BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 Jan; 18 (1): 67-70

Effects of dexamethasone, ibuprofen, and ligustrazini on lipopolysaccharides-induced tumor necrosis factor α production

QIU Hai-Bo, PAN Jia-Qi[1], DU Bin, ZHAO Yong-Qiang[1], CHEN De-Chang
(*Department of Critical Care Medicine, [1]Department of Hematology Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China*)

KEY WORDS lipopolysaccharides; tumor necrosis factor; gene expression; dexamethasone; ibuprofen; ligustrazini; blood

AIM: To study the influence of dexamethasone (Dex), ibuprofen (Ibu), and ligustrazini (Lig) on lipopolysaccharides (LPS)-induced tumor necrosis factor α (TNF_α) gene expression (both mRNA and protein). **METHODS**: TNF_α in supernatants of human whole blood was measured by ELISA; The TNF_α mRNA was assessed by slot blot analysis. **RESULTS**: LPS-induced TNF_α production was in a dose-dependent manner. TNF_α levels in the whole blood increased markedly at 3 h and peaked at 6 h. The induction of TNF_α mRNA was very rapid, peaking at 2 h after LPS challenge. Dex exerted inhibitory effects on TNF_α production in a dose-dependent manner. Ibu and Lig had 2-phase effects on TNF_α release. **CONCLUSION**: Dex, Ibu, and Lig affected TNF_α gene expression, so they may be new approaches of anti-TNF_α for treatment of sepsis.

The morbidity and mortality associated with sepsis and septic shock are primarily attributable to the endogenous mediators released during the host's response to bacterial lipopolysaccharides (LPS)[1]. One such mediator is tumor necrosis factor α (TNF_α), a cytokine released primarily by monocytes and tissue macrophages. TNF_α is produced in a rapid burst in response to LPS, causing direct functional alterations in a wide variety of cell types, as well as activating a cascade of additional mediators[2,3]. It is believed that conventional therapy can not decrease the mortality of sepsis. To block the cascade of mediators at early stage may be an approach of effective therapy.

The whole blood culture system provides a much needed bridge between the *in vitro* experiments with isolated, cultured cells, and *in vivo* studies[4]. It was used in our study to observe the anti-TNF_α effects of some drugs.

In this study the kinetics of LPS-induced TNF_α expression in human whole blood culture system and the effects of dexamethasone (Dex), ibuprofen (Ibu) and ligustrazini (Lig) on TNF_α production were investigated to obtain more approaches of the anti-TNF_α therapy.

Received 1995-08-28 Accepted 1996-08-08

MATERIALS AND METHODS

Reagents LPS (*E coli* 0127:B8) was from Difco Co; TNF_α ELISA kits were from Boehringer (Mannheim, Germany); h-TNF_α cDNA plasmid (PAW711) was generously provided by Immunological Lab of PUMC Hospital; Dex and Ibu were from Sigma Co; Lig was from Beijing Fourth Pharmaceutical Works.

Experimental design Blood from normal male volunteers ($n = 4$) was drawn into heparinized syringes (20 kU heparin/L). Blood was placed in 24-well plates, 1 mL blood/well, and then LPS was added in the wells. After 24-h incubation at 37 ℃ in a 5 % CO_2 atmosphere, the blood was spinned (600 × g, 5 min). The supernatants were assayed for TNF_α and the cells in precipitate was dissolved in RNA extraction solution (solution D), and stored at −70 ℃.

The blood was cultured with Dex, Ibu, or Lig, LPS 10 $mg \cdot L^{-1}$ was added 1 h later. At different time points (0 to 24 h) after LPS stimulation, supernatants and cells were processed as above.

TNF_α assay The TNF_α concentrations in supernatants were determined by ELISA capable of detecting > 10 $ng \cdot L^{-1}$. The TNF_α levels were calculated from a standard curve obtained by recombinant TNF_α provided by kit.

Slot blot analysis mRNA levels were assessed by slot blot analysis. The blots were hybridized with [α-^{32}P]dCTP labeled h-TNF_α-cDNA probe. Autoradiograms were scanned with a laser densitometer and area-integrated in order to quantitate the relative mRNA levels.

The data were expressed as $\bar{x} \pm s$. The significance was evaluated by group comparison of t test.

RESULTS

Induction of TNF_α gene expression by LPS LPS induced TNF_α production in a dose-dependent manner (Tab 1).

Tab 1. Effects of LPS on TNF_α production in human whole blood *in vitro*. $n = 4$, $\bar{x} \pm s$.

LPS/mg·L^{-1}	TNF_α levels/μg·L^{-1}
0	0
10^{-4}	0
10^{-3}	2.4±0.9
10^{-2}	3.6±0.8
10^{-1}	5.2±1.8
1	9.3±3.8
2	14.5±4.2
10	16.2±3.8
10^2	15.8±4.6

Supernatants from LPS-stimulated blood were collected at different time points during 24-h incubation for test of TNF_α levels. The results showed that LPS markedly induced TNF_α release within 3 h. TNF_α levels peaked at 6 h, remained high for at least 12 h, and essentially returned to baseline by 24 h. The induction of TNF_α mRNA was very rapid, peaking at 2 h after LPS challenge. At 2 h after LPS 1 μg·L^{-1} stimulation, TNF_α was not detected in the supernatant, but the TNF_α mRNA increased markedly in the precipitate cell (Fig 1, 2).

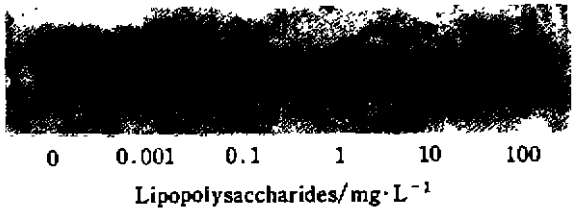

Fig 1. Autoradiogram densitometry of slot blot analysis.

Effect of drugs on TNF_α production Dex $>1\times10^{-8}$ mol·L^{-1} markedly inhibited TNF_α expression (Tab 2).

Dex $1\cdot10^{-7}$ mol·L^{-1} resulted in the TNF_α peak level decreased by 82 % (16.2 ± 7.9 *vs* 3.0 ± 2.5 μg·L^{-1}, $P<0.05$) compared to LPS (10 mg·L^{-1}) control. The TNF_α levels peaked at 8 h. At 4, 6, 8, and 12 h, TNF_α levels were lower markedly than that of LPS control ($P < 0.05$) (Fig 3).

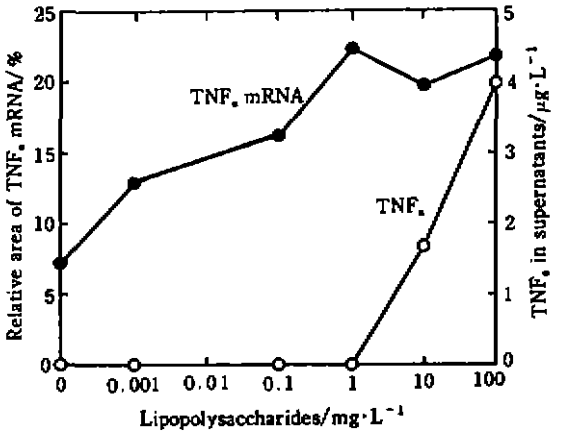

Fig 2. Slot blot analysis of TNF_α gene expression at 2 h after LPS stimulation.

Tab 2. Effects of dexamethasone, ibuprofen, and ligustrazini on LPS-induced TNF_α production *in vitro*. $n = 4$, $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* LPS control.

mol·L^{-1}	TNF_α levels/μg·L^{-1}		
	Dexamethasone	Ibuprofen	Ligustrazini
0	15.4±3.3	14.7±6.2	19.8±4.4
10^{-9}	14.9±2.7^a	8.5±3.0^b	–
10^{-8}	6.2±0.6^c	4.8±2.1^b	29.7±10.0^b
10^{-7}	3.8±0.6^c	4.1±2.5^b	4.5±2.8^b
10^{-6}	1.5±0.5^c	10.3±2.7^a	4.5±3.7^b
10^{-5}	1.8±0.6^c	15.8±3.3^a	2.3±1.5^b
10^{-4}	2.3±0.5^c	24.8±6.3^b	4.3±3.3^b
10^{-3}	1.1±0.4^c	20.7±4.9^b	3.1±1.2^b
10^{-2}	–	–	1.2±1.1^b

After Dex 1×10^{-7} mol·L^{-1} treated at 1 h before and 1 h after LPS stimulation, the TNF_α mRNA level decreased about 50 % at 2 h after LPS stimulation *vs* LPS control (Tab 3).

Ibu had 2-phase effects on TNF_α production (Tab 2). Ibu 1×10^{-7} – 1×10^{-9} mol·L^{-1} decreased TNF_α production, whereas 1×10^{-3} – 1×10^{-4} mol·L^{-1} showed a stimulating activity (Tab 2). Ibu 10^{-7} mol·L^{-1} markedly decreased the peak level of TNF_α by 51 % (16.2 ± 7.9 *vs* 7.9 ± 1.8 μg·L^{-1}, $P<0.05$) compared to LPS (10 mg·L^{-1}) control (Fig 3). At 4, 6, 8, and 12 h,

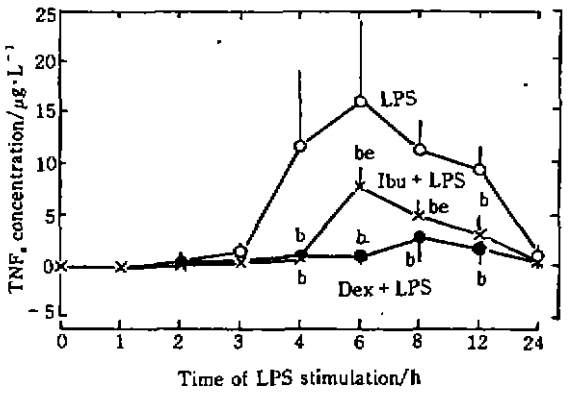

Fig 3. Effects of Dex (10^{-7} mol·L^{-1}) and Ibu (10^{-7} mol·L^{-1}) on LPS-induced TNF$_\alpha$ production. $n = 4$, $\bar{x} \pm s$. $^bP < 0.05$ *vs* LPS (10 mg·L^{-1}) control; $^cP < 0.05$ *vs* Dex + LPS.

Tab 3. Effects of Dex, Ibu, and Lig on LPS-induced TNF$_\alpha$ mRNA expression.

	Area of mRNA/%	
	Before	After
Lipopolysaccharides	12.6	–
Dexamethasone	6.1	7.2
Ibuprofen	7.6	13.8
Ligustrazini	10.7	12.6

the TNF$_\alpha$ levels were lower than LPS control ($P < 0.05$). But Ibu had less inhibitory effect than that of Dex ($P < 0.05$). The TNF$_\alpha$ mRNA level decreased about 40 % at 2 h, compared with LPS control (Tab 3).

Lig also had 2-phase effects on TNF$_\alpha$ production (Tab 2). Compared with LPS (10 mg·L^{-1}) control, Lig 10^{-8} mol·L^{-1} stimulated TNF$_\alpha$ production ($P < 0.05$), whereas Lig $1 \times 10^{-2} - 1 \times 10^{-7}$ mol·L^{-1} decreased LPS-induced TNF$_\alpha$ production at 6 h after LPS stimulation ($P < 0.05$), but Lig 10^{-7} mol·L^{-1} had no effect on LPS-induced TNF$_\alpha$ mRNA expression (Tab 3).

DISCUSSION

The present study demonstrated that *in vitro* LPS-stimulated TNF$_\alpha$ release peaked at 6 h, and returned to baseline at 24 h. But Wilson BMG, *et al* reported that TNFα release peaked at 10 – 12 h in whole blood culture system[4]. Several factors may contribute to this difference. First, different species of LPS may have different effect on TNF$_\alpha$ expression. Wilson BGM *et al* used the LPS from *Salmonella minnesota* (wild type), but LPS from *E coli* 0127 : B8 was used in our study. Second, whole blood was diluted 5-fold and especially cultured overnight before treatment in the study of Wilson BGM *et al*, which could have activated inflammatory cells and changed the response of blood to LPS.

Both LPS-induced TNF$_\alpha$ release and mRNA expression were inhibited by Dex in a dose-dependent manner in our study. Waage A *et al* reported that effect of Dex on TNF$_\alpha$ depended on the time of Dex given[5], and Dex blocked the inducing action of LPS only if Dex administered at 1 or 24 h prior to LPS stimulation. Our experiment showed that Dex administered at 1 h after LPS stimulation also had inhibitory effect on TNF$_\alpha$ gene expression (Tab 3). Since TNF$_\alpha$ is believed to play a central role in the pathophysiology of sepsis[1,2]. Our results suggested that Dex might be effective in sepsis therapy if Dex was given at early stage of sepsis. Of course, there is still much controversy about the application of Dex in sepsis therapy.

Our work showed that Ibu had 2-phase effects on TNF$_\alpha$ production. As a cyclooxygenase inhibitor, Ibu might affect TNF$_\alpha$ gene expression through its inhibitory effect on PGE_2. TNF$_\alpha$ production is up-regulated by cGMP and down-regulated by cAMP[6], and PGE_2 may display dual activities in that low concentrations stimulate whereas higher concentrations suppress TNF$_\alpha$ synthesis[7]. It may be speculated that high dose Ibu suppress PGE_2 strongly, and the very low level PGE_2 would induce TNF$_\alpha$ production by activating guanosine cyclase (cGMP increase)[6]. Low dose Ibu with a slight inhibition of PGE_2 would result in down-regulation of TNF$_\alpha$ expression by activating adenyl cyclase (cAMP increase). It should be noted in anti-TNF$_\alpha$ therapy that the dose of Ibu would affect its effect on TNF$_\alpha$ production.

The present study revealed that a Chinese traditional medicine, Lig which is a selective inhibitor of TXA_2 synthetase, had dual effect on TNF$_\alpha$ release. We wonder that high dose Lig inhibits TXA_2 synthetase, and consequently

increases PGE_2 production which induces cAMP release, and then inhibits TNF_α release. We also found Lig did not inhibit LPS-induced TNF_α mRNA, suggesting that Lig suppressed only TNF_α translational activation, but not transcriptional activation. The work demonstrates that Dex inhibits LPS-induced TNF_α expression, and Ibu and Lig have dual effects on TNF_α expression, suggesting that Ibu and Lig, like Dex, may exhibit a possible protective effect against the toxicity of LPS.

REFERENCES

1 Shapiro L, Gelfand JA. Cytokines and sepsis: pathophysiology and therapy. New Horizons 1993; 1: 13 - 22

2 Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, *et al*. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteriaemia
Nature 1987; **330**: 662 - 4.

3 DeForge LE, Remick DG. Kinetics of TNF, IL-6, and IL-8 gene expression in LPS-stimulated human whole blood.
Biochem Biophys Res Commun 1991; **174**: 18 - 24.

4 Wilson BMG, Severn A, Rapson NT, Chana J, Hopkins P. A convenient human whole blood culture system for studying the regulation of tumor necrosis factor release by bacterial lipopolysaccharide. J Immunol Methods 1991; **139**: 233 - 40.

5 Waage A, Bakke O. Glucocorticoids suppress the production of tumour necrosis factor by lipopolysaccharide-stimulated human monocytes Immunology 1988; **63**: 299 - 302

6 Gong JH, Renz H, Sprenger H, Nain M, Gemsa D.
Enhancement of tumor necrosis factor-α gene expression by low doses of prostaglandin E_2 and cyclic GMP.
Immunobiology 1990; **182**: 44 - 55

7 Renz H, Gong JH, Schmidt A, Nain M, Gemsa D. Release of tumor necrosis factor-α from macrophages. Enhancement and suppression are dose-dependently regulated by prostaglandin E_2 and cyclic nucleotides. J Immunol 1988; **141**: 2388 - 93

67-70

地塞米松、布洛芬和川芎嗪对脂多糖诱导肿瘤坏死因子α产生的影响

R977.6

邱海波，潘家绮[1]，杜 斌，赵永强[1]，陈德昌
（中国医学科学院中国协和医科大学北京协和医院加强医疗科，[1]血液科，北京 100730，中国）

R977.1

关键词 脂多糖；肿瘤坏死因子；基因表达；地塞米松；布洛芬；川芎嗪；血液

目的：研究地塞米松(Dex)、布洛芬(Ibu)和川芎嗪(Lig)对脂多糖(LPS)诱导的肿瘤坏死因子(TNF_α)表达的影响. **方法**：上清 TNF_α 含量用 ELISA 测定；狭缝杂交对 TNF_α mRNA 表达量相对定量. **结果**：全血上清 TNF_α 浓度随 LPS 刺激剂量增加而增加，刺激后 6 小时 TNF_α 达峰值，TNF_α mRNA 能较早检出，LPS 刺激后 2 小时达峰值. Dex 明显抑制 LPS 诱导的 TNF_α 释放，呈剂量依赖性. Ibu 和 Lig 对 TNF_α 释放表现为双向效应. **结论**：Dex、Ibu 和 Lig 能不同程度抑制 TNF_α 表达，可能成为严重感染的抗 TNF_α 治疗手段.