higher than that of patients with pneumonia and the sepsis infants with positive blood culture[10]. There are no reports so far about the effect of glycine on serum IL-1 and IL-6 of NEC rats induced by endotoxin-hypoxia. Our results showed that glycine could alleviate the serum levels of IL-1 and IL-6 in NEC rats induced by endotoxin-hypoxia. Glycine was administrated prior to LPS in this study. Lu demonstrated that glycine has an inhibitory effect on endotoxin both in vivo and in vitro[8,11,12]. We observed that glycine could reduce the levels of serum IL-1 and IL-6 in NEC rats by inhibiting endotoxin and thus protect NEC rats.

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