International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

International Journal of Pharmacology 6 (2): 81-88, 2010
ISSN 1811-7775

Protective Effect of Baicalein Extracted from *Scutellaria baicalensis* against Lipopolysaccharide-Induced Glomerulonephritis in Mice

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Abstract: This study was to evaluate the therapeutic effect of baicalein, extracted from *Scutellaria baicalensis*, on lipopolysaccharide (LPS)-induced glomerulonephritis in mice. For this purpose, bacterial LPS (2.5 mg/kg/day) was injected intraperitoneally (i.p.) for 14 days to induce glomerulonephritis; then mice were treated with baicalein (150 mg/kg/day) by oral gavage for 14 days. In comparison to samples from mice receiving only LPS injection, oral administration of baicalein significantly attenuated the rise of serum levels of blood urea nitrogen and creatinine, prtoeinuria, kidney wet-to-dry weight ratio as well as renal glomerular cell proliferation induced by LPS. Moreover, treatment with baicalein decreased the urine NOx (nitrite+nitrate) concentration and serum levels of proinflammatory cytokine including tumor necrosis factor-α, interleukin-1β and interleukin-6 accompanied by suppression of inducible nitric oxide synthase and cyclooxygenase-2 expression and prostaglandin E_2 production in renal tissue relative to LPS-injected alone mice. Similarly, the LPS-induced increase of urine 8-iso-prostaglandin $F_2\alpha$, a marker of reactive oxygen species, was also markedly inhibited by baicalein. The present results demonstrate that baicalein possesses antiinflammatory and antioxidant properties that may be of benefit against the deleterious actions of LPS in glomerulonephritis.

Key words: Baicalein, lipopolysaccharide, glomerulonephritis, inflammation, nitric oxide

INTRODUCTION

Sepsis is generally considered a systemic inflammatory disease and still represents the leading cause of renal injury (Foster, 2008). Clinical evidence has shown that glomerulonephritis (GN) is a common cause of chronic kidney disease and end stage renal failure. The glomerulonephritis (GN) is defined as inflammation and cell proliferation in the glomerulus accompanied by common clinical finding including hematuria, proteinuria, edema and renal dysfunction (Foster, 2008). In response to endotoxin (lipopolysaccharide, LPS), a constituent of gram-negative bacteria wall, the pathophysiological cascade of GN is triggered by a variety of factors including proinflammatory cytokines. Among these cytokines, tumor necrosis factor-α (TNF-α) is proposed to play an early and crucial role in the cascade (Jansen *et al*., 1996) and neutralizing TNF-α exhibited a protective effect against LPS-induced renal failure (Knotek *et al*., 2001).

The inducible nitric oxide synthase (iNOS)-derived NO overproduction stimulated by LPS and TNF-α plays a critical role in the pathogenesis of septic shock and multiple organ injury (Yang *et al*., 2002). Similarly, the cyclooxygenase-2 (COX-2)-derived overproduction of prostaglandin E_2 (PGE_2), an arachidonic acid metabolite, often found in LPS-treated animals is also an important mediator causing inflammatory responses (Ejima *et al*., 2003). Previous studies have shown that suppression of iNOS or COX-2-mediated process leads to an attenuation of LPS-induced renal dysfunction and renal injury (Wang *et al*., 2000, 2006). In addition, the glomerular Reactive Oxygen Species (ROS) also plays a key role in renal diseases by its cytotoxic effect on mesangial and glomerular endothelial cells (Rodrigo and Bosco, 2006). Thus, the agents that inhibit induction of iNOS and

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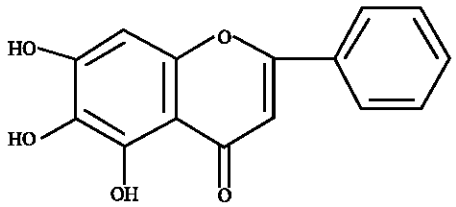

Fig. 1: Structure of baicalein

COX-2, as well as production of proinflammatory cytokines and ROS may prevent and treat endotoxemic GN.

Baicalein (5, 6, 7-trihydroxyflavone) (Fig. 1), a flavonoid, isolated from the root of *Scutellaria baicalensis*, is often used as herbal medicine in many Asian countries due to its beneficial value, including antibacterial, antiviral, antiinflammatory and antioxidative actions (Min, 2009; Cheng *et al*., 2007). Furthermore, previous studies have reported that baicalein suppressed LPS-induced iNOS and COX-2 expression and TNF-α formation in RAW 264.7 macrophages (Cheng *et al*., 2007; Woo *et al*., 2006). On the basis of these properties, we postulated that baicalein may protect against endotoxemic GN. However, there is no report on the effect of baicalein on endotoxemic GN. Therefore, this study was designed to investigate whether baicalein protects against LPS-induced GN.

MATERIALS AND METHODS

Materials: Baicalein was synthesized by Professor Wen-Hsin Huang in July, 2007 according to the protocol described previously (Huang *et al*., 2003). The synthetic baicalein was further purified by column chromatography with silica gel in acetone/hexane (9/1). The purity was identified by HPLC and the purity of synthetic baicalein was over 98%, comparable to commercial baicalein. Baicalein suspended in vehicle (0.5% carboxymethylcellulose) was used to treat animals by oral administration. All the chemicals and reagents used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated.

Animal experimental protocol: The study was approved by the Ethical Committee of Animal Experiments of National Defense Medical Center. C57BL/6 male mice aged 8-10 weeks, weighting between 25-30 g, were used throughout the study. Mice were maintained on a standard rodent chow and filtered tap water on an *ad libitum* manner. To establish GN animal model, the mice were injected intraperitoneally (i.p.) with LPS (*Escherichia coli* serotype O26:B6, 2.5 mg/kg/day) dissolved in sterile saline for 14 days. In our preliminary test, different doses of baicalein ranging from 50-150 mg kg^{-1} were administrated to evaluate their effects on renal function and kidney wet-to-dry weight ratio. Present results showed that 50 or 100 mg kg^{-1} baicalein had no significantly protective effect in this endotoxemic GN model. Thus, a dose of 150 mg kg^{-1} baicalein was chosen for this study. The animals were divided into three experimental groups. (1) Normal group. Mice were injected with the same volume of saline only without LPS and were given vehicle (0.5% carboxymethylcellulose) alone by oral gavage for 14 days. (2) LPS-treated group. Mice were injected with LPS for 14 days and followed by receiving vehicle (0.5% carboxymethylcellulose) by oral gavage for 14 days. (3) Baicalein-treated group. After a successive injection with LPS for 14 days, the mice were treated with baicalein (150 mg/kg/day) by oral gavage for 14 days. After finishing the treatment, samples were taken from these mice for subsequent tests.

Measurement of biochemical parameters and kidney edema: After finishing the treatment as the experimental protocol, a blood sample was collected from the mice and centrifuged (2000 g for 3min) to separate serum and the serum was stored at -80°C until assay. The levels of serum blood urea nitrogen (BUN), creatinine and urine albumin used as indicators of renal function were commercially measured. After the weight of wet right kidney was measured, the same kidney was dried in an oven for 48 h to remove any gravimetrically detectable water and weighed it again to calculate the kidney wet-to-dry weight ratio, an indicator of renal edema.

Measurement of cytokines, PGE_2 and urine 8-iso-prostaglandin $F_2\alpha$: The serum levels of TNF-α, interleukin-1β (IL-1β) interleukin-6 (IL-6) and interleukin-10 (IL-10) were measured by ELISA Kits (R and D Systems, Inc., Minneapolis, MN), respectively. The level of PGE_2 and 8-iso-prostaglandin $F_2\alpha$ was measured by EIASA kit (Cayman Chemical).

Measurement of urine NOx (nitrite+nitrate) level: Aliquots of 30 μL sample were deproteinated with 100 μL 95% alcohol for 30 min (4°C) and samples were centrifuged for 6 min at 12,000 g. The supernatant (6 μL) was injected into a collection chamber containing 5% VCl_3. In this strong reducing environment, both nitrate and nitrite were converted to NO. A constant stream of helium gas was used to carry the output into a NO analyzer (Sievers 280NOA; Sievers Instruments Inc., Boulder, CO, USA), where the NO reacts with ozone (O_3),

resulting in the emission of light. Light emission is proportional to the NO formed. Standard amounts of sodium nitrate were used for calibration.

Immunohistochemical staining: Paraffin sections of kidney tissue were deparaffinized and the endogenous peroxidase activity was blocked with methanol containing 0.3% hydrogen peroxide for 15 min at room temperature. Sections were washed in 0.01 M Phosphate-Buffered Saline (PBS), pH 7.2, containing 10% normal goat serum and incubated overnight at 4°C with rabbit anti-iNOS or anti-COX-2 antibody. Horseradish-conjugated goat anti-rabbit Ig antibody was used at 1:200 after washing. Immune complexes were detected with a solution of 3, 3-diaminobenzidine (0.2 mg mL^{-1}) and hydrogen peroxide in 0.05 M Tris-HCl buffer. Sections were counterstained with methyl green. In negative control sections, rabbit antiserum against ovalbumin (1 : 500) was used as an irrelevant antibody.

Histopathological studies: The kidney tissues were removed, placed in formalin and embedded in wax according to a standard protocol. Four micrometer sections were cut and stained with hematoxylin and eosin for light microscopy and the histological alteration was evaluated by a pathologist who was blind to the experimental conditions.

Statistical analysis: Data were expressed as Means±SE of the Mean (SEM) All results were analyzed by one-way ANOVA followed by a multiple comparison test (Scheffe test). A p-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Baicalein improved renal function and kidney wet-to-dry weight ratio in endotoxemic mice: Injection of LPS (2.5 mg/kg/day, i.p.) for 14 days induced a renal dysfunction reflected by increased serum BUN and creatinine concentrations, proteinuria and kidney wet-to-dry weight ratio. Oral administration of baicalein (150 mg kg^{-1}) for 14 days significantly improved these alterations (Fig. 2a-c, 3) compared to those in the LPS-treated group.

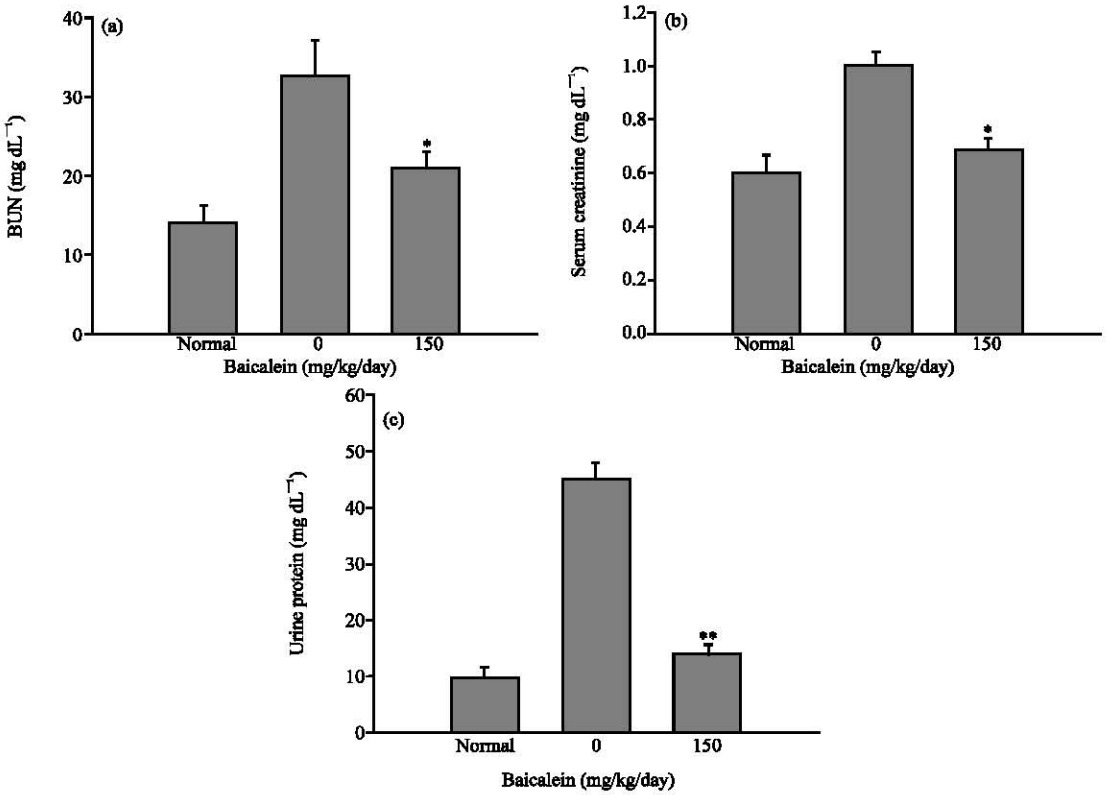

Fig. 2: (a-c) Effect of baicalein on serum BUN, creatinine and urine protein levels in LPS-treated mice. The mice were injected with LPS (2.5 mg/kg/day, i.p.) for 14 days followed by treatment with baicalein (150 mg/kg/day, p.o.) for 14 days and the blood and urine were taken for measurement of these parameters to evaluate renal function. Each data was expressed as Mean±SEM (n = 5). *p<0.05, **p<0.01 vs. LPS-treated group

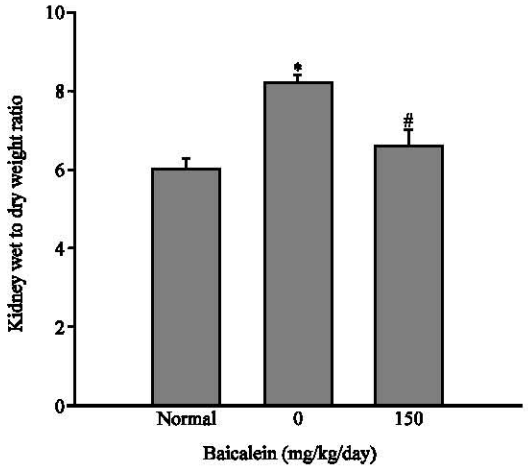

Fig. 3: Effect of baicalein on kidney edema in LPS-treated mice. The mice were injected with LPS (2.5 mg/kg/day, i.p.) for 14 days followed by treatment with baicalein (150 mg/kg/day, p.o.) for 14 days and the kidneys were photographed and measured the kidney wet-to-dry weight ratio. Each data was expressed as Mean±SEM (n = 5). *p<0.05 vs. normal mice. #p<0.01 vs. LPS-treated group

Baicalein inhibited serum cytokine concentrations in endotoxemic mice: Injection of LPS for 14 days induced a marked increase in serum TNF-α IL-β and IL-6 levels compared to that in normal group. Oral administration of baicalein for 14 days significantly decreased the increased cytokine concentrations compared to those in the LPS-treated group (Fig. 4a-c).

Baicalein reduced urine 8-iso-prostaglandin $F_2\alpha$ level in endotoxemic mice: After injection of LPS for 14 days the concentration of urine 8-iso-prostaglandin $F_2\alpha$ was markedly increased compared to that in normal group and this enhancement was significantly attenuated by treatment with baicalein (Fig. 5).

Bicalein inhibited urine NOx level and renal PGE_2 formation in endotoxemic mice: The urine NOx level and PGE_2 formation in renal tissue were markedly increased after injection of LPS for 14 days. Treatment with baicalein significantly inhibited the rise of urine NOx and renal PGE_2 formation compared to that in LPS-treated group (Fig. 6a, b).

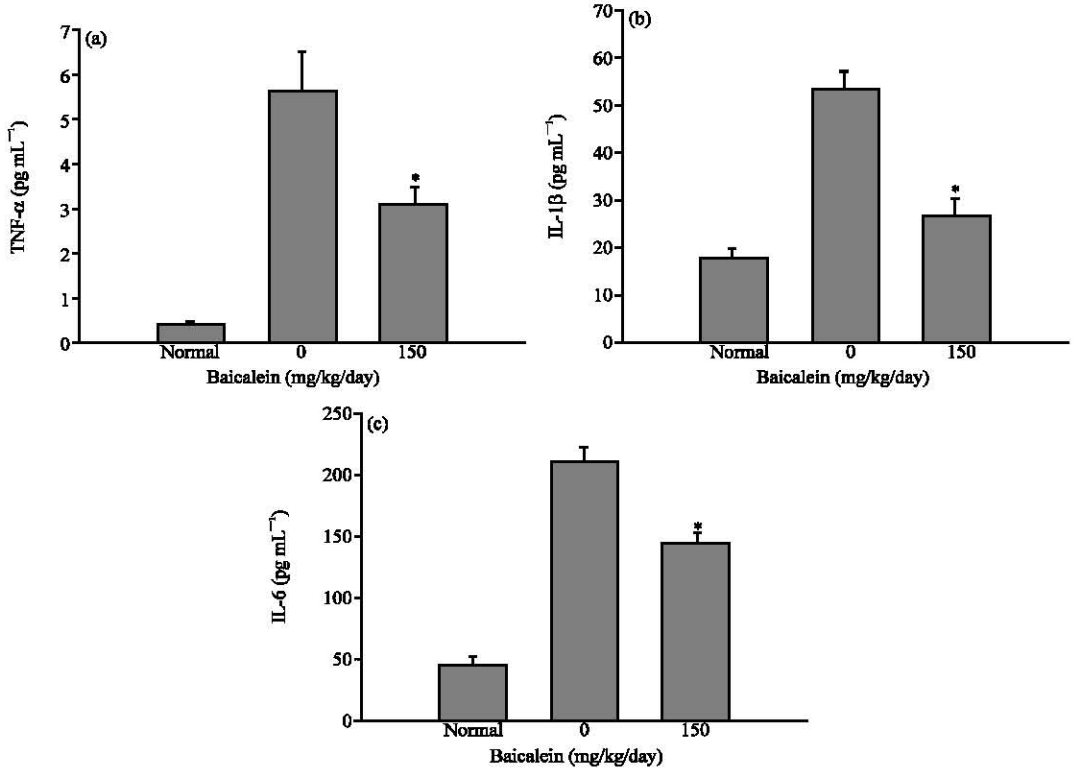

Fig. 4: (a-c) Effect of baicalein on serum TNF-α IL-1β and IL-6 levels in LPS-treated mice. The mice were injected with LPS (2.5 mg/kg/day, i.p.) for 14 days followed by treatment with baicalein (150 mg/kg/day, p.o.) for 14 days and the blood was taken for measurement of cytokines. Each data was expressed as Mean±SEM (n = 5). *p<0.05 vs. LPS-treated group

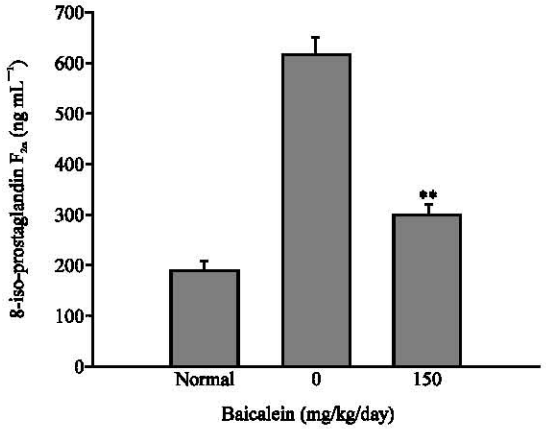

Fig. 5: Effect of baicalein on urine 8-iso-prostaglandin $F_2\alpha$ level in LPS-treated mice. The mice were injected with LPS (2.5 mg/kg/day, i.p.) for 14 days followed by treatment with baicalein (150 mg/kg/day, p.o.) for 14 days and the urine was taken to measure 8-iso-prostaglandin $F_2\alpha$ level. Each data was expressed as Mean±SEM (n = 5). **p<0.01 vs. LPS-treated group

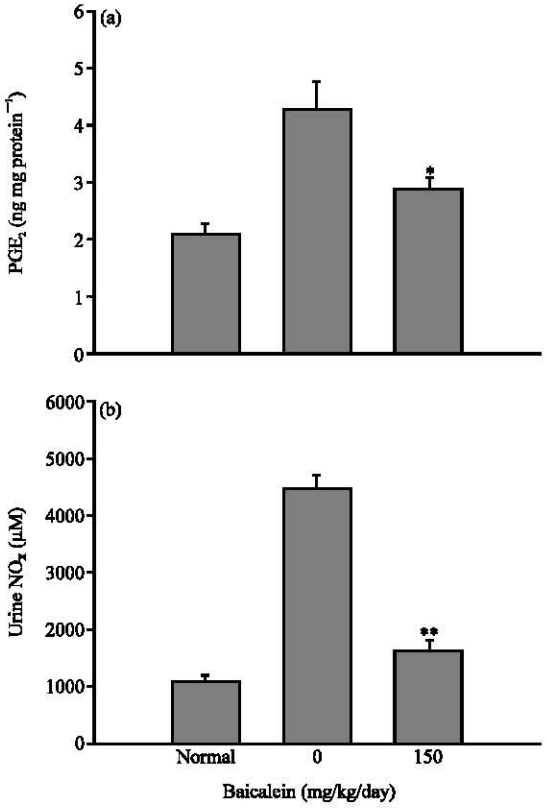

Fig. 6: (a, b) Effect of baicalein on urine NOx level and kidney PGE_2 production in LPS-treated mice. The mice were injected with LPS (2.5 mg/kg/day, i.p.) for 14 days followed by treatment with baicalein (150 mg/kg/day, p.o.) for 14 days and the urine and kidney were taken to measure NOx and PGE_2 level. Each data was expressed as Mean±SEM (n = 5). *p<0.05, **p<0.01 vs. LPS-treated group

Baicalein inhibited iNOS and COX-2 expression in renal: The iNOS and COX-2 expression in renal tissue evaluated by immunohistochemical staining was enhanced by LPS and the increased expression of iNOS and COX-2 was reduced by baicalein (Fig. 7).

Baicalein improved renal histological alterations in endotoxemic mice: Histological examinations showed that there was an appearance of mesangial cell proliferation and synthesis of extracellular matrix proteins in LPS-treated group, which was markedly ameliorated by treatment with baicalein (Fig. 8).

This study demonstrates that oral administration of baicalein protects against LPS-induced GN. This conclusion is based on the significant attenuation of the increased serum BUN and creatinine concentrations, proteinuria, as well as kidney wet-to-dry weight ratio after LPS administration in baicalein-treated group compared to those in LPS-treated group. In response to LPS, various inflammatory mediators, such as proinflammatory cytokines, ROS, NO and PGE_2 are produced, which are thought to contribute to the LPS-induced pathological symptoms in renal diseases (Wang *et al*., 2003). In this study, LPS-induced increase of serum levels of TNF-α IL-β and IL-6 was significantly inhibited by baicalein, indicating that its protective effect in GN may be through reducing proinflammatory cytokine production. However, the serum level of IL-10, an antiinflammatory cytokine (Sanjabi *et al*., 2009), was not affected by baicalein (data not shown), suggesting that the beneficial effect of baicalein in GN might be not mediated by enhanced IL-10 formation; whether the effect of baicalein is a result of reduction in proinflammatory cytokines remains to be determined.

During endotoxemia, overproduction of ROS may lead to leukocyte activation, mesangial cell apoptosis and glomerular damage (Moreno *et al*., 2000). Therefore, the inhibition of LPS-induced urine 8-iso-prostaglandin $F_2\alpha$ level, a useful clinical biomarker of oxidative stress (Lim *et al*., 2002), by baicalein, may be also involved in the beneficial effects in endotoxemic GN. In addition, the large amount of NO produced by iNOS in response to LPS can further form highly reactive species, most notably peroxynitrite, by interaction with superoxide anion (Kukreja and Hess, 1992). Then, peroxynitrite decomposes to produce hydroxyl radical. Both hydroxyl

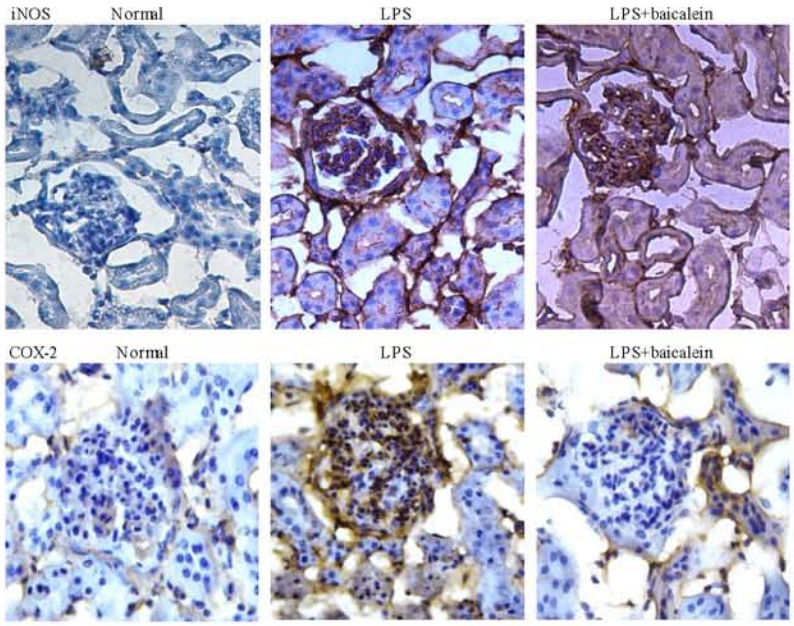

Fig. 7: Effect of baicalein on the expression of iNOS and COX-2 of kidney in LPS-treated mice. The mice were injected with LPS (2.5 mg/kg/day, i.p.) for 14 days followed by treatment with baicalein (150 mg/kg/day, p.o.) for 14 days and the kidneys were taken to measure iNOS and COX-2 expression by immunohistochemical staining method

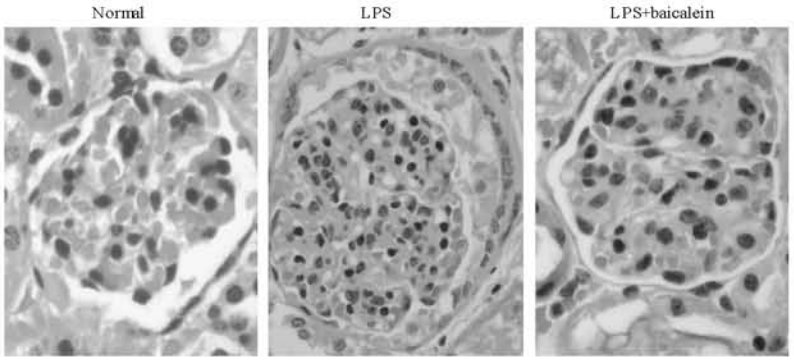

Fig. 8: Effect of baicalein on the histological changes of kidney in LPS-treated mice. The mice were injected with LPS (2.5 mg/kg/day, i.p.) for 14 days followed by treatment with baicalein (150 mg/kg/day, p.o.) for 14 days and the histological examination of the kidney was preformed

radical and peroxynitrite results in lipid peroxidation, protein oxidation, mitochondrial dysfunction and subsequently leads to tissue damage and cell death. Thus, the inhibition of iNOS/NO pathway in renal tissue by baicalein is an important mechanism contributing to attenuation of pathogenesis of endotoxemic GN.

A lot of studies have suggested that the COX-2/PGE_2-mediated pathway is involved in the renal dysfunction of GN including reduction of glomerular filtration rate and tubular function through down regulation of Organic Anion Transporters (OATs) especially OAT1 and OAT3 gene expression (Hocherl *et al*., 2009; De Vriese, 2003). Accordingly, the beneficial effect of baicalein on improving renal function in endotoxemic GN may be associated with the inhibition of COX-2-mediated downregulation of OATs. Once activation by LPS, the nuclear factor kappa B (NF-κB), a nuclear transcription factor, translocates into nucleus and in turn activates several inflammatory gene transcription including iNOS, COX-2 and proinflammatory cytokines (Pasparakis, 2009). Based on the results of previous study showing baicalein blocked the activation of NF-κB by inhibiting the degradation of IκBα in LPS-treated RAW 264.7 macrophages (Cheng *et al*., 2007), we proposed that

the antiinflammatory activities of baicalein in GN may be mediated by suppressing the activation of NF-κB.

In clinical practice, GN is characterized by the appearance of mesangial cell proliferation and synthesis of extracellular matrix proteins. It has been demonstrated that several factors including enhanced iNOS and COX-2 expression, platelet-derived growth factor (PDGF) and ROS all stimulate mesangial cell proliferation (Tokuyama *et al*., 2008; Sheu *et al*., 2005; Budisavljevic *et al*., 2003). Furthermore, in glomerular mesangial cells, the synthesis of 12 (S)-hydroxyeicosatetraenoic acid (12 (S)-HETE), a product of 12-lipoxygenases (12-LOX), also can directly induce mesangial cell hypertrophy and matrix production by activating mitogen-activated protein kinase (MAPK) pathway (Reddy *et al*., 2002). Importantly, it is well known that baicalein is a potent inhibitor of 12-LOX. Therefore, the inhibition of LPS-induced glomerular cell proliferation by baicalein in endotoxemic GN may be, at least in part, due to attenuation of iNOS and COX-2 induction, free radical formation, PDGF-A expression and 12-LOX-mediated process.

CONCLUSION

The results obtained in this study first demonstrates that baicalein protects against LPS-induced GN in mice, which is probably mediated by inhibition of various proinflammatory mediator formation, iNOS and COX-2 induction.

ACKNOWLEDGMENTS

This study was supported by a research grant from the Committee on Chinese Medicine and Pharmacy, Department of Health, Taiwan, Republic of China (CCMP94-RD-022) and partly from Veterans General Hospital-Kaohsiung (VGHKS96-68).

REFERENCES

Budisavljevic, M.N., L. Hodge, K. Barber, J.R. Fulmer and R.A.D. Arvizu*et al*., 2003. Oxidative stress in the pathogenesis of experimental mesangial proliferative glomerulonephritis. Am. J. Physiol. Renal Physiol., 285: 1138-1148.

Cheng, P.Y., Y.M. Lee, Y.S. Wu, T.W. Chang, J.S. Jin and M.H. Yen, 2007. Protective effect of baicalein against endotoxic shock in rats *in vivo* and *in vitro*. Biochem. Pharmacol., 73: 793-804.

De Vriese, A.S., 2003. Prevention and treatment of acute renal failure in sepsis. J. Am. Soc. Nephrol., 14: 792-805.

Ejima, K., M.D. Layne, I.M. Carvajal, P.A. Kritek and R.M. Baron *et al*., 2003. Cyclooxygenase-2-deficient mice are resistant to endotoxin-induced inflammation and death. FASEB. J., 17: 1325-1327.

Foster, M.H., 2008. Novel targets for immunotherapy in glomerulonephritis. Biologics, 2: 531-545.

Hocherl, K., C. Schmidt and M. Bucher, 2009. COX-2 inhibition attenuates endotoxin-induced downregulation of organic amion transporters in the rat renal cortex. Kidney Int., 75: 373-380.

Huang, W.H., P.Y. Chien, C.H. Yang and A.R. Lee, 2003. Novel synthesis of flavonoids of scutellaria baicalensis GEORGI. Chem. Pharm. Bull., 51: 339-340.

Jansen, M.J., T. Hendriks, M.T. Vogels, J.W. van der Meer and R.J. Goris, 1996. Inflammatory cytokines in an experimental model for the multiple organ dysfunction syndrome. Crit. Care Med., 24: 1196-1202.

Knotek, M., B. Rogachev, W. Wang, T. Ecder and V. Melnikov *et al*., 2001. Endotoxemic renal failure in mice: Role of tumor necrosis factor independent of inducible nitric oxide synthase. Kidney Int., 59: 2243-2249.

Kukreja, R.C. and M.L. Hess, 1992. The oxygen free radical system: From equations through membrane protein interactions to cardiovascular injury and protection. Cardiovasc. Res., 26: 641-655.

Lim, P.S., Y.M. Chang, L.M. Thien, N.P. Wang, C.C. Yang, T.T. Chen and W.M. Hsu, 2002. 8-iso-prostaglandin F2 alpha as a useful clinical biomarker of oxidative stress in ESRD patients. Blood Purif., 20: 537-542.

Min, L.W., 2009. New therapeutic aspects of flavones: The anticancer properties of Scutellaria and its main active constituents Wogonin, Baicalein and Baicalin. Cancer Treat. Rev., 35: 57-68.

Moreno, M.V., Y. Ishikawa, J.L. Cazana and M. Kitamura, 2000. Selective involvement of superoxide anion, but not downstream compounds hydrogen peroxide and peroxynitrite, in tumor necrosis factor-alpha-induced apoptosis of rat mesangial cells. J. Biol. Chem., 275: 12684-12691.

Pasparakis, M., 2009. Regulation of tissue homeostasis by NF-B signalling: implications for inflammatory diseases. Nat. Rev. Immunol., 9: 778-788.

Reddy, M.A., S.G. Adler, Y.S. Kim, L. Lanting and J. Rossi *et al*., 2002. Interaction of MAPK and 12-lipoxygenase pathways in growth and matrix protein expression in mesangial cells. Am. J. Physiol. Renal Physiol., 283: 985-994.

Rodrigo, R. and C. Bosco, 2006. Oxidative stress and protective effects of polyphenols: Comparative studies in human and rodent kidney. A review. Comp. Biochem. Physiol. C Toxicol. Pharmacol., 142: 317-327.

Sanjabi, S., L.A. Zenewicz, M. Kamanaka and R.A. Flavell, 2009. Anti-inflammatory and pro-inflammatory roles of TGF-beta, IL-10 and IL-22 in immunity and autoimmunity. Curr. Opin. Pharmacol., 9: 447-453.

Sheu, M.L., K.F. Chao, Y.J. Sung, W.W. Lin, S.Y.L. Shiau and S.H. Liu, 2005. Activation of phosphoinositide 3-kinase in response to inflammation and nitric oxide leads to the up-regulation of cyclooxygenase-2 expression and subsequent cell proliferation in mesangial cells. Cel. Signal., 17: 975-984.

Tokuyama, H., D.J. Kelly, A. Cox, Y. Zhang, K. Thai, D.J.N. Paterson and R.E. Gilbert, 2008. Tranilast ameliorates experimental mesangial proliferative glomerulonephritis. Nephron. Exp. Nephrol., 109: 1-7.

Wang, J.L., H.F. Cheng, S. Shappell and R.C. Harris, 2000. A selective cyclooxygenase-2 inhibitor decreases proteinuria and retards progressive renal injury in rats. Kidney Int., 57: 2334-2342.

Wang, W., S. Jittikanont, S.A. Falk, P. Li and L. Feng *et al*., 2003. Interaction among nitric oxide, reactive oxygen species and antioxidants during endotoxemia-related acute renal failure. Am. J. Physiol. Renal Physiol., 284: 532-537.

Wang, W., E. Zolty, S. Falk, V. Basava, L. Reznikov and R. Schrier, 2006. Pentoxifylline protects against endotoxin-induced acute renal failure in mice. Am. J. Physiol. Renal Physiol., 291: 1090-1095.

Woo, K.J., J.H. Lim, S.I. Suh, Y.K. Kwon and S.W. Shin *et al*., 2006. Differential inhibitory effects of baicalein and baicalin on LPS-induced cyclooxygenase-2 expression through inhibition of C/EBPbeta DNA-binding activity. Immunobiology, 211: 359-368.

Yang, F., A.S. Comtois, L. Fang, N.G. Hartman and G. Blaise *et al*., 2002. Nitric oxide-derived nitrate anion contributes to endotoxic shock and multiple organ injury/dysfunction. Crit. Care Med., 30: 650-657.