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Effects of dexamethasone, cyproheptadine, anisodamine, and dinoprostone on TNF_α production in endotoxic shock[1]

WANG Li-Zan[2], LIU Yu-Qin, CUI Yun-He[3], ZHU Fan-He, WANG Bao-Sheng, LUN Ning[4]
(*Department of Pathophysiology*; [3]*Department of Histology and Embryology*;
[4]*Department of Biochemistry, Jining Medical College, Jining 272113, China*)

KEY WORDS septic shock; tumor necrosis factor; gene expression; lipopolysaccharides; dexamethasone; cyproheptadine; anisodamine; dinoprostone; atropine derivatives; Northern blotting

ABSTRACT

AIM: To study the effects of dexamethasone (Dex), cyproheptadine (Cyp), anisodamine (Ani), and dinoprostone (Din) on lipopolysaccharides (LPS)-induced tumor necrosis factor alpha (TNF_α) gene expression and antishock effects of inhibiting TNF_α production. **METHODS**: Endotoxic shock in rats was produced by iv injection of LPS (*E coli* O111B4, 5 $mg \cdot kg^{-1}$). TNF_α mRNA accumulation was assessed by Northern blot. Plasma TNF_α contents were determined by radioimmunoassay. **RESULTS**: The TNF_α mRNA levels in rat liver at 2 h after LPS challenge was increased obviously (autoradiograms analyzed by scanning were 38 ± 10 *vs* saline control 11 ± 8, $P < 0.01$). The plasma TNF_α contents were markedly increased [(22 ± 3) $\mu g \cdot L^{-1}$ *vs* saline control (2.2 ± 1.0) $\mu g \cdot L^{-1}$, $P < 0.01$]. Dex 5, Cyp 5, Ani 10, or Din 2 $mg \cdot kg^{-1}$ immediately injected after iv LPS markedly decreased the TNF_α mRNA levels in rat liver and plasma TNF_α contents. The Dex, Cyp, Ani, and Din improved the mouse survival rate 24 h after LPS 20 $mg \cdot kg^{-1}$ challenge. **CONCLUSION**: Dex, Cyp, Ani, and Din strongly inhibit LPS-induced TNF_α gene expression, and have a beneficial antishock effects.

INTRODUCTION

Tumor necrosis factor alpha (TNF_α) is a polypeptide cytokine synthesized primarily by monocytes and macrophages in response to endotoxin. It has been found to occupy a pivotal role in the development of shock and tissue injury during septicemia[1-3]. Infusion of rats with recombinant TNF_α results in a syndrome of shock and tissue injury that was pathologically similar to septic shock. Rats succumbing to TNF-induced shock developed hypotension, tachycardia, tachypnea, and a profound metabolic acidosis. At necropsy there was evidence of diffuse hemorrhagic necrosis in bowel, acute renal tubular necrosis, pulmonary leukocyte margination, and edema[4,5]. Clinical studies have demonstrated that TNF levels predict morbidity and mortality in clinical septic shock[6]. Anti-TNF monoclonal antibodies prevent septic shock during lethal bacteremia[7,8].

Although TNF_α may mediate much of the clinical pathology, the role of TNF_α especially its gene regulation in septic shock has not been clearly elucidated. The purpose of the present work was to study the effects of dexamethasone (Dex), cyproheptadine (Cyp), anisodamine (Ani), and dinoprostone (Din, prostaglandin

[1] Project supported by Shandong Committee of Science and Technology, No 970116.
[2] Correspondence to Prof WANG Li-Zan.
Phn 86-537-225-3911, ext 2155. Fax 86-537-225-2776.
E-mail tianshw@ji-public.sd.cninfo.net
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E_2) on LPS-induced TNF_α gene expression and antishock effects of inhibiting TNF_α production in endotoxic shock.

MATERIALS AND METHODS

Endotoxin/LPS (*E coli* O111B4, Institute of Medicine and Biological Products Appraisal, China); total RNA isolation kit and random primed DNA labeling kit (Promega Co); mouse TNF_α cDNA plasmid (Institute of Tumor Research, Beijing); [α-^{32}P] dCTP (Beijing Furui Biotech Co); mouse TNF_α RIA kit (Institute of Basic Medicine of PLA General Hospital, Beijing); Dex (Qingdao Third Pharmaceutical Factory); Cyp (Ji-nan Yongning Pharmaceutical Co); Ani (Taixing Pharmaceutical Factory); Din (Sigma Co).

Experimental protocol Wistar rats (♂, weighing 200 – 250 g) supplied by the Animal Center of Shandong Medical University (Certificate № 970101) were anesthetized with ip urethane 1 g·kg^{-1} and were randomly divided into 6 groups: (1) control group, saline 5 mg·kg^{-1} iv; (2) LPS + saline group, LPS 5 mg·kg^{-1} + saline, iv; (3) LPS + Dex group, LPS 5 + Dex 5 mg·kg^{-1}, iv; (4) LPS + Cyp group, LPS 5 + Cyp 5 mg·kg^{-1}, iv; (5) LPS + Ani group, LPS 5 + Ani 10 mg·kg^{-1}, iv; (6) LPS + Din group, LPS 5 + Din 2 mg·kg^{-1}, iv. LPS was iv injected, immediately followed by iv injection of drug or saline in groups 2 – 6. The TNF_α mRNA levels in rat liver were assessed by Northern blot at 2 h after iv LPS.

Isolation of RNA and Northern blot Total mRNA was isolated[9]. For Northern blot, RNA was denatured by 1 % formaldehyde gel electrophoresis and then RNA was transferred from the formaldehyde gel to a nitrocellulose filter by blotting. All the membranes bound RNA were hybridized for 24 h to [α-^{32}P] dCTP labeled TNF_α probes. Autoradiograms were scanned with a laser densitometer to quantitate the relative mRNA levels.

Plasma TNF_α assay Blood samples were taken from the carotid artery of rats before and after iv LPS 2 h. The contents were determined by radioimmunoassay.

Survival trial The mice (♀, weighing 20 – 30 g) were provided by the Animal Center of Shandong Medical University (Ceritificate № 970101). LPS (20 mg·kg^{-1}) was iv injected, immediately followed by iv injection of Dex 5, Cyp, Ani 10, Din 2 mg·kg^{-1}, or saline. Survival rate 24 h after LPS challenge was assessed.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and compared by t-test.

RESULTS

TNF_α gene expression TNF_α mRNA expression in rat liver was increased at 2 h after LPS 5 mg·kg^{-1} challenge. The signal of hybridization was analyzed with laser densitometer scanning: 38 ± 10 *vs* saline control 11 ± 8, $P < 0.01$. Dex 5, Cyp 5, Ani 10, or Din 2 mg·kg^{-1} inhibited LPS-induced TNF_α mRNA expression in liver. TNF_α mRNA levels at 2 h after iv LPS were decreased by 62 %, 52 %, 47 %, and 53 %, respectively as compared with LPS + saline group. (Fig 1)

Plasma TNF_α content The plasma TNF_α levels in rats were remarkably increased at 2 h after iv LPS 5 mg·kg^{-1}. Compared with the saline control, the difference was quite significant ($P < 0.01$). Dex 5, Cyp 5, Ani 10, or Din 2 mg·kg^{-1} markedly decreased LPS-induced TNF_α prodction. (Tab 1).

Survival rate Dex 5, Cyp 5, Ani 10, or Din 2 mg·kg^{-1} obviously increased the mouse survival rate 24 h after LPS (20 mg·kg^{-1}, ip) challenge. (Tab 2)

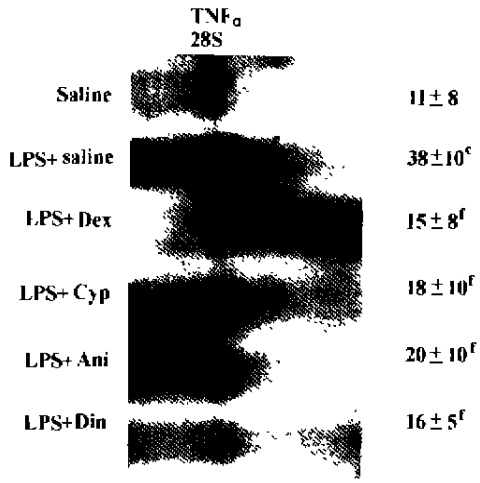

Fig 1. Effects of Dex, Cyp, Ani, and Din on LPS-induced TNF_α mRNA expression in rat liver as assessed by Northern blot. Total RNA from rat liver was extracted at 2 h after iv LPS. Autoradiograms were scanned with a laser densitometer to quantitate the relative TNF_α mRNA levels. $n = 8$ rats. $\bar{x} \pm s$. $^cP < 0.01$ *vs* saline control. $^fP < 0.01$ *vs* LPS + saline group.

Tab 1. Effect of Dex, Cyp, Ani, and Din on LPS-induced TNF_α production in rats. $n = 11$ rats. $\bar{x} \pm s$. $^cP < 0.01$ *vs* saline control. $^fP < 0.01$ *vs* LPS + saline group.

Group/ mg·kg^{-1}	Before endotoxin challenge/μg·L^{-1}	After endotoxin challenge 2 h/μg·L^{-1}
Saline control	2.1 ± 0.8	2.2 ± 1.1
LPS + Saline	2.2 ± 1.0	22 ± 3^c
LPS + Dex 5	2.2 ± 0.7	4.7 ± 1.9^f
LPS + Cyp 5	2.0 ± 0.9	7.8 ± 2.4^f
LPS + Ani 10	2.4 ± 0.9	8.1 ± 3.0^f
LPS + Din 2	2.1 ± 0.5	5.7 ± 2.1^f

Tab 2. Effect of dexamethasone, cyproheptadine, anisodamine, and dinoprostone on 24-h suivival rate in endotoxin challeneged mice. $\bar{x} \pm s$. $^cP < 0.01$ *vs* LPS + saline group.

Group/mg·kg^{-1}	Alive	Dead	Total	Survival rate
Saline control	20		20	100 %
LPS + Saline	3	17	20	15 %
LPS + Dex 5	19	1	20	95 %^{c}
LPS + Cyp 5	16	4	20	80 %^{c}
LPS + Ani 10	15	5	20	75 %^{c}
LPS + Din 2	13	7	20	65 %^{c}

DISCUSSION

Septic shock faced a high risk of death from diffuse tissue injury and multiple organ failure. TNF_α as an important mediator of septic shock is supported by laboratory and clinical studies. In the present study, the results showed that TNF_α mRNA expression in liver and the plasma TNF_α levels in rats markedly increased at 2 h after LPS challenge. The Dex, Cyp, Ani, or Din markedly decreased the TNF_α mRNA levels in rat liver and plasma TNF_α contents at 2 h after iv LPS. It suggested that Dex, Cyp, Ani, and Din strongly inhibited LPS-induced TNF_α gene expression, and protected against shock and tissue injury in septicemia.

Early investigators presumed that septic shock was directly mediated by the endotoxin of bacterium. The endotoxin induces the sympathetic excitement and circulation failure. But the mortality of septic shock has not decreased significantly in patients with septic shock using broad spectrum antibiotics and vasodilator drugs. The results showed that Dex, Cyp, Ani, and Din obviously improved survival rate at 24 h after iv LPS in this studiy. It suggested that the principle mediators of septic shock be actually endogenous factors. When LPS-induced endogenous factor (TNF_α) production was inhibited by Dex, Cyp, Ani, or Din, the shock state and survival rate was remarkably improved.

TNF_α gene expression is regulated by a variety of factor. Dex inhibited TNF production at the levels of both gene transcription and translation was demonstrated by laboratory studies[10]. This paper first reports the inhibiting action of Cyp and Ani on TNF_α gene expression. Whilom found that the mechanism of antishock effect of Ani was to dilate the vasculature, improve the microcirulation, and protect cellular membrane[11]. The results proved that Dex, Cyp, Ani, and Din inhibited

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TNF_α production and had a beneficial antishock action, giving an experimental basis for treating septic shock.

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地塞米松、噻庚啶、山莨菪碱和地诺前列酮对内毒素休克 TNF_α 产生的影响[1]

王立赞[2], 刘玉琴, 崔运河[3], 朱凡河, 王保生, 论 宁[4]

(济宁医学院病理生理学教研室, [3]组织学与胚胎学教研室, [4]生物化学教研室, 济宁 272113, 中国)

关键词 败血症性休克; 肿瘤坏死因子; 基因表达; 脂多糖类; 地塞米松; 噻庚啶; 山莨菪碱; 地诺前列酮; 阿托品衍生物; RNA 印迹

目的: 研究地塞米松(Dex)、噻庚啶(Cyp)、山莨菪碱(Ani)和地诺前列酮(Din)对脂多糖(LPS)诱导的肿瘤坏死因子(TNF_α)基因表达的影响和抑制 TNF_α 产生的抗休克作用. **方法**: Wistar 大鼠静脉注射 LPS (*E coli* O111B4, 5 $mg \cdot kg^{-1}$)复制内毒素休克模型. Northern 印迹杂交分析肝脏 TNF_α mRNA 表达, 放射免疫法测定血浆 TNF_α 的含量. **结果**: LPS 攻击后 2 h 肝脏 TNF_α mRNA 表达水平显著增高(放射性自显影扫描分析 38 ± 10 *vs* 盐水对照组 11 ± 8, $P < 0.01$); 血浆 TNF_α 水平明显升高 [(22 ± 3) $\mu g \cdot L^{-1}$ *vs* 盐水对照组(2.2 ± 1.0) $\mu g \cdot L^{-1}$, $P < 0.01$]. 静脉注射 LPS 后立即静脉注射 Dex 5、Cyp 5、Ani 10 及 Din 2 $mg \cdot kg^{-1}$均能显著降低大鼠肝脏 TNF_α mRNA 水平和血浆 TNF_α 含量, 提高 LPS 20 $mg \cdot kg^{-1}$攻击的小鼠 24 h 的存活率. **结论**: Dex、Cyp、Ani 和 Din 均能显著抑制 LPS 诱导的 TNF_α 基因表达, 具有较强的抗休克作用.

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