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Journal of Ethnopharmacology 101 (2005) 68–74
Journal of ETHNO-PHARMACOLOGY
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San-Huang-Xie-Xin-Tang attenuates inflammatory responses in lipopolysaccharide-exposed rat lungs

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Received 5 January 2005; received in revised form 21 February 2005; accepted 24 March 2005
Available online 6 May 2005

Abstract

In this study, the potential anti-inflammatory effect of San-Huang-Xie-Xin-Tang (SHXT) and its main component baicalin on LPS-induced lung injury were investigated and compared to the profile of dexamethasone (DEXA) in a pre-clinical animal model. Post-treatment with SHXT (75 mg/kg), baicalin (1.5 mg/kg) and DEXA (0.5 mg/kg), significantly inhibited LPS-induced hypotension, lung edema and acute survival rates. Western blotting analysis results indicated that all of them significantly inhibited LPS-induced iNOS, TGF-β, p38MAPK, and ICAM-1 expressions in the lung tissues. Results from ELISA analysis showed that SHXT, baicalin and DEXA all decreased plasma levels of IL-1β, TNF-α, and MCP-1 caused by LPS. Based on these findings, SHXT and baicalin decreased plasma concentrations of IL-1β, TNF-α, MCP-1, and expressions of TGF-β, ICAM-1, phosphorylated p38 MAPK, and iNOS, which were associated with lung injury and lethality. These evidences indicated that SHXT and baicalin showed strong anti-inflammatory activity, similar to that observed for DEXA, and therefore implicated that herbal SHXT might be therapeutically useful for the treatment of endotoxic lung injury.

Keywords: San-Huang-Xie-Xin-Tang; Baicalin; Lipopolysaccharide; Anti-inflammatory effect; Hypotension; Lung injury

1. Introduction

Adult respiratory distress syndrome (ARDS) is a major lethal complication observed in sepsis or septic shock. This syndrome is characterized by severe hypoxemia, diffused infiltration of the lung, reduction in respiratory compliance, increase in pulmonary arterial pressure, and pulmonary resistance (McLean and Byrick, 1993; Kollef and Schuster, 1995).

It is well recognized that lipopolysaccharides (LPS), which are structural components of the outer membranes of Gram-negative bacteria, play a pivotal role in the sepsis syndrome (Rietschel et al., 1994). LPS given intravenously is a model often used for studying ARDS (Martin and Silverman, 1992). Various inflammatory mediators have been implicated in LPS-induced pulmonary damage. These include cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α, eicosanoids, platelet-activating factor, complement components, reactive oxygen species, and adhesion molecules (Salzer and McCall, 1990). The actions of IL-1 and TNF-α are thought to have key roles in the pathogenesis of endotoxin-induced lung injury (Ohlsson et al., 1990). The administration of a specific antibody against TNF-α (Tracey et al., 1987) or a receptor antagonist for IL-1 (Ohlsson et al., 1990) improved survival rates of the subject animals after lethal LPS administration. p38 mitogen-activated protein kinase (MAPK) pathway has been investigated its roles in the initiation of pulmonary inflammation factors, including TNF-α, transforming growth factor (TGF)-β (Branton and Kopp, 1999), monocyote chemoattractant protein (MCP)-1 (Chensue et al., 1996; Hannigan et al.,

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doi:10.1016/j.jep.2005.03.015

1998), intercellular adhesion molecule 1 (ICAM-1) (Kasper et al., 1995; Tamura et al., 1998) and nuclear factor (NF)-κB (Abraham, 2003). Binding elements for the transcriptional regulatory factor NF-κB are present in the enhancer/promoter regions of cytokine genes as well as in other immunoregulatory molecules that participate in the development and progression of acute lung injury, such as ICAM-1 and inducible nitric oxide synthase (iNOS) (Abraham, 2000). Blockade of NF-κB activation inhibits the development of acute lung injury after LPS administration, as shown by decreased edema, diminished neutrophil infiltration, and suppression of proinflammatory cytokine expression in the lungs (Liu et al., 1997).

Glucocorticosteroids remain the most effective therapy for inflammatory disorders, which inhibit many functions of activated macrophages, including secretion of cytokines such as IL-1β and TNF-α from cells. However, despite the rapid and proven efficacy of topical glucocorticosteroids, the side effects of glucocorticosteroids limit their clinical usefulness (Belvisi et al., 2001). Therefore, other agents are being sought for the treatment of pulmonary and other inflammatory diseases.

San-Huang-Xie-Xin-Tang (SHXT), a traditional oriental medicinal formula containing *Coptidis rhizoma* (*Coptis chinesis* Franch), *Scutellariae radix* (*Scutellaria baicalensis* Georgi) and *Rhei rhizoma* (*Rheum officinale* Baill), has been used to treat gastritis, gastric bleeding and peptic ulcers (Lin and Tan, 1994). In our previous study, we have analyzed its components and, in spite of other unknown chemical components included in SHXT, baicalin is the most abundant one. The preventive effects of SHXT on LPS-induced reactions such as generation of cytokines, iNOS, COX-2, PGE_2 and arterial hypotension were studied in Wistar rats and RAW 264.7 macrophages (Lo et al., 2005). However, therapeutic effect of SHXT on lung injury has not been elucidated. Here we investigated the therapeutic effects of SHXT and baicalin on LPS-induced lung injury. The plasma levels of cytokines and lethality in rats were also examined on their therapeutic but not preventive effects reported in our previous study (Lo et al., 2005).

2. Materials and methods

2.1. Materials

The blended mixture of *C. chinesis* Franch, root of *S. baicalensis* Georgi and rhizome of *R. officinale* Baill, in a 1:1:2 ratio. The voucher specimen and method for extraction and analysis of SHXT were described the same as that described previously (Lo et al., 2005). Antibodies for Western blotting were purchased from Transduction Lab (USA) and Serotec (UK). Tissue protein extraction reagent was purchased from Pierce (USA). Cytokine immunoassay kits were purchased from Endogen (USA). All other chemicals were purchased from Sigma Chemical Company (USA). The working solution of SHXT, baicalin and dexamethasone were diluted with saline and used in animal experiments.

2.2. Experimental design

This study was approved by the Animal Care and Use Committee at the Kaohsiung Medical University. Male Wistar rats, weighing 250–350 g, were provided by the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). They were housed under conditions of constant temperature and controlled illumination (lights on between 7:30 and 19:30). Rats were allowed food and water ad libitum.

Rats were anesthetized with an intraperitoneal (i.p.) injection of pentobarbital sodium (50 mg/kg). Following tracheal cannulation, systemic arterial blood pressure and heart rate were recorded from the femoral artery with a pressure transducer (Spectramed, Model P10EZ, USA). Body temperature was maintained at 37 °C by an electric heating pad. A femoral vein was cannulated for the administration of LPS (*E. coli* serotype 026:B6), SHXT, baicalin, or dexamethasone (DEXA), respectively. Rats were injected intravenously with either saline (0.1 ml/kg) or LPS (10 mg/kg) for control and experimental purposes, respectively. All the animals were assigned to one of the following groups: saline only, LPS–saline, LPS/SHXT (75 mg/kg), LPS/baicalin (1.5 mg/kg), and LPS/DEXA (0.5 mg/kg) groups. LPS was injected at a dose of 10 mg/kg, after 10 min, SHXT, baicalin and DEXA were given, respectively. A single injection of LPS (10 mg/kg, i.v.) produced a biphasic arterial hypotension; an initial and transient decrease in arterial mean blood pressure (about 10 min), followed by a delayed and prolonged hypotension (Lo et al., 2005). And thus we choose to administer the SHXT, baicalin and DEXA 10 min after LPS administration in this study. The used dose of baicalin is according to the content of baicalin in SHXT by high-performance-liquid-chromatography (Gilson, France) method (Lo et al., 2005). Separate groups were used for acute survival study ($n = 10$ each), lung tissue sampling ($n = 10$ each), and blood sampling ($n = 10$ each). The animals that supplied pulmonary tissues or blood samples were killed under anesthesia.

2.3. Acute survival studies

Survivors were monitored per 30 min for 7 h after LPS administration. The blood pressure (BP) changes were recorded within 5 h after LPS injection. Ten minutes after LPS (10 mg/kg) administration, SHXT, baicalin and DEXA were intravenously injection, respectively.

2.4. Edema index

The water content of the lungs was determined by calculating the wet/dry weight ratio of lung tissues at 5 h after LPS administration. After LPS intravenously administration 10 min, SHXT, baicalin and DEXA were given, respectively. The left lung was dissected free from non-pulmonary tissues,

rinsed free from blood, and weighed (wet weight). Dry weight was determined after the lung was dried at 80 °C for 72 h, and the edema index (wet/dry weight ratio) was calculated by dividing the wet weight by the dry weight (Yoshinari et al., 2001).

2.5. Western blot analysis

The right middle and accessory lobes of the lung were harvested at 5 h after LPS administration, cleared of non-pulmonary tissues, and frozen at −80 °C until homogenization. Tissue protein extraction reagent was used and the lysate was centrifuged at 15,000 × *g* for 30 min. Then supernatant was freeze-dried. Eight percent SDS-polyacrylamide minigels was used, and transferred to immobilon polyvinylidene difluoride membranes (Millipore, Germany). The membrane was incubated overnight at 4 °C with 1% BSA and then incubated with anti-iNOS, anti-TGF-β, anti-ICAM-1, anti-P-p38 MAP kinase and anti-β-actin antibodies. Expression of protein was detected by enhanced chemiluminescence using Hyperfilm and ECL reagent (Amersham, UK).

2.6. Measurement of plasma cytokine by ELISA

Blood was collected from a venous cannula, injected into ice-cold heparinized Eppendorf tubes and centrifuged at 1500 rpm for 10 min at 4 °C. Plasma supernatant was stored at −80 °C until analyzed. Solid phase sandwich enzyme-linked immunosorbent assay (ELISA) kits that specifically detected IL-1β, TNF-α and MCP-1 were used (Lo et al., 2004).

2.7. Statistics

Results are expressed as mean ± S.E.M. Statistical differences were determined by independent and paired Student's *t*-test in unpaired and paired samples. Whenever a control group was compared with more than one treated group, the one-way ANOVA or two-way repeated measures ANOVA was used. When the ANOVA manifested a statistical difference, both Dunnett's and Student–Newman–Keuls test were applied when needed. $P < 0.05$ was considered to be significant. Analysis of data and plotting of figures were done with the aid of software (SigmaStat and SigmaPlot, Version 5.0, San Rafael, CA, USA; GraphPad PRISM™, Version 2.0, San Diego, CA, USA) run on an IBM-compatible computer.

3. Results

3.1. Inhibition of LPS-induced arterial hypotension

A single injection of LPS (10 mg/kg, i.v.) produced a biphasic arterial hypotension; an initial and transient decrease in arterial mean blood pressure, which partially recovered within 15 min, followed by a delayed and prolonged hypotension. In this study, 10 min after LPS administration, post-treatment with SHXT (75 mg/kg, i.v.), baicalin (1.5 mg/kg, i.v.), and DEXA (0.5 mg/kg, i.v.) significantly attenuated LPS-induced prolonged arterial hypotension (Fig. 1).

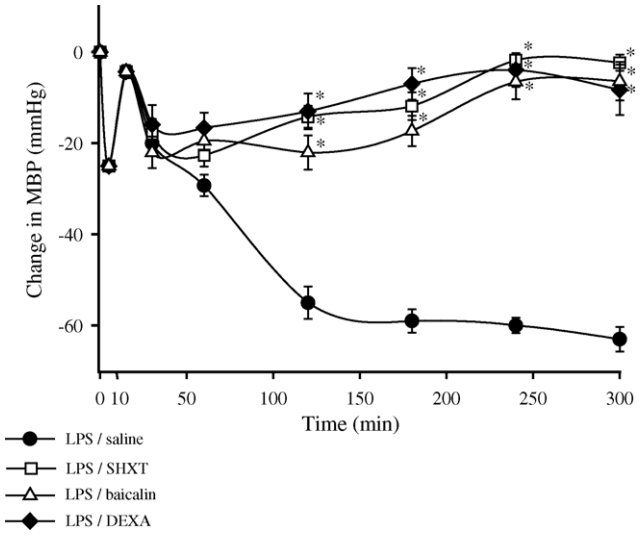

Fig. 1. Effects of post-treatment with San-Huang-Xie-Xin-Tang (SHXT), baicalin and dexamethasone (DEXA) on LPS (10 mg/kg)-induced hypotension on mean arterial blood pressure (MBP) in Wistar rat anesthetized with pentobarbital. Rats were post-treated with SHXT (75 mg/kg), baicalin (1.5 mg/kg) and DEXA (0.5 mg/kg) 10 min after LPS administration, respectively. Each point represents the mean ± S.E.M. of 10 rats. *Significantly different from LPS/saline group, $p < 0.05$, ANOVA followed by Dunnett's test.

3.2. Survival studies

In the LPS–saline group, 1 of the 10 (90%) survived for 330 min and 0 of the 10 (0%) survived for 7 h. Both the LPS/SHXT group and LPS/baicalin group, 9 of the 10 (90%) survived for 7 h. And the LPS/DEXA group 10 of the 10 (100 %) survived for 7 h. LPS/SHXT group, LPS/baicalin group and LPS/DEXA group all showed significantly better survival rate than the LPS–saline group (Fig. 2).

3.3. Therapeutic effects in LPS-induced lung edema

The edema index indicated the water content of the lung, which was determined by calculating the wet/dry weight ratio of lung tissues at 5 h after LPS administration. Lung wet/dry weight ratios were significantly higher at 5 h after LPS administration in the LPS–saline group compared with the control (saline only) group. SHXT, baicalin and DEXA administration significantly attenuated LPS-induced lung edema (Fig. 3).

3.4. Inhibited LPS-induced iNOS, phosphorylated p38 MAP kinase, TGF-β and ICAM-1 in lung tissues

In the LPS–saline group, the expressions of iNOS, phosphorylated p38 MAP kinase, TGF-β and ICAM-1 were

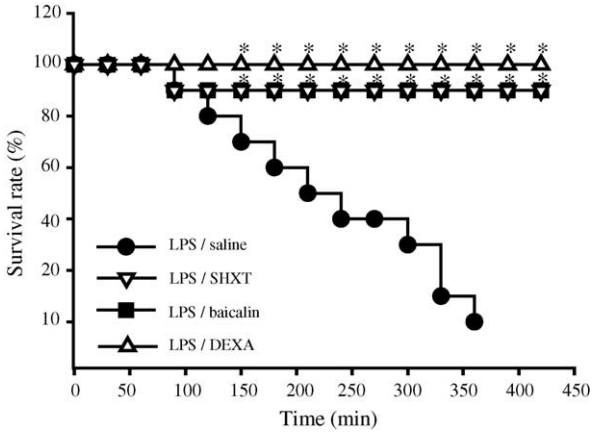

Fig. 2. Effects of post-treatment with San-Huang-Xie-Xin-Tang (SHXT), baicalin and dexamethasone (DEXA) on survival of anaesthetized rats exposed to LPS (10 mg/kg). Rats were post-treated with SHXT (75 mg/kg), baicalin (1.5 mg/kg) and DEXA (0.5 mg/kg) 10 min after LPS administration, respectively. *Significantly different from LPS/saline group, $p < 0.05$, ANOVA followed by Dunnett's test.

all significantly increased than the control (saline) group in the lung tissues, respectively. However, LPS/SHXT, LPS/baicalin and LPS/DEXA groups showed significantly inhibited LPS-induced phosphorylated iNOS, p38 MAP kinase, TGF-β and ICAM-1 expressions in the lung tissues, respectively (Fig. 4).

3.5. Inhibition of LPS-induced plasma cytokine immunoreactivities

In the LPS–saline group, LPS administration markedly elevated plasma TNF-α concentration within the first 2 h than the control (saline) group. However, the elevations of TNF-α in the LPS/SHXT, LPS/baicalin and LPS/DEXA groups were significantly lower than LPS/saline group. Moreover, plasma concentrations of IL-1β and MCP-1 increased with time up to 5 h after LPS administration. The elevations of IL-1β and MCP-1 were lower in LPS/SHXT, LPS/baicalin and LPS/DEXA groups, compared with LPS–saline group (Fig. 5).

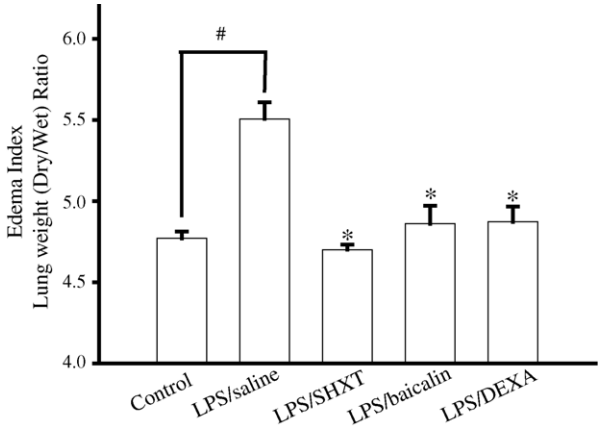

Fig. 3. The water content of the lungs was determined by calculating the wet/dry weight ratio of lung tissues at 5 h after LPS (10 mg/kg) administration. San-Huang-Xie-Xin-Tang (SHXT, 75 mg/kg), baicalin (1.5 mg/kg) and dexamethasone (DEXA, 0.5 mg/kg) were post-treated 10 min after LPS administration. Each bar represents the mean ± S.E.M. of 10 rats. #Significantly different from control (saline only) group, *significantly different from LPS/saline group, $p < 0.05$, ANOVA followed by Dunnett's test.

4. Discussion

In the present study, we have shown the therapeutic effect of SHXT and baicalin on LPS-induced lung edema and lethality in a pre-clinical animal model. This study confirms that SHXT possessed not only preventive effect (Lo et al., 2005) but also therapeutic effect on LPS-induced hypotension and inflammatory mediators. This is also the first demonstration that SHXT and baicalin have the ability to inhibit LPS-induced lung edema. The action mechanism of these benefits produced by post-treatment with SHXT and baicalin includes attenuating expression of iNOS, ICAM-1 TGF-β and phosphorylation of p38 MAP kinase in lung tissues and decreasing MCP-1, IL-1β and TNF-α in plasma levels of LPS-exposed rats.

Endotoxic shock is characterized by systemic hypotension, hyporeactiveness to vasoconstrictors and acute lung edema. It has already been shown that NO generated from iNOS in endotoxin shock plays an important role in vascular hyporeactivity and tissue damage through its cytotoxic function. Therefore, drugs that inhibit iNOS expression resulting in decrease of NO generation may have beneficial therapeutic effects in the treatment of disease due to over production of NO (Warren et al., 1992). In this study, the post-treatment with SHXT and baicalin reversed the LPS-induced severe hypotension sustained for up to 7 h. Moreover, NO production mediated by the iNOS pathway is responsible for endotoxin-induced lung injury (Wang et al., 1999; Heremans et al., 2000). In the present study, SHXT and baicalin decreased LPS-induced iNOS expression in lung tissues, thereby they might inhibit overproduction of NO and vascular hyper-permeability associated edema. It is also noteworthy that SHXT and baicalin drastically improved the survival rate in our experimental model of endotoxic shock (Fig. 2).

The p38 MAP kinase plays an important role in endotoxemia-induced lung injury (Nick et al., 2000; Arcaroli et al., 2001). p38 MAP kinase is necessary for LPS-induced production of TNF-α and IL-1 in monocytes (Hannigan et al., 1998). There is also evidence that p38 MAPK can participate directly in increasing LPS-induced nuclear translocation of NF-κB in neutrophils and enhanced expression of proinflammatory cytokines (e.g. IL-1β and TNF-α) whose transcription is dependent on NF-κB (Abraham, 2003). Inhibition of p38 MAP kinase was found to reduce mortality rate in a murine model of endotoxin shock (Badger et al., 1996). Furthermore, inhibition of p38 MAP kinase decreased TNF-α

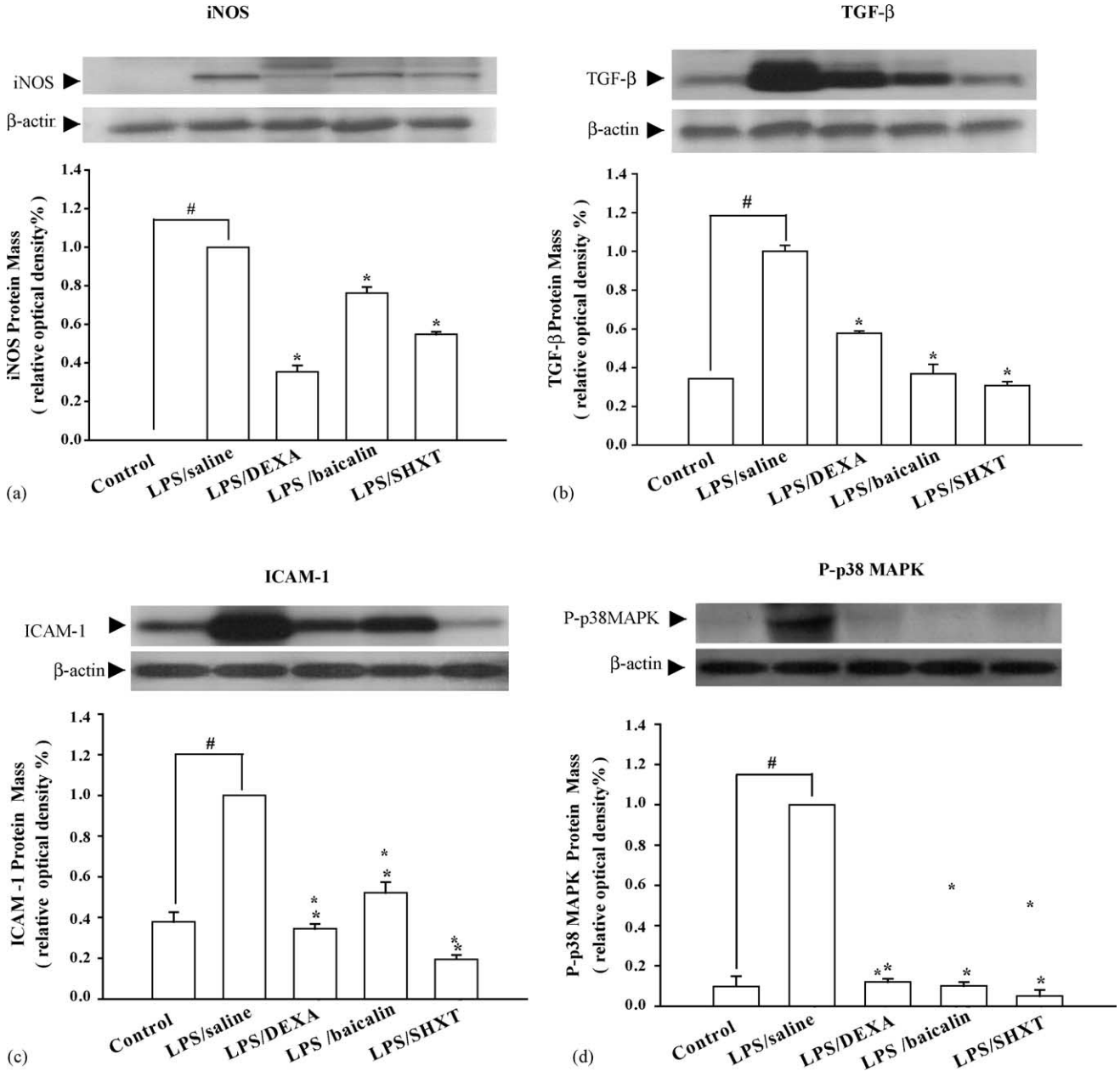

Fig. 4. Inhibitory effects of San-Huang-Xie-Xin-Tang (SHXT), baicalin and dexamethasone (DEXA) on the iNOS (a), TGF-β (b), ICAM-1 (c) and P-p38 MAPK (d) expressions in lung tissues at 5 h after LPS (10 mg/kg) administration. Wistar rats were post-treated with SHXT (75 mg/kg), baicalin (1.5 mg/kg) and DEXA (0.5 mg/kg) 10 min after LPS administration, respectively. Densitometries analyses are presented as the relative ratio of P-p38 MAPK/β-actin. Data were obtained from three independent experiments and expressed as mean ± S.E.M. #Significantly different from control (saline only) group, *significantly different from LPS/saline group, $p < 0.05$, ANOVA followed by Dunnett's test.

levels and neutrophil recovery in the airspaces following intratracheal administration of LPS (Nick et al., 2000). In the present study, the phosphorylation of p38 MAP kinase was increased in LPS–saline group and significantly inhibited by post-treatment with SHXT and baicalin. SHXT and baicalin also significantly inhibited the production of TNF-α and IL-1 induced by LPS. Thus, we suggest that SHXT and baicalin suppresses TNF-α and IL-1 production might mediate via blocking the phosphorylation of p38 MAP kinase.

Baicalin has been used as anti-inflammatory agents for a number of years (Kubo et al., 1984; Huang, 1999; Li et al., 2000), this is the first observation that baicalin has the ability to inhibit the LPS-induced lung edema. At acute phase of lung injury, TGF-β plays an important role in pathogenesis of disease (Hurst et al., 1999). At late phase of lung injury, TGF-β leads to pulmonary fibrosis by inducing collagen synthesis and inhibit collagenase production. In this study, SHXT and baicalin significantly attenuated LPS-induced TGF-β

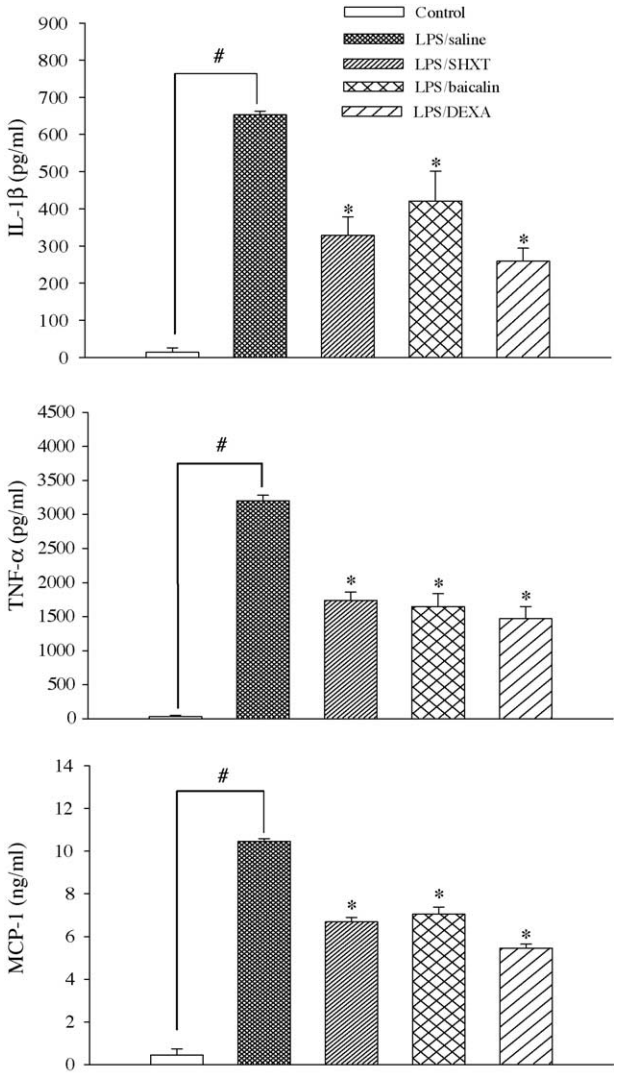

Fig. 5. Effects of San-Huang-Xie-Xin-Tang (SHXT), baicalin and dexamethasone (DEXA) on the plasma levels of IL-1β, TNF-α and MCP-1 in LPS-treated Wistar rats anesthetized with pentobarbital. Wistar rats were post-treated with SHXT (75 mg/kg), baicalin (1.5 mg/kg) and DEXA (0.5 mg/kg) 10 min after LPS (10 mg/kg) administration, respectively. Data were obtained from ten independent experiments and expressed as mean ± S.E.M. #Significantly different from control (saline only) group, *significantly different from LPS/saline, $p < 0.05$, ANOVA followed by Dunnett's test.

expression in lung tissues. In this study, SHXT and baicalin significantly attenuated LPS-induced TGF-β expression in lung tissues. Chemokines (e.g. MCP-1) contribute to the initiation of inflammatory processes and recruitment of leucocytes since they are expressed at early time points (Standiford et al., 1991; Rollins, 1997). TNF-α and IL-1 stimulate MCP-1 and ICAM-1 production by bronchial epithelial cells. The generation of MCP-1 and upregulation of ICAM-1 might then help in the recruitment and migration of inflammatory and activated cells into target tissue. Therefore, inhibition of adhesion molecules and chemotactic activities can down-regulate recruitment of inflammatory cells in the airway microenvironment and represent potential targets for anti-inflammatory therapies in airway disorders (Polito and Proud, 1998). This study indicated that SHXT and baicalin could decrease the plasma levels of TNF-α, IL-1β and MCP-1 induced by LPS. Furthermore, the current results demonstrated SHXT and baicalin decreased LPS-induced TGF-β and ICAM-1 expression. The therapeutic effect of SHXT or baicalin on LPS-induced lung edema was similar to that of DEXA, a traditional glucocorticosteroid. Although glucocorticosteroids are most commonly used for lung inflammation, they caused several serious adverse effects (Belvisi et al., 2001). However, no serious side effects of baicalin have been reported to date. These results suggest SHXT and baicalin might be alternatively useful, other than glucocorticosteroids, in LPS-induced lung injury.

5. Conclusion

Post-treatment with SHXT and its main component baicalin attenuated LPS-induced hypotension, lung edema and lethality by inhibiting the production of TNF-α, IL-1β and MCP-1 and the expression of TGF-β, iNOS, ICAM-1 and the activation of p38 MAP kinase. Therefore, SHXT or baicalin might be therapeutically beneficial in the treatment of the LPS-induced pulmonary inflammatory diseases.

Acknowledgement

This work was supported by grants No. NSC-92-2745-B-037-001 from the National Science Council, Taiwan, ROC.

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