International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2016.597.604

Research Article

A Study on Triptolide Protects Against Lipopolysaccharide-induced Endotoxemia by Anti-inflammation Effect

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Abstract

Background and Objective: Triptolide (TPL) is an extracts of the root of Chinese herb *Tripterygium*, which have anti-carcinogenesis effects, immunosuppressive and anti-inflammatory response. The present study was designed to evaluate the anti-inflammation effect of triptolide. **Methodology:** The endotoxemia models were established by intraperitoneally (i.p.) injecting 30 mg kg^{-1} purified LPS in BALB/c mice. The treatment groups were injected intraperitoneally different doses (1.0, 2.0 and 5.0 mg kg^{-1}) of TPL 30 min before LPS infusion. Survival rate was monitored for 7 days and serum TNF-α, ALT and BUN levels were determined. *In vitro*, endotoxemia cell models were established by given 1 μg mL^{-1} LPS in RAW 264.7 murine macrophage cell. The concentration of TNF-α and IL-1β was detected and the protein levels of TNF-α and IL-1β were determined. **Results:** A rise TNF-α and IL-1β level in serum and the increase of BUN and ALT level. While TPL treatment significantly increased survival rate in LPS-induced endotoxemia mice model, decreased the levels of ALT, BUN and serum TNF-α and IL- 1β. *In vitro*, LPS-induced TNF-α and IL-1β production was inhibited by TPL. **Conclusion:** These findings suggested that TPL had the ability of antiendotoxemia and protecting LPS-induced organ injuries by decreasing TNF-α and IL-1β level.

Key words: Triptolide, lipopolysaccharide, anti-endotoxemia, anti-inflammatory activity

Received: March 23, 2016 Accepted: April 19, 2016 Published: July 15, 2016

Citation: Zheng Wu, Cheng Lu, Yunjian Liao, Jiahong Chen, Jun Liu, Youwei Huang and Xi Lin, 2016. A study on triptolide protects against lipopolysaccharide- induced endotoxemia by anti-inflammation effect. Int. J. Pharmacol., 12: 597-604.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lipopolysaccharide (LPS) is the toxic component of endotoxin of cell wall constituents in Gram-negative bacteria and release of LPS *in vivo* would cause endotoxemia called LPS-induced endotoxemia[1]. On the cellular level, Gram-negative bacteria infection would release endotoxin, which would activate macrophages to secrete high level of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) and gradually lead to overwhelming inflammatory response, resulting in endotoxemia[2]. On the individual level, LPS leads to activation of blood coagulation system, increase of fibrin formation and degradation of fibrin and these changes cause clinical cases of Disseminated Intravascular Coagulation (DIC). The TNF-α is one of the most important early releasing inflammatory cytokines in endotoxemia. Numbers of treatments of endotoxemia studies showed protective effect by suppressing TNF-α level[3-5]. The IL-1β is another inflammatory cytokine, which also secret by activated macrophages and can stimulate cell proliferation and cell differentiation in immunoreaction[6-8]. Since inflammatory cytokines were thought to be an major way that cause injury in endotoxemia, inhibiting release of cytokines or decreasing cytokines level would a possible method for resisting endotoxemia.

In recent years, traditional Chinese medicine extracts thought to be a novel drug in control inflammation[9]. Previous studies have shown that the role of triptolide (TPL), extracts of the root of Chinese herb *Tripterygium* have anti-carcinogenesis effects, immunosuppressive and anti-inflammatory response[10,11]. The TPL used to treat different kinds of autoimmune and inflammation-related conditions by inhibiting release of inflammatory cytokines such as TNF-α, interleukin-2 (IL-2), inducible Nitric Oxide Synthase (NOS), cyclooxygenase-2 (COX-2) and interferon-gamma (IFN-gamma)[12]. Since deterioration of endotoxemia could result in accumulation of inflammatory cytokines, TPL may be a novel and effective agent for endotoxemia.

To have a better understanding of TPL anti-endotoxemia effect, here the authors report TPL on LPS-induced endotoxemia model in mice, both survival rate and organ injuries were monitored. The changes of TNF-α and IL-1β level both *in vivo* and *in vitro* under the effect of TPL was also observed.

MATERIALS AND METHODS

Experimental animals: The BALB/c mice weigh 18-22 g were supplied by Medical Experimental Animal Center of Guangdong Province, China. All mice were kept in cages before experiment with water and food *ad libitum*. This animal experiments were conducted according to the ethical guidelines of National Guide for the Care and Use of Laboratory Animals and approved by Jinan University Animal Care and Use Committee (Guangzhou, China).

Materials: Dulbeccos Modified Eagles Medium (DMEM) and Fetal Bovine Serum (FBS) were gained from BD Bioscience Franklin lakes NJ and LPS (from *Escherichia coli* serotype 0111-B4) was obtained from Sigma (Madrid, Spain). The TNF-α and IL-1β ELISA kit were purchased from RapidBio Lab (Calabasas, CA).

Animal models and drug treatments: Mice were randomly divided into five groups, each of one contains 10. The LPS-induced endotoxemia models were established by intraperitoneally (i.p.) injecting 30 mg kg^{-1} purified LPS in BALB/c mice. In the LPS group, the mice are with no treatment. Three TPL established groups are as follows: Low-dose group: with 1.0 mg kg^{-1} TPL, medium-dose group: with 2.0 mg kg^{-1} TPL and high-dose group: 5.0 mg kg^{-1} TPL, respectively, 30 min before LPS infusion[13]. The AS for the control group, mice were injected with saline.

Sample collection and detection of inflammatory cytokines: Blood were taken through catheterization of abdominal aorta at 0 and 30 min and 1, 2, 4 and 8 h following the treatment of LPS. Subsequently, blood were collected in 3.8% sodium citrate (1:10 v/v citrate/blood) and centrifuged at 2000×g for 15 min at 4°C. Then the concentration of serum samples were isolated from clotted blood and stored at -20°C for further until assayed. Serum samples were collected at 8 h after the treatment of LPS and stored at -20°C to determine the levels of alanine aminotransferase (ALT) and Blood Urine Nitrogen (BUN).

Histopathology examination of liver and kidney: To determine the extent of liver and renal tissue injury in LPS-induced endotoxemia and the protective effects of TPL. The chest cavities of animals were opened and the livers and kidneys were excised prior to collecting blood samples. After that, livers and kidneys were fixed in 4% paraformaldehyde overnight, dehydrated, embedded in paraffin and then sliced into 5 mm thick sections. After deparaffinization with different concentration gradient of alcohol, slices were stained by Hematoxylin and Eosin (H and E). Pathological changes of the liver and renal tissue were observed using an inverted microscope.

ALT and BUN assay *in vivo*: The assay of serum levels of ALT (the marker of liver injury) and BUN (the marker of renal injury) was performed, using a 7170A automatic analyzer (HITACHI, Japan).

Serum TNF-α and IL-1β assay: To assay the TNF-α and IL-1β serum was collected from the five groups at 0 and 30 min and 1, 2, 4 and 8 h. And the concentrations were determined by using quantitative sandwich enzymelinked immunosorbent assay (ELISA) kits (RapidBio Lab., Calabasas, CA) using commercial reagents following the manufactory's instruction.

TPL treatment on rat peripheral blood macrophages: In order to evaluate the effect of TPL on inflammatory cytokines, a LPS-induced endotoxemia model was established *in vitro*. To obtain macrophages, monocytes were induced, which were isolated from rat Peripheral Blood Mononuclear Cells (PBMC)[14]. Then the macrophages were cultured in DMEM contained 10% heat-inactivated FBS at 37°C under a humidified atmosphere of 5% CO_2 for further study.

Macrophages were seeded in 96-well plates at the density of 1500 cells/well and DMEM contained 10% (v) fetal bovine serum was added to make the total volume of each well be 200 μL. After being treated with or without 2.0 mg kg^{-1} TPL for 12 h and the cell-free supernatants were collected for further ELISA each 3 h. On the other group, macrophages were pre-cultured for 12 h without treatment prior to incubating with 0, 1.0, 2.0 and 5.0 mg kg^{-1} for another hour and then correct the cell-free supernatants as well.

Statistical analysis: All data were present as Mean±Standard Deviation. Differences between two groups were analyzed by one way ANOVA analysis. The survival rate was analyzed by Gehan-Breslow test. A p-value of <0.05 was considered significant. Statistical analysis were done using SigmaPlot software.

RESULTS

Protective effect on LPS-induced endotoxemia: To evaluate the protective effect of TPL on endotoxemia, a survival experiment was performed for 7 days in LPS-induced endotoxemia BALB/c mice treated with TPL. As the result showed (Fig. 1), only six mice in the LPS-control group were survived. In the TPL treatment group, the specific survival rate after 7 days were 30% (6/20), 45% (9/20), 70% (14/20) according to low, medium and high-dose TPL group. To be

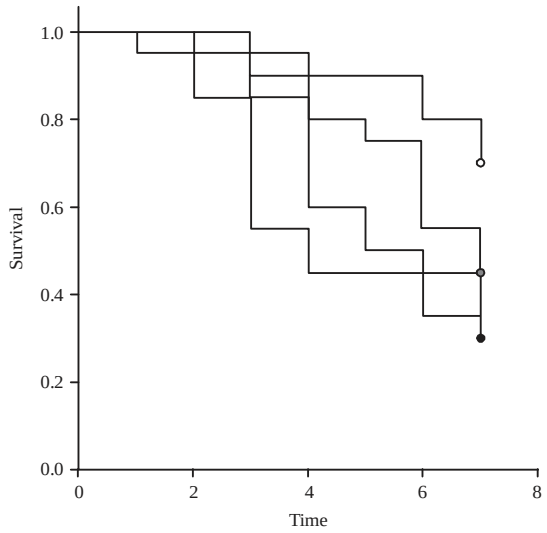

Fig. 1: Survival analysis of BALB/c mice after injected LPS and TPL. Death number of each group was monitored every 24 h in 7 days. Survival rate = total numbers-death numbers/total number

specific, the first three days after LPS infusion the survival rate of each group dropped abruptly, especially six mice of the LPS group died on day 3. Then the trend turned stable on day 4. Overall, TPL treatment showed protective effect on LPS-induced endotoxemia.

Effect of TPL on liver and renal histological damage in LPS-induced endotoxemia: In the control group, normal liver histology showed that hepatic cord arranged was organized (Fig. 2a), but after LPS administration liver histology result showed a number of acidophile and vacuolation of hepatocyte (Fig. 2b). Besides, under the treatment of 0.3 and 1 mg kg^{-1} TPL, respectively only a little inflammation and cell injury situation was seen in liver compared with the LPS-induced group (Fig. 2c, d). No obvious differences were recognized between the 3 mg kg^{-1} TPL treatment group and the control group at the same time (Fig. 2e).

As for the pathological analysis of renal, normal renal histology showed clear structures of renal tubules and glomeruli (Fig. 3a), however, in the LPS group these structures were destroyed and degraded (Fig. 3b). The staining results of the 0.3 and 1 mg kg^{-1} TPL treatment group showed that several structures of renal tubules and glomeruli were blurry compared to the control (Fig. 3c, d). Morphologically, there were no significant differences between the 3 mg kg^{-1} TPL treatment group (Fig. 3e) and the control group.

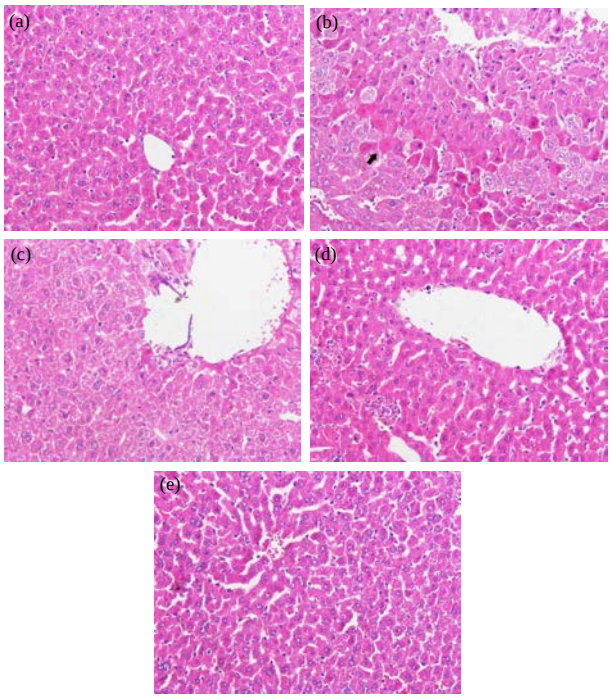

Fig. 2(a-e): Pathological detection was stained by hematoxylin and eosin in the liver tissue, (a) Microphotograph of liver of the control group , (b) Microphotograph of liver of the LPS-induced group. The increase of acidophile, severe inflammation and vacuolation of hepatocytes were highlighted by black arrows in the liver and (c-e) Microphotograph of liver of the TPL treatment groups (1.0, 2.0 and 5.0 mg kg^{-1}), all figures magnification was 200×

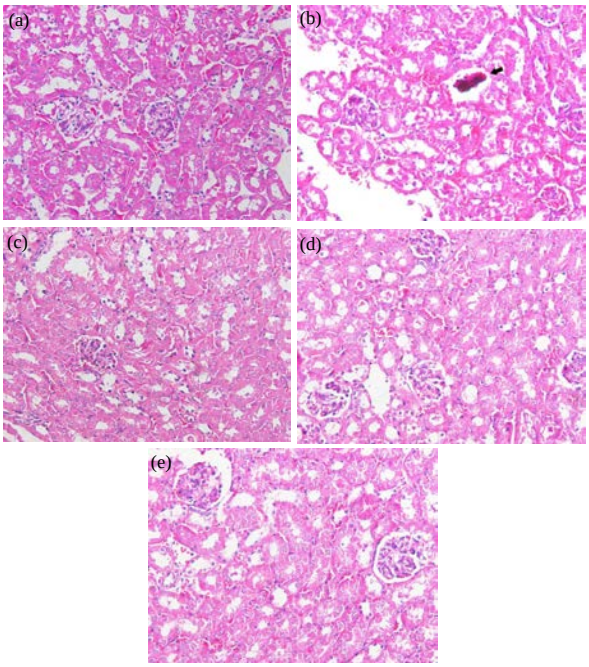

Fig. 3(a-e): Pathological detection was stained by hematoxylin and eosin in the renal tissue, (a) Microphotograph of renal of the control group, (b) Microphotograph of renal of the LPS-induced group. The structures of renal tubules and renal glomeruli were destroyed and degraded, which were highlighted by black arrows in the renal and (c-e) Microphotograph of renal of the TPL treatment groups (1.0, 2.0 and 5.0 mg kg^{-1}), all figures magnification was 200×

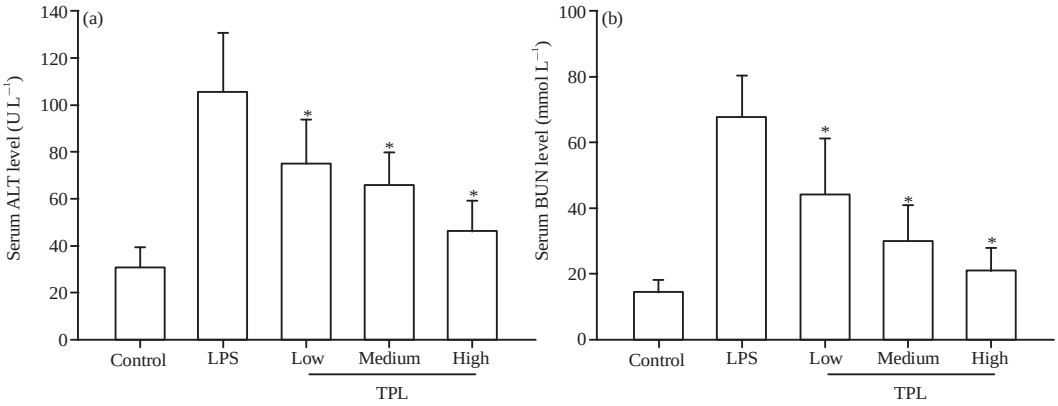

Fig. 4(a-b): Monitoring of organ function, (a) Alanine aminotransferase (ALT, a sign of liver injury) level in serum after 8 h administration of LPS and TPL and (b) Blood urine nitrogen (BUN, a sign of renal injury) level in serum after 8 h administration of LPS and TPL. Data of ALT and BUN level were expressed as Mean±SD, *p<0.05 versus the LPS group (n = 10), Control: Saline control group, LPS: L PS-induced group, Low TPL: 1.0 mg kg^{-1} TPL+LPS group, Medium TPL: 2.0 mg kg^{-1} TPL+LPS and High TPL: 5.0 mg kg^{-1} TPL+LPS

Effect of TPL on serum ALT and BUN level in LPS-induced endotoxemia: The ALT and BUN level in serum were tested to further evaluate the effect of TPL on LPS-induced liver and renal damage. The result indicated that there were a significant attenuation of ALT and BUN in TPL treatment group comparing with LPS-induced group ($p<0.05$, Fig. 4).

Serum ALT level in LPS-induced group (105.74±25.19 U L^{-1}) was notably elevated compared with control group (30.84±8.69 U L^{-1}). Treatment with TPL (low-does, middle-dose and high-dose) significantly reduced serum ALT level (75.31±18.49, 66.23±13.81 and 46.31±12.94 U L^{-1}, respectively) compared with the LPS-induced group ($p<0.05$, Fig. 4a).

The similar improvement was also observed in the assay of serum BUN level. In the LPS-induced group (67.81±12.63 mmol L^{-1}) and serum BUN level significantly increased compared with the control group (14.58±3.77 mmol L^{-1}). Importantly, treatment with TPL (middle-dose and high-dose) significantly reduced the serum level of BUN (44.16±17.34, 30.19±10.83 and 21.37±6.75 mmol L^{-1}, respectively) compared with the LPS-induced group ($p<0.05$, Fig. 4b).

Effect of TPL on serum inflammatory cytokines *in vivo*: To investigate the inhibition of TPL on inflammatory cytokines production of LPS-induced mice, TNF-α and IL-1β were detected. After LPS infusion, TNF-α and IL-1β levels elevated in a short time, while the control group was maintained in a low and stable level ($p<0.05$). But TNF-α and IL-1β levels were effectively inhibited after 1 h ($p<0.05$ vs. LPS group) (Fig. 5).

Effect of TPL on rat peripheral blood macrophages *in vitro*: To further investigated the influence of TPL on inflammatory cytokines, a rat peripheral blood macrophages cellular inflammation model was used and TNF-α and IL-1β production were determined. It can see that the secretions (TNF-α and IL-1β) of peripheral blood macrophages accumulated over the course of culture without TPL ($p<0.05$ vs. control group) (Fig. 6). It is obviously that TPL inhibited TNF-α and IL-1β secretion compared with the LPS-induced group ($p<0.05$).

DISCUSSION

The LPS stimulate cells to over release inflammatory factors and lead to endotoxemia. Besides, the symptom of endotoxemia would appear in various diseases, usually would cause lethal septic shock, multiple organ failure or disseminated intravascular coagulation. Therefore, one of the strategies of curing endotoxemia was to reduce the over production of pro-inflammatory factors and protect organs from over inflammation caused by injuries. In the present study, the effect of TPL on survival rate and organ injuries were verified in mice model. To have a better understanding about the effect of TPL to suppress inflammatory factors, the study was also monitored TNF-α and IL-β level. It is speculated that TPL may have therapeutic potential in curing endotoxemia, through inhibiting inflammatory factors.

In this study, LPS-induced endotoxemia was simulated by injecting LPS in BALB/c mice. The LPS is the toxic component of endotoxin, which would lead to severe inflammatory

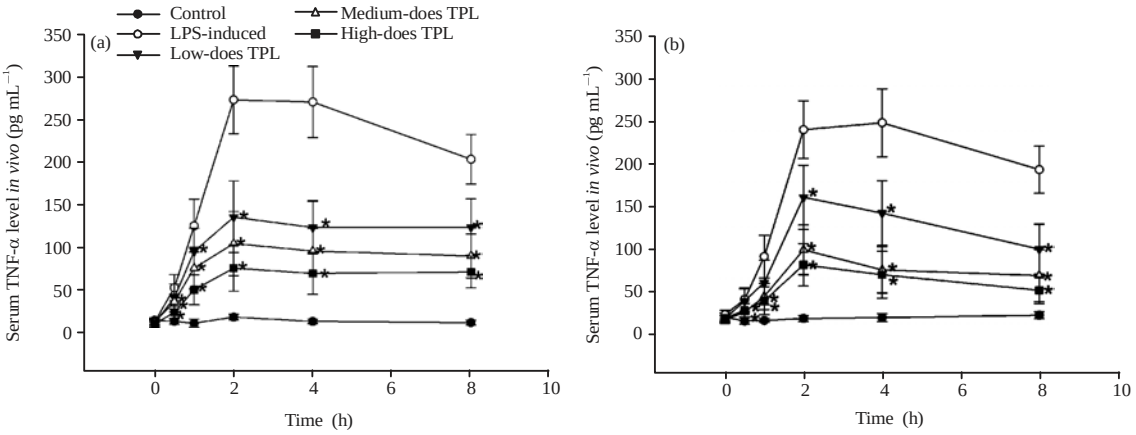

Fig. 5(a-b): Trend of serum inflammatory factors *in vivo* with time, (a) Effect of intraperitoneal injections of saline, LPS, 1.0 mg kg^{-1} TPL+LPS, 2.0 mg kg^{-1} TPL+LPS and 5.0 mg kg^{-1} TPL+LPS on TNF-α level in serum in 8 h and (b) Effect of intraperitoneal injections of saline, LPS, 1.0 mg kg^{-1} TPL+LPS, 2.0 mg kg^{-1} TPL+LPS and 5.0 mg kg^{-1} TPL+LPS on IL-1β level in serum in 8 h. Data are expressed as Mean±SD, the differences of respective groups from the LPS-induced group were analyzed by one way ANOVA at the exact time point, *p<0.05 is considered significant (n = 10)

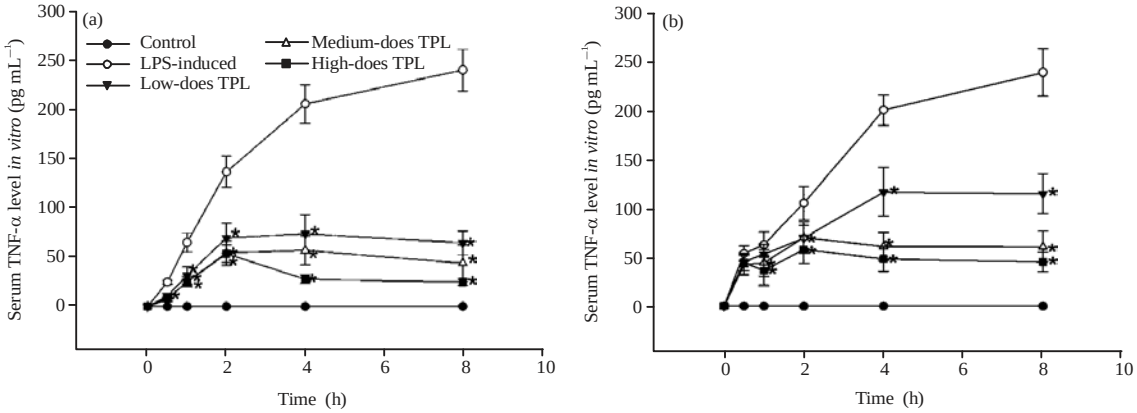

Fig. 6(a-b): Trend of serum inflammatory factors *in vitro* with time, (a) Effect of culture in saline, LPS, 1.0 mg kg^{-1} TPL+LPS, 2.0 mg kg^{-1} TPL+LPS and 5.0 mg kg^{-1} TPL+LPS on TNF-α level in serum in 8 h and (b) Effect of culture in saline, LPS, 1.0 mg kg^{-1} TPL+LPS, 2.0 mg kg^{-1} TPL+LPS and 5.0 mg kg^{-1} TPL+LPS on IL-1β level in serum in 8 h. Data are expressed as Mean±SD, the differences of respective groups from the LPS-induced group were analyzed by one way ANOVA at the exact time point, *p<0.05 is considered significant (n = 5)

reaction finally form endotoxemia. And LPS-induced endotoxemia has a high mortality rate that exceeded 15% clinical patient died for this[15]. *In vivo* experiment, high mortality was an major sign of LPS-induced endotoxemia models[13] and a recent clinical study reported that the survival rate of endotoxemia could only maintain at low level[16]. Saito *et al.*[17] found that the mortality of mice exceeded 30% after injected LPS. So that high mortality and severe organ damage of the LPS-induced group indicated an endotoxemia model was created successfully (Fig. 1). On the other hand, the survival rate in the TPL treatment groups was far more higher than that in the LPS-induced group, which showed that TPL had protective effect on LPS-induced endotoxemia.

Results of ALT and BUN level demonstrated that TPL decreased LPS-induced endotoxemia mortality by protecting liver and renal function. Liver and renal are the detoxification organs and so would damaged first when inflammation comes out. Therefore, it was easy to find obvious morphological changes such as glomerular atrophy in endotoxemia models (Fig. 2 and 3). Organ injuries caused by endotoxemia had been observed in many studies[1,18,19]. Besides, multi-organ dysfunction usually came with mortality. So, it was of great

importance to protect organ functions in the cure of endotoxemia. Both morphological results and the decrease of ALT and BUN level in administration groups showed the effects of TPL in organs protection. It was reasonable to believe that TPL successfully improved the survival rate by protecting LPS-induced organ injuries.

On the molecular level, release of endotoxin would activate macrophages to secrete high level of TNF-α and IL-1β, resulting in endotoxemia. It was reported that the accumulation of TNF-α would lead to the secondary inflammatory cascades[20]. Simultaneously, release of IL-1β *in vivo* would also lead to hyperalgesia and mediator neuroinflammation[21,22]. The TPL can inhibit the expression of these inflammatory cytokines[23]. The reduction of TNF-α and IL-1β *in vivo* improved LPS-induced organ injury. As the accumulation of pro-inflammatory cytokines could lead to inflammatory cascades and results in organ failure and mortality, many of them have been chosen as therapeutic targets for endotoxemia. Speaking from the result, TPL showed anti-inflammatory effect *in vivo* and successfully suppressed the over production of TNF-α and IL-1β, weakening the inflammation in liver and renal.

CONCLUSION

The TPL showed protective effect on LPS-induced endotoxemia and it showed improvement of liver and kidney function. Moreover, TPL significantly decreased pro-inflammatory cytokines TNF-α and IL-1β level.

ACKNOWLEDGMENT

This project was financially supported by National High Technology Research and Development Program of China (2014AA020534), Science and Technology Planning Project of Guangdong Province (2013B010404030, 2014A010105029, 2014A020211022) and Science and Technology Planning Project of GuangZhou Canton (201510010074).

REFERENCES

1. Yan, Y.J., Y. Li, B. Lou and M.P. Wu, 2006. Beneficial effects of ApoA-I on LPS-induced acute lung injury and endotoxemia in mice. Life Sci., 79: 210-215.
2. Wang, Y., Z. Qin, S. Shen, N. Xiang and J. Liu *et al*., 2015. A novel fibrinogenase from Agkistrodon acutus venom protects against LPS-induced endotoxemia via regulating NF-κB pathway. Immunopharmacol. Immunotoxicol., 37: 413-420.
3. Rim, H.K., W. Cho, S.H. Sung and K.T. Lee, 2012. Nodakenin suppresses lipopolysaccharide-induced inflammatory responses in macrophage cells by inhibiting tumor necrosis factor receptor-associated factor 6 and nuclear factor-κB pathways and protects mice from lethal endotoxin shock. J. Pharmacol. Exp. Therapeut., 342: 654-664.
4. Yang, M., L. Cao, M. Xie, Y. Yu and R. Kang *et al*., 2013. Chloroquine inhibits HMGB1 inflammatory signaling and protects mice from lethal sepsis. Biochem. Pharmacol., 86: 410-418.
5. Baker, A.K., R. Wang, N. Mackman and J.P. Luyendyk, 2009. Rapamycin enhances LPS induction of tissue factor and tumor necrosis factor-α expression in macrophages by reducing IL-10 expression. Mol. Immunol., 46: 2249-2255.
6. Satoh, T., A. Otsuka, E. Contassot and L.E. French, 2015. The inflammasome and IL-1β: Implications for the treatment of inflammatory diseases. Immunotherapy, 7: 243-254.
7. Piccioli, P. and A. Rubartelli, 2013. The secretion of IL-1β and options for release. Semin. Immunol., 25: 425-429.
8. Voronov, E., Y. Carmi and R.N. Apte, 2014. The role IL-1 in tumor-mediated angiogenesis. Front. Physiol., Vol. 5. 10.3389/fphys.2014.00114
9. Qiu, D. and P.N. Kao, 2003. Immunosuppressive and anti-inflammatory mechanisms of triptolide, the principal active diterpenoid from the Chinese medicinal herb *Tripterygium wilfordii* Hook. f. Drugs R and D, 4: 1-18.
10. Wang, W., X.P. Mei, L. Chen, J. Tang and J.L. Li *et al*., 2012. Triptolide prevents and attenuates neuropathic pain via inhibiting central immune response. Pain Physician, 15: E995-E1006.
11. Li-Weber, M., 2013. Targeting apoptosis pathways in cancer by Chinese medicine. Cancer Lett., 332: 304-312.
12. Brinker, A.M., J. Ma, P.E. Lipsky and I. Raskin, 2007. Medicinal chemistry and pharmacology of genus *Tripterygium* (Celastraceae). Phytochemistry, 68: 732-766.
13. Luo, S., R. Wang, W. Jiang, X. Lin, P. Qiu and G. Yan, 2010. A novel recombinant snake venom metalloproteinase from *Agkistrodon acutus* protects against taurocholate-induced severe acute pancreatitis in rats. Biochimie, 92: 1354-1361.
14. Qureshi, T., J.W. Templeton and L.G. Adams, 1996. Intracellular survival of *Brucella abortus*, *Mycobacterium bovis* BCG, *Salmonella dublin* and *Salmonella typhimurium* in macrophages from cattle genetically resistant to *Brucella abortus*. Vet. Immunol. Immunopathol., 50: 55-65.
15. Hurley, J.C., B. Guidet, G. Offenstadt and E. Maury, 2012. Endotoxemia and mortality prediction in ICU and other settings: underlying risk and co-detection of Gram negative bacteremia are confounders. Crit. Care, Vol. 16. 10.1186/cc11462.
16. Szeto, C.C., B.C.H. Kwan, K.M. Chow, K.B. Lai and W.F. Pang *et al*., 2010. Endotoxemia is associated with better clinical outcome in incident Chinese peritoneal dialysis patients: A prospective cohort study. Peritoneal Dialysis Int., 30: 178-186.

17. Saito, R., T. Shimosawa, T. Ogihara, N. Maruyama, T. Fujita, N. Okamura and K. Nakahara, 2012. Function of adrenomedullin in inflammatory response of liver against LPS-induced endotoxemia. APMIS, 120: 706-711.
18. Guo, Y., Y. Zhang, K. Hong, F. Luo, Q. Gu, N. Lu and A. Bai, 2014. AMPK inhibition blocks ROS-NFκB signaling and attenuates endotoxemia-induced liver injury. PloS ONE, Vol. 9. 10.1371/journal.pone.0086881.
19. Aslami, H., C.J. Beurskens, F.M. de Beer, M.T. Kuipers and J.J.T.H. Roelofs *et al*., 2013. A short course of infusion of a hydrogen sulfide-donor attenuates endotoxemia induced organ injury via stimulation of anti-inflammatory pathways, with no additional protection from prolonged infusion. Cytokine, 61: 614-621.
20. Lehmann, C., R. Scheibe, M. Schade, K. Meissner and M. Grundling *et al*., 2008. Effects of activated protein C on the mesenteric microcirculation and cytokine release during experimental endotoxemia. Can. J. Anesthesia, 55: 155-162.
21. McNamee, E.N., E.W. Griffin, K.M. Ryan, K.J. Ryan, S. Heffernan, A. Harkin and T.J. Connor, 2010. Noradrenaline acting at β-adrenoceptors induces expression of IL-1β and its negative regulators IL-1ra and IL-1RII and drives an overall anti-inflammatory phenotype in rat cortex. Neuropharmacology, 59: 37-48.
22. Eliav, E., R. Benoliel, U. Herzberg, M. Kalladka and M. Tal, 2009. The role of IL-6 and IL-1β in painful perineural inflammatory neuritis. Brain Behav. Immunity, 23: 474-484.
23. Zhou, H.F., X.Y. Liu, D.B. Niu, F.Q. Li, Q.H. He and X.M. Wang, 2005. Triptolide protects dopaminergic neurons from inflammation-mediated damage induced by lipopolysaccharide intranigral injection. Neurobiol. Dis., 18: 441-449.