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Effects of Serum TNF-α and NO on Morphology of Myocardial Tissues in Neonatal Rats with Endotoxic Shock

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Abstract: Objective To study the changes of serum TNF-α and NO in neonatal rats with endotoxic shock and its relationship with myocardial cells damge and to explore the protection effect of dexamethasone (DXM) on neonatal rats with endotoxic shock. **Methods** Nine of the 117 seven-day-old healthy neonatal Wistar rats were used as the pre-experimental base value control group, and the other 108 rats were randomly divided into control group, endotoxic shock group (LPS group, LPS 5 mg/kg) and treatment group (DXM group, LPS 5 mg/kg + DXM 5 mg/kg). Before and after injection of LPS (2, 4, 6 and 24 hs), 9 rats in each group were sacrificed and blood samples were collected to detect TNF-α by ELISA and NO by nitrate reductase. Myocardial super-microstructure was observed under an electron microscope. **Results** ① In the LPS group, the concentration of serum TNF-α peaked at 2 h, and it decreased to the level of control group after 6 hs. In the DXM group, the level of TNF-α at 2 h was higher than that of control group ($P<0.05$), but lower than that of the LPS group ($P<0.05$). At 4 hs the level TNF-α in DXM group was lower than that of the LPS group ($P<0.05$), but was not different than that of the control group ($P>0.05$). From 6 h after injection, the differences of TNF-α levels in 3 groups were not obvious ($P>0.05$). ② In the LPS group, the concentration of NO rose after 2 h ($P<0.01$), and peaked at 24 h ($P<0.01$). After 2 hs, the levels of NO in the DXM group were lower than those of the LPS group ($P<0.01$). ③ Six hours after the injection of LPS, a little mitochondria of myocardial cells in the LPS group appeared to develop vacuolae-like degeneration. At 24 h, most mitochondria of myocardial cells in the LPS group presententecl with vaculea-like degeneration and necrosis. The myocardial fibers were broken. While in the DXM group, the changes in the super-microstructure at 24 h were not as serious as those which took place in the LPS group. **Conclusions** TNF-α and No were involved in the damage of myocardial cells in neonatal rats with endotoxic shock. DXM could partly protect the myocardial cells from damage by inhibiting the production of TNF-α and NO. [**Chin J Contemp Pediatr, 2003, 5(5): 403-406**]

Key words: TNF-α; NO; Dexamethasone; Endotoxic shock; Myocardial damage

新生大鼠内毒素休克时血清TNF-α和NO对心肌超微结构的影响

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[摘 要] 目的 观察新生大鼠内毒素休克时血清TNF-α和NO的动态变化以及与心肌细胞损害的关系,探讨地塞米松(DXM)对心肌的保护作用。**方法** 健康7 d Wistar大鼠117只,随机取9只作实验前基础值组,余108只随机分成对照组、休克组(LPS 5 mg/kg)及治疗组(LPS 5 mg/kg+DXM 5 mg/kg)。各组于注射LPS前(0 h)及注射后2,4,6,24 h分别断头取血并留取心脏组织。双抗夹心ELISA方法测定血清TNF-α浓度,硝酸盐还原酶法测定血清NO浓度,以透射电镜观察心肌超微结构改变。**结果** ①休克组TNF-α于注射LPS 2 h达高峰,6 h后下

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降到对照组水平。注射LPS 2 h后,DXM组TNF-α高于对照组($P<0.05$),但明显低于休克组($P<0.05$);注射LPS 4 h,DXM组TNF-α含量低于休克组($P<0.05$),但与对照组差异无显著性($P>0.05$)。LPS注射后6 h起,3组间TNF-α差异均无显著性($P>0.05$)。②休克组血清NO从注射LPS 2 h起升高($P<0.01$),24 h达高峰($P<0.01$);从注射LPS 2 h起,DXM组血清NO均低于同时间点休克组($P<0.01$)。③心肌超微结构改变:休克组于注射LPS 6 h心肌细胞少数线粒体有空泡变性,24 h出现心肌纤维断裂、大量线粒体空泡变性、坏死。而DXM组在注射LPS 24 h仅少数线粒体有空泡变性,心肌纤维完整。**结论** 内毒素休克新生大鼠通过释放炎症介质损害心肌组织,DXM通过抑制炎症介质分泌,稳定机体内环境,对心肌起部分保护作用。

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[关 键 词] TNF-α;NO;地塞米松;内毒素休克;心肌损害

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Multiple organs failure can take place among the neonates with septic shock. Once myocardial depression happens, the treatment becomes more difficult. The mechanism of damage of myocardial cells is very complicated. Studies in recent years have found that some mediators such as endotoxin and tumor necrosis factor-alpha (TNF-α) exerted a similar proinflammatory effect on neonatal rats and resulted in cardiodepression[1]. The cardiodepression effects of endotoxin and cytokines on mammals might be related to the induction of nitric oxide synthesis[2]. Overseas researches have found that TNF-α and interleukin-1 (IL-1) may cause the coronary injury[3], but few references have been found about the relationship between the morphological changes in myocardial cells and the effect of serum TNF-α and nitric oxide (NO) on neonatal rats with endotoxic shock. This study aimed at exploring the relationship between TNF-α, NO and the morphological changes in myocardial cells in neonatal rats with endotoxic shock and discussing the protective mechanism of dexamethasone (DXM) on cardiac tissues.

Materials and methods

Animal model

One hundred and seventeen 7 day old healthy neonatal Wistar rats (provided by the Experimental Animal Center of the Second Affiliated Hospital of China Medical University), weighing (17 ± 3) g, were used in this study. Nine were used as the pre-experimental base value control group and the rest were randomly divided into 3 groups: control group, which were injected intraperitoneally with 0.1 ml 0.9% sodium chloride (NS); an endotoxic shock group (LPS group) which were injected with LPS 5 mg/kg (Sigma USA) and a treatment group (DXM group), which were given DXM 5 mg/kg immediately after injection of LPS 5 mg/kg. The endotoxic shock model was established according to the reference[4]. Before and 2, 4, 6 and 24 hs after injection, the rats in each group were sacrificed and the blood samples were collected and placed in a liquid nitrogen container. Three pieces of 1 mm^3 myocardial tissue from each group were taken to be examined by electron-microscope.

Assay of TNF-α and NO

TNF-α was assayed by ELISA(Kit from Diaclone, France). NO was assayed by the method of nitrate reductase (Kit from Jiancheng organism and engineering, Nanjing, China). The absorption value of visible violet light at 500 nm, ID 0.5 cm was detected by a French made S. 500P continuous spectrophotometer.

The myocardial super-microstructure

The myocardial tissues were soaked in 2.5% glutaral and stored at 4℃. After being fixed by 2.5% glutaral and osmic acid, they were desiccated gradually by acetone and embedded with resin. After ultrathin sectioning they were stained by acetic acid uranium and lemon acid and observed under H600 transmission electron microscope.

Statistical analysis

All the data were expressed with mean ± standard deviation. The q-test and ANOVA were used to analyze the differences.

Results

The changes of TNF-α

In the control group, the difference among vari-

ous time points was not significant ($P > 0.05$). In the LPS group, the concentration of TNF-α at 2 h was the highest, and it was higher than that of the control ($P < 0.01$). It decreased 2 hours later but from 6 hours onwards after injection the difference compared with that of control group was insignificant. In the DXM group the TNF-α level at 2 hours was the highest. Although it decreased compared with that of the LPS group ($P < 0.05$), it was still higher than that of the control group ($P < 0.05$). Four hours after injection the TNF-α level in the DXM group was lower than that of the LPS group ($P < 0.05$) but there was no significant difference when compared with the control group ($P > 0.05$). From 6 hours onwards after injection, the difference among the 3 groups were insignificant. See Table 1.

Table 1 Contents of TNF-α at various time points in each group ($n=9$, $\bar{x} \pm s$, pg/ml)

Groups	0 h	2 h	4 h	6 h	24 h
Control	14.9±5.0	14.4±2.8	15.2±9.6	14.9±4.2	14.7±5.2
LPS	14.6±4.6	378.6±24.4^{b,d}	30.4±3.6^{b}	16.9±4.6	15.3±4.9
DXM	15.8±5.0	19.8±4.4^{a,c,d}	14.2±2.8^{c}	14.9±2.6	14.8±5.5

Note: a vs control $P < 0.05$; b vs control $P < 0.01$; c vs LPS $P < 0.05$; d vs the same group at 0 h $P < 0.01$

Variations of serum NO

In the control group, there was no significant difference among various time points ($P > 0.05$). In the LPS group, the level of NO began to rise 2 hours after injection and peaked 24 hours after injection. While in the DXM group the level of NO rose from 4 hours onwards after injection and peaked at 24 hours, but they were all lower than that of the LPS group ($P < 0.01$) and higher than that of the control group ($P < 0.01$ or 0.05). See Table 2.

Table 2 Variations of serum NO at various time points in each group ($n=9$, $\bar{x} \pm s$, pg/ml)

Group	0 h	2 h	4 h	6 h	24 h
Control	28.6±5.8	28.3±4.9	28.0±5.2	29.2±5.8	30.2±6.2
LPS	28.2±4.8	40.5±7.6^{b,d}	108.4±16.8^{b,d}	153.6±20.1^{b,d}	362.2±16.2^{b,d}
DXM	28.4±5.2	28.2±2.2^{c,d}	40.5±10.6^{a,c,d}	114.8±10.2^{b,c,d}	225.4±20.2^{b,c,d}

Note: a vs control $P < 0.05$; b vs control $P < 0.01$; c vs LPS $P < 0.01$; d vs the same group at 0 h $P < 0.01$

The changes of the morphology in myocardial cells

In the control group myocardial fibers were arranged regularly and Z lines and mitochondria were clear. Six hours after injection, a little mitochondria of myocardial cells in the LPS group presented with vacuolae-like degeneration. Twenty-four hours after injection, most mitochondria of myocardial cells in the LPS group developed vacuolae-like degeneration and necrosis. The myocardial fibers grew wider and ranked irregularly. Six hours after injection, the super-microstructure of myocardial tissues in the DXM group had almost regained their normal status. Twenty-four hours after injection, only a little mitochondria of myocardial cells in the DXM group developed vacuolae-like degeneration. The myocardial fibers were complete.

Discussion

During the endotoxic shock, LPS interacts with the host immune system and stimulates mycrophage and other inflammatory cells, which results in inflammation material being released. TNF-α is the initial factor in the inflammatory chain reaction, leading to

IL-1, IL-6, IL-8 and other factors such as prostaglandins and NO being released. As a result of this endothelial cells are damaged, platelets assemble and more oxide free radicals are released. Finally a cascade reaction takes place and the tissue impairment is increases. In the clinical experiment, the concentration of serum TNF-α increased in neonates with septic shock, which was related to the degree of shock[5]. In this study, the concentrations of TNF-α in neonatal rats peaked at 2 hours after injection of LPS, which was similar to the study of adult rats[6], suggesting that the reactions to excessive inflammatory factors in neonatal and adult rats are similar.

NO is a small inorganic molecule, a proper amount of which can modulate blood vessel tension, maintain blood pressure and organs' reperfusion. But excessive NO will result in persistent irreversible hypotention[7]. Meanwhile, NO can act with the enzymes in mitochondria[4], and play an important role in endotoxic shock. In this study the myocardial superstructure 6 hours after injection of LPS mainly presented with mitochondria necrosis, which was parallel with the time of shock and the degree of metabolic acidosis and the concentration of TNF-α, NO. The mechanism of myocardial cells damage by NO can be summarised as follows: Persistently released NO stimulates resolvable guanylyl cyclase, which raises the level of cGMP and stimulates the cGMP-depended protein enzyme, then L-type calcic cannel is closed and myocardial depression is resulted[8]. NO acts upon $O_2^{-}\cdot$ and make $ONOO^{-}$ to be release which induces peroxidation of lipid[9]. NO released from myocardial cells can inhibit mitochondria activity and further reduce the production of ATP. In this study the TNF-α level peaked at 2 hours after injection of LPS, then gradually decreased but the NO level increased from 2 hours onwards after injection of LPS and kept increasing to 24 hours. It indicated that NO was induced by TNF-α and was one of the final media of inflammation cascade reaction. In the DXM group the TNF-α level increased from 2 hours onwards but was lower than that of the LPS group, while the NO level increased from 4 hours and was lower than that of the LPS group. It implied that DXM protected myocardial tissue by reducing TNF-α and the NO level.

To sum up, TNF-α and NO were representative inflammatory factors, which are involved in the damage of myocardial cells in neonatal rats with endotoxic shock. DXM could partly protect the myocardial tissues from damage by inhibiting the production of TNF-α and NO.

[References]

[1] Muller-Werdan U, Schumann H, Fuchs R, Darmer D, Stadler J, Loppow H, et al. Endotoxin and tumor necrosis factor alpha exert a similar proinflammatory effect in neonatal rat cardiomyocytes, but have different cardio-depressant profiles [J]. J Mol Cell Cardiol, 1998, 30(5): 1027-1036.

[2] Stein B, Frank P, Schmitz W, Scholz H, Thoenes M. Endotoxin and cytokines induce direct cardio-depressive effects in mammalian cardiomyocytes via induction of nitric oxide synthase [J]. J Mol Cell Cardiol, 1996, 28(8): 1631-1639.

[3] Hohlfeld T, Klemm P, Thiemermann C, Warner TD, Schror K, Vane JR. The contribution of tumor necrosis factor-alpha and endothelin-1 to the increase of coronary resistance in head from rats treated with endotoxin [J]. Br J Pharmacal, 1995, 116(8): 3309-3315.

[4] Wright CE, Ress DD, Moncada S. Protective and pathological roles of nitric oxide in endotoxin shock [J]. Cardiov Res, 1992, 26(1): 48-57.

[5] Wu Y-B, Huang Y, Han Y-K. The assays and sinificance of serum TNF-α in newborns with septic shock [J]. China Crit Care Med (in Chinese), 1998, 10(8): 484-486.

[6] Huang Q-Q, Yang Y, Wan L-J, Su M-X, Wen S-M. Protective effects of composite salvia miltiorrhiza injection on endotoxic shock in rabbits [J]. Chin Crit Care Med (in Chinese), 1998, 10(4): 227-230.

[7] Kinugawa K, Takahashi T, Kohmoto O, Yao A, Aoyagi T, Momomura S, et al. Nitric oxide mediated effects of interleukin-6 on $[Ca^{2+}]$ I and cell contraction in cultured chick ventricular myocytes [J]. Circ Res, 1994, 75(2): 285-290.

[8] Shah AM, Lewis MJ, Hendson AH. Effects of 8-bromocyclin GMP on contraction and on inotropic response of ferret cardiac muscle [J]. J M C, 1991, 23(1): 55-61.

[9] Goode HF, Webster NR. Free radicals and antioxidants in sepsis [J]. Crit Care Med, 1993, 21(11): 1770-1776.

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