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161-165

Staurosporine 阻断正常细胞于 G_1/S 时相的边界

R977

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关键词 staurosporine；核分裂间期；S期；胸腺嘧啶核苷激酶；成纤维细胞；培养的细胞；胃癌；流式细胞光度术；磷酸化

目的：通过使用非特异性激酶抑制剂 staurosporine (5 $\mu g \cdot L^{-1}$)，揭示正常与肿瘤细胞之间在 G_1/S 时相过渡阶段调控的不同。方法：流式细胞光度术，点杂交，激酶活性分析，电泳。结果：18 小时 staurosporine (5 $\mu g \cdot L^{-1}$) 的处理，可以阻断正常细胞系 2BS 细胞（人胚胎肺成纤维细胞，5 - 20 代）于 G_1 期，降低其胞内胸腺嘧啶核苷激酶（TK）的转录和活性水平，同时也去磷酸化胞内一个 107 kDa 的蛋白。在 2BS 细胞系中，所有以上现象在洗去 staurosporine 后都可以得到逆转。但是，所有以上结果在 BGC-823 细胞内（人胃癌细胞）未曾出现。结论：在 G_1/S 时相过渡阶段，正常与肿瘤细胞的调节存在差别。在正常细胞中，参与这一调节的激酶对 staurosporine (5 $\mu g \cdot L^{-1}$) 比在肿瘤细胞内的激酶敏感得多。

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Effects of dexamethasone and ibuprofen on LPS-induced gene expression of TNF_α, IL-1β, and MIP-1α in rat lung

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KEY WORDS Wistar rats; lipopolysaccharides; tumor necrosis factor; interleukin-1; gene expression; dexamethasone; ibuprofen; respiratory distress syndrome

AIM: To study the kinetics of tumor necrosis factor α (TNF_α), interleukine-1β (IL-1β), and macrophage inflammatory protein-1α (MIP-1α) gene expression in rat lung after ip lipopolysaccharides (LPS) and the effect of dexamethasone (Dex) and ibuprofen (Ibu) on the cytokines gene expression. **METHODS**: The amount of Evans blue in lung was measured by fluorescence method. The mRNA levels of TNF_α, IL-1β, and MIP-1α in rat lung were assessed by slot blot analysis. **RESULTS**: The mRNA levels of TNF_α, IL-1β, and MIP-1α in rat lung after ip LPS increased in a dose-dependent manner, and peaked at 2, 6, and 12 h, respectively. Both Dex 50 mg $\cdot kg^{-1}$ and Ibu 90 mg $\cdot kg^{-1}$ injected at 1 h before ip LPS markedly decreased the content of Evans blue in lung at 1 h after ip LPS. After Dex or Ibu pretreatment, the peak levels of TNF_α, IL-1β, and MIP-1α mRNA decreased markedly compared with LPS alone. **CONCLUSION**: The gene expression of TNF_α, IL-1β, and MIP-1α in rat lung increased

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after ip LPS. Dex and Ibu prevented LPS-induced lung injury through inhibiting the cytokines gene expression.

The morbidity and mortality associated with severe sepsis and related disorders remain high despite prompt development of bactericidal antibiotics and intensive care support[1]. The tissue damage caused by uncontrolled overproduction of inflammatory mediators accounts for the pathophysiology of sepsis and acute lung injury[1,2]. Although the role of tumor necrosis factor-α (TNF_α), interleukin-1β (IL-1β), and macrophage inflammatory protein-1α (MIP-1α) has not been clearly elucidated[1,2], there is substantial evidence suggesting that the inflammatory cytokines are important mediators of acute lung injury in sepsis[3]. We raised that possibility that the pharmacologic modulation of the cytokine expression may be of utility in the treatment of acute lung injury. This paper was to study the changes of lung vascular permeability in rat lung in response to LPS, the kinetics of TNF_α, IL-1β, and MIP-1α gene expression in lung, as well as the effects of dexamethasone (Dex) and ibuprofen (Ibu) on lung vascular permeability and the cytokines expression.

MATERIALS AND METHODS

Reagents LPS (*E coli* 0127: B8) was from Difco; Evans blue was from Fluka; Dex and Ibu were from Sigma; [α-^{32}P]dCTP was provided by China Nuclear Physical Institute. The plasmid of m-TNF_α, m-IL-1β, and m-MIP-1α cDNA were generously provided by Dr Sander van Deventer (Amsterdam University).

Rats and Evans blue measurement Wistar rats, supplied by the Experimental Animal Institute of Chinese Academy of Medical Science, were divided into 10 groups (5 rats/group): 1) saline, 2) LPS 0.5 mg·kg^{-1}, 3) LPS 5 mg·kg^{-1}, 4) LPS 12.5 mg·kg^{-1}, 5) LPS 12.5 mg·kg^{-1} + Ibu 90 mg·kg^{-1}, 6) LPS 12.5 mg·kg^{-1} + Dex 50 mg·kg^{-1}, 7) saline, 8) LPS 0.5 mg·kg^{-1}, 9) LPS 5 mg·kg^{-1}, 10) LPS 12.5 mg·kg^{-1}. Rats were anesthetized with sodium pentobarbital (40 mg·kg^{-1}). Saline, Dex 50 mg·kg^{-1} or Ibu 90 mg·kg^{-1} was injected iv, LPS was injected ip at 1 h later. Evans blue (1 %, 2 mL·kg^{-1}) was iv injected 1 min before ip LPS. The rats in groups 1 – 6 were killed by exsanguination at 1 h, while the other rats were killed at 8 h after ip LPS. The amount of Evans blue dye extravasated from the 100 mg lung tissue was determined by fluorescence analysis[4], and then expressed as μg·g^{-1} of dry weight of tissue which reflected the lung vascular permeability.

Isolation of RNA and Northern, slot blot analysis The rat lung (about 1 g) was harvested and RNA was then isolated from homogenized lung tissue using ice-cold guanidinium thiocyanate/sodium citrate/β-mercaptoethanol solution according to the single-step method (Chomoczynki and Sacchi). Approximately 20 μg of total RNA was applied to nylon membrane for slot blot analysis. The membrane was baked at 80 ℃ for 2 h. For Northern blot, RNA was evaluated by Northern gel electrophoresis and then RNA was transferred from the formaldehyde gel to a nylon membrane by blotting. All the membranes bound RNA were hybridized for about 20 h to [α-^{32}P]dCTP labeled TNF_α, IL-1β, and MIP-1α probes. Autoradiograms were scanned with a laser densitometer, and area-integrated to quantitate the relative mRNA levels.

TNF activity after LPS injection by different routes After iv or ip LPS 5 mg·kg^{-1}, the rats blood was drawn into heparinized syringe at 0, 0.5, 1, 2, 4, 6, 12, and 24 h. The plasma was seperated and then assayed for TNF activity by L929 cytotoxicity method as previously described[5].

Statistical analysis Data were expressed as $\bar{x} \pm s$. t test was used to evaluated the difference between 2 groups.

RESULTS

Effects of Dex and Ibu on LPS-induced lung injury As an index of pulmonary microvascular permeability, the contents of Evans blue in lung increased in a dose-dependent manner at 1 h after ip LPS. But there was no difference between ip LPS groups and saline at 8 h ($P>0.05$). Both Dex 50 mg·kg^{-1} and Ibu 90 mg·kg^{-1} injected at 1 h before LPS 12.5 mg·kg^{-1} stimulation markedly decreased the content of Evans blue as compared with LPS alone ($P < 0.05$). There was no difference between Dex and Ibu ($P>0.05$) (Tab 1).

Effects of LPS on TNF_α, IL-1β, and MIP-1α gene expression in lung The mRNA levels of TNF_α, IL-1β, and MIP-1α in lung of rats were increased in a dose-dependent manner at 2 h after ip LPS (0, 0.5, 2, 5, 12.5 mg·kg^{-1}) as compared to the saline control. After ip LPS (5 mg·kg^{-1}), TNF_α, IL-1β, and MIP-1α mRNA expression was induced at 1, 2, 6 h and peaked at 2, 6, 12 h respectively, and the contents of mRNA were 3, 1.5, and 6 times over saline (Fig 1).

Tab 1. Contents of Evans blue (μg/g dry weight) in rat lung at 1 and 8 h after ip LPS. $n = 5$, $\bar{x} \pm s$. $^bP<0.05$ *vs* saline (1 h). $^eP<0.05$ *vs* LPS (12.5 mg·kg^{-1}, 1 h). $^hP<0.05$ *vs* 1 h.

Drugs (mg·kg^{-1})	1 h	8 h
Saline	94 ± 16	94 ± 32
LPS (0.5)	124 ± 52	142 ± 43
LPS (5)	175 ± 9^b	101 ± 30^h
LPS (12.5)	260 ± 63^b	144 ± 68^h
LPS (12.5) + Ibu (90)	155 ± 59^{be}	
LPS (12.5) + Dex (50)	178 ± 43^{be}	

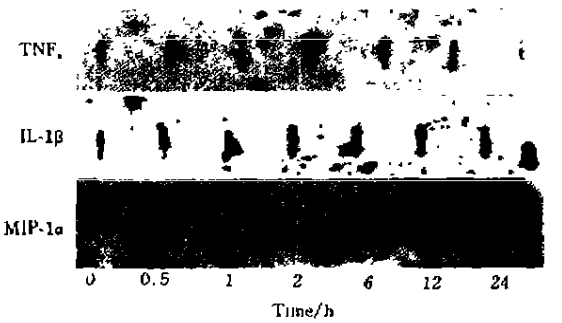

Fig 1. Autoradiograms of slot blot sequentially hybridized for TNF$_\alpha$, IL-1β, and MIP-1α probes. Total RNA from rat lung was extracted at different time (0 to 24 h) after ip LPS.

Effects of Dex and Ibu on TNF$_\alpha$, IL-1β, and MIP-1α expression in lung After pretreatment with Dex 50 mg·kg^{-1} or Ibu 90 mg·kg^{-1}, the LPS (5 mg·kg^{-1})-induced mRNA expression in lung was markedly decreased, the peak levels of TNF$_\alpha$, IL-1β, and MIP-1α mRNA were decreased by 70 % and 25 %, 50 % and 30 %, 70 % and 15 % respectively as compared with ip LPS alone. The results of Northern blot (Fig 2) were same as the slot blot.

Effects of the pathway of *LPS* injection on plasma TNF activity The rats plasma TNF activity peaked at 2 h and was not detected at 4 h after iv LPS. After ip LPS, the TNF level peaked at 6 h, and was not detected at 12 h (Fig 3).

DISCUSSION

Acute lung injury in rats was produced by ip LPS in this study. The results showed that the

Fig 2. Northern blot showing effect of Dex and Ibu on LPS-induced TNF$_\alpha$ mRNA expression in rat lung. Total RNA from rat lung was extracted at 2 h after ip LPS.

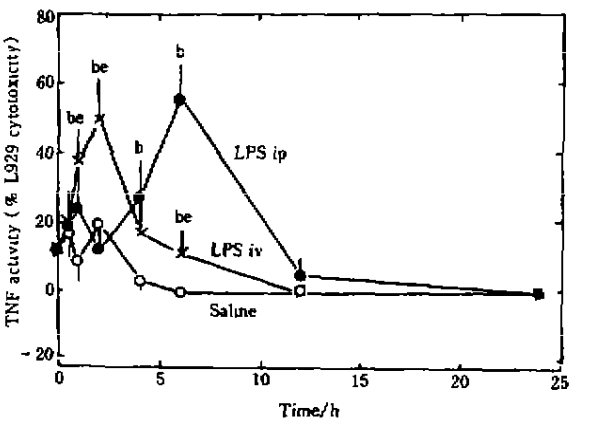

Fig 3. TNF activity in rat plasma after iv or ip LPS. $n = 4$, $\bar{x} \pm s$. $^bP<0.05$ *vs* saline, $^eP<0.05$ *vs* LPS ip group.

contents of Evans blue in lung tissue increased in a dose-dependent manner after ip LPS. It suggested that the lung vascular permeability increased after LPS stimulation. The mRNA expression of TNF$_\alpha$, IL-1β, and MIP-1α in lung began at 1, 2, 6 h, and peaked at 2, 6, 12 h respectively. These marked differences in kinetics of cytokine expression undoubtedly provide a reflection of the functional differences of these mediators. TNF$_\alpha$ may play a role in triggering release of IL-1β and MIP-1α. It is suggested that TNF$_\alpha$ appears to play a central role in the pathogenesis of acute lung injury. There are also many evidences to support that IL-1β may also play an important role in lung injury[6]. The role of MIP-1α in acute lung injury is less clear, but appears to possess potent chemotactic activity for

human neutrophils *in vitro* and elicit a localized leukocyte infiltration *in vivo*[7,8]. Thus the increase in MIP-1α mRNA raises the possibility that this cytokine may have mediated localization of neutrophils to the lungs.

The reason for ip LPS in the study was that ip LPS resembled intraperitoneal sepsis in patients[5]. The present study showed that the TNF release after iv LPS was more rapid than that by ip. It suggests that the profile of cytokines expression be related to the route of LPS administration.

Our study showed that LPS-induced gene expression TNF_α, IL-1β, and MIP-1α in lung may be markedly inhibited by Dex and Ibu and the contents of Evans blue in lung decreased if pretreatment with Dex or Ibu. It suggested that Dex and Ibu prevented LPS-induced lung injury by strong inhibitory effects upon TNF_α, IL-1β, and MIP-1α gene transcriptional activation. Glucocorticoids suppressed IL-1β synthesis by two distinct mechanisms, blocking transcription of IL-1β mRNA and blocking post-transcriptonal IL-1β synthesis via cAMP[9]. The inhibitory effects of Ibu on TNF_α, IL-1β, and MIP-1α gene transcription may be mediated by intracellular PGE_2 and cAMP/cGMP pathway[10], but the mechanism is less clear.

This work demonstrates that gene expression of TNF_α, IL-1β, and MIP-1α in rat lung increases after ip LPS, and to inhibit expression of the cytokines by Dex and Ibu may be an effective therapeutic approach for acute lung injury.

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地塞米松和布洛芬对脂多糖诱导的大鼠肺中 TNF_α、IL-1β 和 MIP-1α 基因表达的影响

R977.1

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R971.1

关键词 Wistar 大鼠；脂多糖；肿瘤坏死因子；白细胞介素-1；基因表达；地塞米松；布洛芬；呼吸窘迫综合症

IL-1

目的：研究地塞米松(Dex)和布洛芬(Ibu)对脂多糖(LPS)诱导的大鼠肺 TNF_α、IL-1β 和 MIP-1α 基因表达的影响. 方法：荧光法测定肺中伊文斯蓝含量，Slot blot 对细胞因子 mRNA 表达相对定量. 结果：腹腔注射 LPS 导致大鼠肺中 TNF_α、IL-1β 和 MIP-1α mRNA 表达明显增加，与 LPS 剂量有依赖关系，峰值分别在 2，6，12 小时. 在 LPS 前 1 小时给药，Dex 50 $mg \cdot kg^{-1}$ 和 Ibu 90 $mg \cdot kg^{-1}$ 均明显降低肺中伊文斯蓝含量，同时 TNF_α、IL-1β 和 MIP-1α mRNA 表达量亦明显减少. 结论：LPS 诱导大鼠肺中 TNF_α、IL-1β 和 MIP-1α 基因表达，Dex 和 Ibu 通过抑制细胞因子表达而减轻肺损伤.