文章编号:1001-6880(2016)3-0420-05

# 丹参酮 IIA 对脂多糖引起腹膜间皮细胞损伤的保护作用

张 慧,宋 宇*

新乡医学院药学院药理学教研室,新乡 453000

**摘 要**:本研究探讨了丹参酮 IIA 对脂多糖诱导大鼠腹膜间皮细胞(rat peritoneal mesothelial cells,RPMCs)炎症反应、氧化应激及其损伤的影响。采用原代培养大鼠腹膜间皮细胞(RPMCs),分为正常对照组、5 mg/L LPS 作用 RPMC 24 h 组、5 mg/L LPS 分别与 40、80 和 160 μmol/L 丹参酮 IIA 共同作用 24 h 组。MTT 测定各组 RPMCs 增值率。ELISA 法检测细胞培养液中 IL-1β、IL-6 和 TNF-α 表达。流式细胞仪检测活性氧(ROS)水平,试剂盒检测丙二醛(MDA)和超氧化物歧化酶(SOD)表达。RT-PCR 法检测各组 FN、COL I、Bcl-2 和 Bax mRNA 的表达。研究发现丹参酮 IIA + LPS 组的 RPMCs 的增殖率明显高于 LPS 组($P<0.05$)。丹参酮 IIA 可降低 LPS 刺激下 RPMCs 中 IL-1β、IL-6、TNF-α、ROS 和 MDA 的表达,同时 FN、COL I、Bax mRNA 表达也明显下降。但 SOD 水平和 Bcl-2 mRNA 表达明显升高,与 LPS 组相比。实验结果显示,丹参酮 IIA 具有抑制 LPS 所致的氧化应激及炎性反应,减少细胞凋亡及抑制纤维化的作用,进而起到对 RPMCs 的保护作用。

**关键词**:丹参酮 IIA;脂多糖;氧化应激;腹膜间皮细胞

**中图分类号**:R965 **文献标识码**:A DOI:10.16333/j.1001-6880.2016.3.018

# Protective Effect of Tanshinone IIA on Lipopolysaccharide-induced Injury of Rat Peritoneal Mesothelial Cells

ZHANG Hui, SONG Yu*

*College of Pharmacy, Xinxiang Medical University, Xinxiang 453000, China*

**Abstract**: The objective of this study was to investigate the protective effect of tanshinone IIA on rat peritoneal mesothelial cells (RPMCs) treated with lipopolysaccharide (LPS). RPMCs were co-treated with different concentrations of tanshinone IIA (40, 80 and 160 μmol/L) and LPS (5 mg/L) for 24 h. The levels of IL-1β, IL-6, TNF-α in cell culture supernatant were determined by enzyme-linked immunosorbent assay. Reactive oxygen species (ROS) were quantified by flow cytometry. The intracellular malondialdehyde (MDA) level and the activity of superoxide dismutase (SOD) were measured by commercial reagent kits. Relative levels of fibronectin (FN), collagen-I (COL I), Bcl-2 and Bax mRNA were quantified by real-time reverse transcription polymerase chain reaction. The results showed that tanshinone IIA significantly decreased IL-1β, IL-6, TNF-α and ROS, MDA levels in dose-dependent manners. At the same time, the levels of FN, collagen I, Bax mRNA were also significantly decreased by tanshinone IIA. However, SOD levels and Bcl-2 mRNA expressions were significantly increased by tanshinone IIA. In conclusion, tanshinone IIA can inhibit LPS-induced inflammation, oxidative stress in peritoneal mesothelial cells, thus protect peritoneal membrane.

**Key words**: tanshinone IIA; LPS; oxidative stress; RPMCs

## Introduction

Continuous ambulatory peritoneal dialysis (CAPD) has been used as a treatment for chronic renal failure for over three decades [1]. The peritoneal membrane exhibits injuries that correlate with the duration of dialysis. Long-delayed healing of infection is the major reason of peritoneal dysfunction and peritoneal fibrosis. Lipopolysaccharides (LPS), a glycolipid that is produced and secreted by gram-negative bacteria during peritonitis, are caused mainly by catheter-related infections in long-term peritoneal dialysis. LPS is one of the reasons of peritonitis and peritoneal fibrosis [2,3]. Here we use LPS model to stimulate the rat peritoneal mesothelial

Received: November 4, 2015 Accepted: January 5, 2016

Foundation Item: This work was supported by highly educated people funded projects of Xinxiang Medical University

* Corresponding author Tel: 86-373-3029101; E-mail: yl3029101@163.com

cells (RPMCs).

Tanshinone IIA, derived from the dried root of *Salvia miltiorrhiza* Bunge (Lamiaceae), is one of the key components of Danshen and has been widely used for centuries in many Asian countries for the prevention and management of cardiovascular diseases [4]. Studies had demonstrated that tanshinone IIA attenuated the inflammatory response and apoptosis after traumatic injury of the spinal cord in adult rats [5]. Furthermore, previous studies had indicated that tanshinone IIA effectively protected cardiac myocytes against oxidative stress-triggered damage and apoptosis[6].

In the present study, we hypothesized that the administration of tanshinone IIA might have the potential protective effect on RPMCs induced by LPS, thus can attenuate the complication of peritoneal dialysis (PD) patients.

## Material and Methods

### Isolation and primary culture of RPMCs

Male Wistar rats weighing 150-180 g were purchased from animals facility of Experimental animal center of Henan Province, China [Certificate number SYXK (Yu) 2011-0001]. Animals were received an injection of 20 mL 0.25% trypsin (in 0.02% EDTA solution) intraperitoneally, and two hours later, the abdominal fluid of individual rat was collected, followed by centrifuging at 150 × g for 15 min. The cell settling were suspended and cultured in flasks under 5% $CO_2$ at 37 ℃. Cells were treated for 24 h with 5 mg/L LPS in the absence or the presence of tanshinone IIA (40, 80 and 160 μmol/L).

### Methyl thiazolyl tetrazolium analysis

RPMCs were seeded into 96-well plates at a density of $5 \times 10^3$ cells per well 24 h before treatment. Following treatment as above, cell viability was determined using MTT assay. In brief, 15 μL (5 mg/mL) MTT working solution was added to each well and after incubation at 37 ℃ for 4 h, the MTT solution was removed and 200 μL of DMSO was added to dissolve the crystals. The formation of formazan was observed by monitoring the signal at 570 nm using a microplate reader.

### ELISA assay of IL-1β, IL-6 and TNF-α level in supernatant

Levels of IL-1β, IL-6 and TNF-α in the cell culture supernatant were detected using rat IL-1β ELISA kit, rat IL-6 ELISA kit (GenStar BioSolutions Co., Ltd) and rat TNF-α ELISA kit (Beijing BLKW Biotechnology co., Ltd, China), respectively according to the manufacturer's instructions.

### Detection of intracellular ROS level

Intracellular formation of ROS was detected using the reactive oxygen species assay kit (Beyotime, China) according to the manufacturer's instructions. RPMCs ($5 \times 10^6$) were incubated with 10 μM DCFH-DA probes at 37 ℃ for 35 min. DCFH-DA was deacetylated intracellularly by nonspecific esterase, which was further oxidized by ROS to the fluorescent compound 2,7-dichlorofluorescein (DCF). DCF fluorescence was detected by flow cytometer (BD FACScan, USA) with excitation at 488 nm and emission at 525 nm.

### Measurement of intracellular MDA content and SOD activity

The intracellular MDA level and the activity of SOD were measured by commercial reagent kits (Nanjing Jiancheng Bioengineering Institute, China).

### Real-time RT-PCR analysis

RNA expression was evaluated by real-time RT-PCR in cultured RPMCs. Total RNA was extracted using TRIzol reagent (Invitrogen, USA) according to the manufacturer's protocol. The following primer sets were used to amplify: Fibronectin Forward sequence: 5′-AA GCCAGAGTCAGATAACCG-3′ Reverse sequence: 5′-GTTGGCACTGACGAAG AGCC-3′; collagen Ⅰ Forward sequence: 5′ GCGAGGACATGAGGAGTA GC3′ Reverse sequence: 5′ CCTGTGACCAGGGATGATGTCTT3′; Bax Forward sequence: 5′ -GATGCGTCCACCAAGAA -3 ′ Reverse sequence: 5′ -AGTAGAA GAGGGCAACCAC-3 ′, and Bcl-2 Forward sequence: 5′ -CCCAAGGGAAGACGATG-3 ′ Reverse sequence: 5 ′ GAGCGGGTAGGGAAAGA-3 ′, GAPDH Forward sequence 5′-TCCCTCAAGATTGTCAGCA

A-3′ Reverse sequence: 5′-AG ATCCACAACGGATACATT-3′. The cycling program of real-time RT-PCR reaction involved preliminary denaturation at 94 ℃ for 4 min, followed by 35 cycles of denaturation at 94 ℃ for

30 s, annealing at 60 ℃ for 30 s and elongation at 72 ℃ for 30 s. Relative amounts of mRNA were normalized by GAPDH mRNA expression.

**Statistical analysis**

Statistical analysis was carried out with SPSS19. 0. Comparisons among groups were performed by one-way ANOVA. Results were expressed as mean ± SD. $P < 0.05$ was considered statistically significant.

## Results

**Effect of LPS and tanshinone IIA on cell viability**

As shown in Fig. 1, 5 mg/L LPS had significant growth inhibition effects on RPMCs. However, cell viability was increased remarkably after the cells being cotreated with tanshinone IIA for 24 h in a dose-dependent manner.

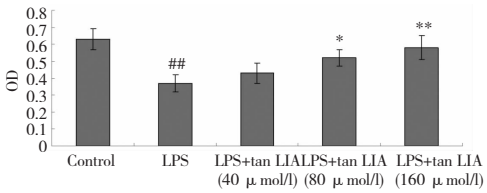


**Fig. 1 Effect of tanshinone IIA on viability of RPMC stimulated by LPS**

Note: $^{*}P < 0.05$, $^{**}P < 0.01$ vs LPS; $^{\#\#}P < 0.01$ vs Control

**Tanshinone IIA suppressed LPS-induced IL-1β, IL-6 and TNF-α expression in RPMCs**

As shown in Fig. 2, the levels of IL-1β, IL-6 and TNF-α increased remarkably in LPS group compared to control group. However, tanshinone IIA suppressed LPS-induced IL-1β, IL-6 and TNF-α expression in dose-dependent manner ($P < 0.05$).

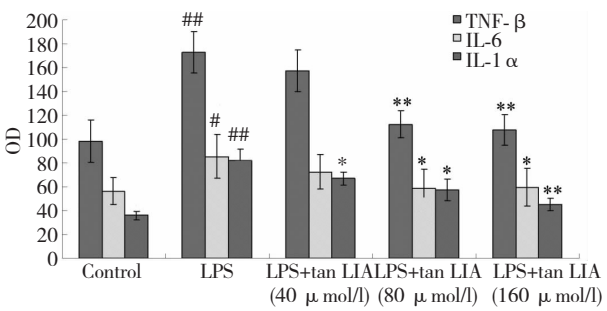


**Fig. 1 Effects of tanshinone IIA on LPS-induced IL-1β, IL-6 and TNF-α expression in RPMCs**

Note: $^{*}P < 0.05$, $^{**}P < 0.01$ vs LPS; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs Control

**Effect of tanshinone IIA on ROS generation induced by LPS in RPMCs**

As shown in Fig. 3, treatment of RPMCs with 5 mg/L LPS for 24 h resulted in marked increases of ROS production compared with control group ($P < 0.01$). But tanshinone IIA decreased LPS-induced production of ROS in RPMCs. Tanshinone IIA at the concentration of 160 μmol/L had the highest effect.

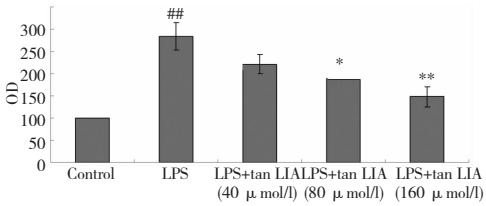


**Fig. 3 Effect of tanshinone IIA on ROS generation induced by LPS in RPMCs**

Note: $^{*}P < 0.05$, $^{**}P < 0.01$ vs LPS; $^{\#\#}P < 0.01$ vs Control

**Effect of tanshinone IIA on SOD activity and MDA content induced by LPS in RPMCs**

Compared with the control group, LPS group showed a significant increase in MDA level and a remarkably decrease in SOD activity, whereas cotreatment with tanshinone IIA increased SOD activity and decreased MDA level (Table 1).

**Table 1 Effect of tanshinone IIA on SOD activity and MDA content induced by LPS in RPMCs**

| Groups | MDA | SOD |
|---|---|---|
| Control | 0.79 ± 0.18 | 42.35 ± 7.25 |
| LPS | 3.14 ± 0.86$^{\#\#}$ | 25.56 ± 5.31$^{\#\#}$ |
| LPS + tan IIA (40 μmol/L) | 2.42 ± 0.34$^{*}$ | 29.35 ± 9.56 |
| LPS + tan IIA (80 μmol/L) | 1.92 ± 0.26$^{**}$ | 32.57 ± 6.54$^{*}$ |
| LPS + tan IIA(160 μmol/L) | 1.63 ± 0.43$^{**}$ | 37.53 ± 5.83$^{**}$ |

Note: $^{*}P < 0.05$, $^{**}P < 0.01$ vs LPS; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs Control.

**Effect of tanshinone IIA on apoptosis-associated mRNA expression in RPMCs exposed to LPS**

Table 2 showed that exposure to 5 mg/L LPS for 24 h caused a marked increase in apoptotic marker Bax mRNA, and a decrease in anti-apoptotic marker Bcl-2 mRNA. Compared with the LPS group, the decrease in Bax mRNA expression and the increase in Bcl-2 mRNA expression were observed in the tanshinone IIA group.

**Table 2 Effect of tanshinone IIA on Bax and Bcl-2 mRNA expression in RPMCs exposed to LPS**

| Groups | Bcl-2 mRNA | Bax mRNA |
|---|---|---|
| Control | 1 | 1 |
| LPS | $0.32 \pm 0.09^{\#\#}$ | $3.28 \pm 0.32^{\#\#}$ |
| LPS + tan IIA (40 μmol/L) | $0.45 \pm 0.08^{*}$ | $2.82 \pm 0.54$ |
| LPS + tan IIA (80 μmol/L) | $0.59 \pm 0.13^{**}$ | $2.12 \pm 0.64^{*}$ |
| LPS + tan IIA (160 μmol/L) | $0.76 \pm 0.17^{**}$ | $1.75 \pm 0.52^{**}$ |

Note: $^{*}P<0.05$, $^{**}P<0.01$ vs LPS; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs Control. All results were shown as mean ± SD and were representative of three independent experiments.

### Effect of tanshinone IIA on fibrosis-associated mRNA expression in RPMCs exposed to LPS

Table 3 showed that exposure to 5 mg/L LPS for 24 h caused a marked increase in FN and COL I mRNA. Compared with the LPS group, the decrease in FN and COL I mRNA expression were observed in the tanshinone IIA group.

**Table 3 Effect of tanshinone IIA on FN and COL Ⅰ mRNA expression in RPMCs exposed to LPS**

| Groups | FN mRNA | COL I mRNA |
|---|---|---|
| Control | 1 | 1 |
| LPS | $2.28 \pm 0.74^{\#\#}$ | $2.17 \pm 0.62^{\#\#}$ |
| LPS + tan IIA (40μmol/L) | $1.95 \pm 0.36$ | $1.65 \pm 0.47^{*}$ |
| LPS + tan IIA (80μmol/L) | $1.64 \pm 0.27^{*}$ | $1.34 \pm 0.34^{**}$ |
| LPS + tan IIA (160 μmol/L) | $1.40 \pm 0.56^{**}$ | $1.24 \pm 0.26^{**}$ |

Note: $^{*}P<0.05$, $^{**}P<0.01$ vs LPS; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs Control. All results were shown as mean ± SD and were representative of three independent experiments.

## Discussion and Conclusion

During long-term PD, loss of peritoneal membrane integrity and peritoneal ultrafiltration capacity are major complications. Bacterial peritonitis is one of the major causes of peritoneal dysfunction and morbidity in CAPD patients. Cycles of inflammation may result in fibrosis and membrane thickening. Inhibition of inflammatory reaction resulted in reduced peritoneal fibrosis[7]. Chronic and prolonged inflammation lead to the occurrence of peritoneal fibrosis from long-term clinical PD[8,9]. In this study, it was demonstrated that tanshinone IIA can attenuate the expression of IL-1$\beta$, IL-6 and TNF-$\alpha$ induced by LPS. In addition, cell viability was increased remarkably by tanshinone IIA compared to LPS group. It suggested that tanshinone IIA might play a part in the local defense of the peritoneal cavity by down-regulating inflammatory mediators, which may play a potential role in preventing peritoneal fibrosis induced by peritonitis.

Bcl-2, an anti-apoptotic protein, is known to be a negative regulator of apoptosis which can prevent cytochrome c release from mitochondria and protect DNA from fragmentation. Conversely Bax, a pro-apoptotic member of the Bax/Bcl-2 family, may be a key factor promoting cytochrome c release. In the present study, it was demonstrated that tanshinone IIA can inhibit LPS-induced down-regulation of Bcl-2 and up-regulation of Bax, thus against LPS-induced RPMCs cell apoptosis and protect RPMCs.

Long term PD may result in oxidative stress, which damages peritoneal mesothelial cells and may cause peritoneal fibrosis and ultrafiltration loss [10,11]. Oxidative stress plays a major role in the development of peritoneal deterioration [12] and apoptosis [13]. Our study found that tanshinone IIA treatment can attenuate LPS-induced ROS generation. SOD, as an important antioxidant enzyme, provides a major defense against ROS generation. MDA is a parameter to measure lipid peroxidation and its increase is a direct result of free radical damage to membrane components of the cells. In the present study, we observed significant increase in the levels of SOD and remarkable decrease in the levels of MDA in RPMCs treated with tanshinone IIA compared to LPS group. The role of tanshinone IIA modulating oxidative stress has been recognized and tanshinone IIA attenuates LPS-induced apoptosis and fibrosis which is related to the inhibition of oxidative stress.

The protective effect of tanshinone IIA can help to maintain peritoneal membrane function longer in patients undergoing CAPD, thus improve peritoneal dialysis outcomes. The potential role of tanshinone IIA as well as signaling molecules, both downstream and upstream, requires further investigation.

**Reference**

1 Zuo L, Wang M. Current burden and probable increasing incidence of ESRD in China. *Clin Nephrol*, 2010, 74 (Suppl 1): S20-22.

(下转第 340 页)