BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Jul; 17 (4): 351 - 353

# Effect of matrine on lipopolysaccharides/*D*-galactosamine-induced hepatitis and tumor necrosis factor release from macrophages *in vitro*

HU Zhen-Lin, ZHANG Jun-Ping, YU Xiang-Bin, LIN Wen, QIAN Ding-Hua (*Research Laboratory of Natural and Synthetic Drugs, College of Pharmacy, Second Military Medical University, Shanghai 200433, China*); WAN Mo-Bin (*Department of Infectious Diseases, Changhai Hospital, Second Military Medical University, Shanghai 200433, China*)

**KEY WORDS** matrine; toxic hepatitis; galactosamine; lipopolysaccharides; tumor necrosis factor; peritoneal macrophages

**AIM**: To study the effects of matrine (Mat) on lipopolysaccharides (LPS)-induced fatal hepatitis in *D*-galactosamine (*D*-GalN)-sensitized mice and tumor necrosis factor (TNF) release from peritoneal macrophages (PMØ). **METHODS**: Mice were pretreated with Mat (10, 50 mg·kg$^{-1}$, ip, bid×3 d), and then injected ip LPS+*D*-GalN. Liver injury was assessed by quantifying plasma activity of alanine aminotransferase (ALT) and histopathological examination. The TNF activities in the supernatants of mouse PMØ stimulated with LPS in the presence of Mat (32.5 - 500 mg·L$^{-1}$) were monitored by the L929 target cells lytic assay. **RESULTS**: Mat pretreatment markedly diminished hepatic injury induced by LPS in combination with *D*-GalN. Mat inhibited LPS-induced TNF release from mouse PMØ *in vitro* in a concentration-dependent manner. **CONCLUSION**: Mat protected the *D*-GalN-treated mice from the development of fatal hepatitis induced by LPS, and inhibited the LPS-induced TNF release from mouse PMØ.

Lipopolysaccharides (LPS) could elicit fatal hepatitis in *D*-galactosamine (*D*-GalN)-sensitized mice. The toxicity of LPS was caused by endogenous mediators released by interaction of LPS with target cells. Tumor necrosis factor (TNF) produced by macrophages (MØ) in response to LPS is a prominent mediator in the pathogenesis of LPS-induced hepatic injury[1-3].

Matrine (Mat), an alkaloid isolated from *Sophora alopecuroides* L, shows anti-inflammatory and immunodepressant effects[4]. This paper was to report the effect of Mat on LPS-induced fatal hepatitis in *D*-GalN-sensitized mice and TNF release from mouse peritoneal macrophages (PMØ) *in vitro*.

Received 1995-04-12 Accepted 1995-09-15

## MATERIALS AND METHODS

**Reagents** Mat (mp 75.5 - 77.5 ℃, purity > 99 %), a pale yellow crystalloid powder from Yan Chi Pharmaceutical Factory, Ningxia, dissolved in PBS for ip injection or RPMI-1640 medium for *in vitro* experiments. *D*-GalN, LPS derived from E coli 0111:B4, calcimycin (Cal), and RPMI-1640 medium were purchased from Sigma Co; Thioglycalate-broth was from Shanghai Academy of Biologic Products.

**Animals** ICR mice, CV, ♀, weighing 22 ± *s* 1.2 g, from the Animal Center of Second Military Medical University (certificate number 28 - 48).

**Induction of fatal hepatitis in mice** ICR mice were injected ip *D*-GalN (800 mg·kg$^{-1}$) and LPS (1 μg·kg$^{-1}$). After 24 h, blood samples were collected for the measurement of plasma ALT activity[5]. For histological examination, the livers were fixed in 10 % formalin solution, and paraffin-embedded sections were stained with hematoxylin and eosin. To investigate the effects of Mat on the development of fatal hepatitis, mice were pretreated with Mat (0 - 50 mg·kg$^{-1}$, ip, bid×3 d). An hour after the last injection, *D*-GalN and LPS were injected ip.

**Induction of TNF from PMØ**[6] Peritoneal exudate cells (PEC) from thioglycalate-primed mice were seeded at 2 × 10$^6$/well for incubation at 37 ℃, in 5 % $CO_2$ to facilitate MØ adherence. The priming agent, Cal (1 μmol·L$^{-1}$) was added to the cells to enhance the TNF release from MØ in response to the stimulation of LPS. After 6-h incubation, the cells were washed with RPMI-1640 medium to remove the agent and the nonadherent cells. The adherent Cal-primed MØ was incubated for 6 h in the presence of LPS (50 μg·L$^{-1}$). TNF activity in the supernatants was monitored by L929 target cells lytic assay. To investigate the effects of Mat on LPS-induced TNF release, Mat (0 - 500 mg·L$^{-1}$) was added together with LPS.

## RESULTS

LPS elevated plasma ALT activity and

mortality rates in a dose-dependent manner, 24 h after ip in combination with *D*-GalN 800 mg·kg$^{-1}$ (Tab 1).

**Tab 1. Plasma ALT activity and mortality in mice 24 h after ip LPS + *D*-GalN 800 mg·kg$^{-1}$.** $\bar{x} \pm s$. $^{c}P < 0.01$ *vs* control.

| LPS/ μg·kg$^{-1}$ | Mice | Plasma ALT/ U·L$^{-1}$ | Mortality/ % |
|---|---|---|---|
| 0 | 10 | 298 ± 105 | 0 |
| 0.1 | 20 | 2 669 ± 2113$^{c}$ | 10 |
| 0.5 | 20 | 4 475 ± 2467$^{c}$ | 40 |
| 1 | 20 | 6 738 ± 2142$^{c}$ | 85 |

The average of plasma ALT activity of mice injected ip LPS 1 μg·kg$^{-1}$ + *D*-GalN 800 mg·kg$^{-1}$ reached more than 6 kU·L$^{-1}$. The liver revealed massive hemorrhagic necrosis (Fig 1, Plate 3).

When Mat 10, 50 mg·kg$^{-1}$ were injected ip before ip LPS 1 μg·kg$^{-1}$ and *D*-GalN 800 mg·kg$^{-1}$, the elevation of plasma ALT activity was reduced in a dose-dependent manner and mortality induced by LPS/*D*-GalN was also prevented (Tab 2).

**Tab 2. Effect of pretreatment of Mat (10 – 50 mg·kg$^{-1}$ ip, bid × 3 d) on hepatitis induced by LPS 1 μg·kg$^{-1}$ + *D*-GalN 800 mg·kg$^{-1}$.** $\bar{x} \pm s$. $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* control.

| Mat/ mg·kg$^{-1}$ | Mice | Plasma ALT/U·L$^{-1}$ | Mortality/ % |
|---|---|---|---|
| 0 | 25 | 6 918 ± 1 913 | 84 |
| 10 | 25 | 3 587 ± 2 540$^{b}$ | 56 |
| 50 | 20 | 1 841 ± 2 456$^{c}$ | 10 |

Liver congestion and necrosis induced by LPS in *D*-GalN-sensitized mice were ameliorated markedly by Mat pretreatment (Fig 1).

The LPS-induced TNF release was inhibited markedly by Mat in a concentration-dependent manner (Tab 3).

**Tab 3. TNF activity in supernatants of Cal-primed PMØ stimulated with LPS (50 μg·L$^{-1}$) + Mat for 6 h.** $n = 3$ homogenates (each pooled from 8 mice). $\bar{x} \pm s$. $^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* control.

| Mat/mg·L$^{-1}$ | TNF activity/kU·L$^{-1}$ |
|---|---|
| 0 | 83 ± 5 |
| 31.2 | 61 ± 27$^{a}$ |
| 62.5 | 42 ± 23$^{b}$ |
| 125 | 33 ± 20$^{c}$ |
| 250 | 22 ± 23$^{c}$ |
| 500 | 4 ± 7$^{c}$ |

## DISCUSSION

The present study demonstrated that LPS showed lethal toxicity and could induce massive hemorrhagic necrosis in the liver in D-GalN-treated mice, which is in accordance with the results[1,2].

Mat pretreatment diminished the sensitivity of *D*-GalN-treated mice to lethal toxicity of LPS and protect the mice from the development of hepatic injury in a concentration-dependent manner.

Mat inhibited the LPS-induced release of TNF from mouse PMØ *in vitro*. Since TNF has been proved to be a prominent mediator in the pathogenesis of LPS-induced hepatic injury, the inhibitory effect of Mat on the TNF production may be involved in the mechanisms of its protection against the hepatotoxicity of LPS.

In conclusion, the present study demonstrated the protective effect of Mat against the LPS-induced development of fatal hepatitis in *D*-GalN-sensitized mice and its inhibitory effects on the LPS-induced release of TNF, a cytokine that plays a crucial role in mediating LPS reaction.

## REFERENCES


1 Hishinuma I, Nagakawa JI, Hirota K, Miyamoto K, Tsukidate K, Yamanaka T, *et al*. Involvement of tumor necrosis factor-α in development of hepatic injury in galactosamine-sensitized mice. Hepatology 1990; **12**: 1187 – 91.

2 Lehmann V, Freudenberg MA, Galanos C. Lethal toxicity of lipopolysaccharide and tumor necrosis factor in normal and *D*-galactosamine-treated mice. J Exp Med 1987; **165**: 657 – 63.

3 Tiegs G, Wolter M, Wendel A. Tumor necrosis factor is a terminal mediator in galactosamine/endotoxin-induced hepatitis in mice. Biochem Pharmacol 1989; **38**: 627 – 31.

4 Tao SC, Wang JZ. Pharmacological effects of alkaloids from *Sophora alopecuroides*. Chin Pharm J 1992; **27**: 201 – 4.

5 Karmen A, Wróblewski F, LaDue JS. Transaminase activity in human blood. J Clin Invest 1955; **34**: 126 – 31.

6 Hu ZL, Zhang JP, Yi YH, Qian DH. Effect of esculenroside H on release of tumor necrosis factor from mouse peritoneal macrophages. Acta Pharmacol Sin 1993; **14**: 550 – 2

## 苦参碱对脂多糖/D-氨基半乳糖诱导的肝炎及离体巨噬细胞释放肿瘤坏死因子的影响

胡振林，张俊平，余祥彬，林 文，钱定华
（第二军医大学药学院中西药研究室，上海 200433，中国）
方莫斌
（第二军医大学长海医院传染病科，上海 200433，中国）

**关键词** 苦参碱；中毒性肝炎；氨基半乳糖；脂多糖；肿瘤坏死因子；腹腔巨噬细胞

**目的**：研究苦参碱（Mat）对脂多糖（lipopolysaccharides，LPS）诱导的 D-氨基半乳糖（D-GalN）致敏小鼠致死性肝炎以及腹腔巨噬细胞（PMØ）释放肿瘤坏死因子（TNF）的影响 **方法**：小鼠 ip Mat 10，50 mg·kg$^{-1}$，bid×3 d，然后 ip LPS（1 μg·kg$^{-1}$）和 D-GalN（800 mg·kg$^{-1}$），通过病理组织学观察及测定血清丙氨酸转氨酶（ALT）活性来评估肝损伤。小鼠 PMØ 培养上清中的 TNF 活性以杀伤 L929 细胞的结晶紫染色法测定 **结果**：Mat 降低了 LPS/D-GalN 引起的血清 ALT 活性升高及小鼠对 LPS/D-GalN 致死毒性的敏感性并抑制 LPS 诱导的小鼠 PMØ 释放 TNF **结论**：Mat 防治 LPS/D-GalN 引起的致死性肝炎，并抑制 LPS 诱导的 TNF 释放



BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Jul; 17 (4): 353 - 356

## Effects of tyrphostins on activity of casein kinase Ⅱ from rat liver

HUANG Cai[1], KANG Tie-Bang, LIANG Nian-Ci
(*Department of Medical Biochemistry, Guangdong Medical College, Zhanjiang 524023, China*)

**KEY WORDS** tyrphostins; caseins; protein kinases; liver; enzyme inhibitors

**AIM**: To investigate the effects of tyrphostins, (AG213, AG1394, AG114, AG1109, AG555) on the activity of casein kinase (CK) Ⅱ. **METHODS**: CK Ⅱ was partially purified from rat livers by sequential DE52 and heparin-Sepharose chromatography. CK Ⅱ activity was assayed by incubating CK Ⅱ with dephosphorylated casein and [γ-$^{32}$P]ATP. **RESULTS**: AG213 inhibited the activity of CK Ⅱ with $IC_{50}$ 44.7 μmol·L$^{-1}$ (41.5 - 47.9 μmol·L$^{-1}$), and AG1394 (144 μmol·L$^{-1}$) strongly inhibited the activity of CK Ⅱ with an inhibitory ratio of 89 %. AG114 (174 μmol·L$^{-1}$) and AG1109 (126 μmol·L$^{-1}$) had inhibitory effects on the activity of CK Ⅱ ($P<0.01$). AG555 (136 μmol·L$^{-1}$) had little effect on CK Ⅱ activity. **CONCLUSION**: Some tyrphostins are potent inhibitors of CK Ⅱ.

[1] Now in *Department of Biology, University of Pennsylvania, Philadelphia PA 19104, USA*.

Received 1994-11-07 Accepted 1995-11-01

Casein kinase (CK) Ⅱ is a ubiquitous protein serine/threonine kinase in the cytosol, nucleus, and membranes of eukaryotic cells. CK Ⅱ purified from various tissues is usually a tetrameric complex with an $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, or $\alpha'_2\beta_2$ structure [1,2]. CK Ⅱ may play an important role in cell proliferation[3-6]. For example, CK Ⅱ phosphorylates a number of nuclear proteins including *Fox*, *Myb*, *Myc*, p53 tumor suppressor protein, and SV40 large T antigen. These proteins are implicated in oncogenic transformation and cell proliferation.

Tyrphostins (AG213, AG114, AG555, AG1394, and AG1109), a series of synthetic chemicals, are inhibitors of tyrosine kinase[7], but the efficacy of AG213 in inhibiting EGF-induced [$^3$H] thymidine uptake in A431 cells does not correlate with its tyrosine kinase inhibitory activity[8]. Their effects on CK Ⅱ are unknown. In the present study, the effects of tyrphostins on the activity of CK Ⅱ from rat liver were investigated.

## MATERIALS AND METHODS

Heparin and phosphatidylserine (PS) (Sigma); ATP