



## Baicalein, an active component of *Scutellaria baicalensis* Georgi, improves cardiac contractile function in endotoxaemic rats via induction of heme oxygenase-1 and suppression of inflammatory responses

Yen-Mei Lee<sup>a,b</sup>, Pao-Yun Cheng<sup>c</sup>, Lih-Shin Chim<sup>d</sup>, Ching-Wen Kung<sup>e</sup>, Shuk-Man Ka<sup>f</sup>, Ming-Tzeung Chung<sup>g</sup>, Joen-Rong Sheu<sup>h,\*</sup>

<sup>a</sup> Graduate Institute of Medical Sciences, Taipei Medical University, Taipei 110, Taiwan

<sup>b</sup> Department of Pharmacology, National Defense Medical Center, Taipei 114, Taiwan

<sup>c</sup> Graduate Institute of Chinese Pharmaceutical Sciences, China Medical University, Taichung 404, Taiwan

<sup>d</sup> School of Medicine, National Defense Medical Center, Taipei 114, Taiwan

<sup>e</sup> Department of Nursing, Tzu Chi College of Technology, Hualien 970, Taiwan

<sup>f</sup> Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, Taipei 114, Taiwan

<sup>g</sup> Department of Obstetrics and Gynecology, Army Forces Tao-Yuan General Hospital, Tao-Yuan 325, Taiwan

<sup>h</sup> Department of Pharmacology, Taipei Medical University, 250 Wu-Hsing Street, Taipei 110, Taiwan

### ARTICLE INFO

#### Article history:

Received 21 July 2010

Received in revised form 20 February 2011

Accepted 3 March 2011

Available online 9 March 2011

#### Keywords:

Baicalein

Sepsis

Myocardial dysfunction

Apoptosis

Inflammation

Oxidative stress

### ABSTRACT

**Aim of the study:** To evaluate the protective effect of baicalein on myocardial dysfunction caused by endotoxaemia in rats and to explore the possible mechanisms.

**Materials and methods:** Baicalein (10 mg/kg, intravenous) was administered to conscious Wistar rats 30 min after lipopolysaccharide (LPS; 10 mg/kg, intravenous) challenge. Six hours after LPS administration, the contractile function of the isolated heart was examined using the Langendorff technique. Cardiac protein expression related to inflammatory responses, superoxide anion production and caspase-3 activity were measured.

**Results:** Post-treatment with baicalein significantly attenuated the LPS-induced hypotension with accompanying tachycardia. The contractile function of isolated heart was significantly preserved 6 h after LPS administration, following treatment with baicalein. Furthermore, baicalein induced the expression of heme oxygenase-1 protein and reduced superoxide anion formation in the myocardium of LPS-treated rats. Cardiac levels of inducible nitric oxide synthase, monocyte chemoattractant protein-1, phospho-IκBα and phospho-p65 protein and caspase-3 activity significantly increased 6 h after LPS challenge but baicalein significantly attenuated these LPS-induced changes.

**Conclusions:** Baicalein improves myocardial contractility in LPS-induced sepsis, which may be related to reductions in oxidative stress, myocardial inflammatory responses and apoptosis.

© 2011 Elsevier Ireland Ltd. All rights reserved.

### 1. Introduction

Myocardial depression is a well-recognized manifestation of organ dysfunction in sepsis. Cardiac dysfunction in sepsis is characterized by decreased contractility, impaired ventricular response to fluid therapy and, in some patients, ventricular dilatation (Zanotti-Cavazzoni and Hollenberg, 2009). Several mediators including cytokines such as tumour necrosis factor (TNF)-α, interleukin (IL)-

1β and IL-6, prostanoids and nitric oxide (NO) have been proposed to be myocardial depressant factors (Merx and Weber, 2007).

Triggering through toll-like receptors by bacterial ligands, such as lipopolysaccharide (LPS), initiates signalling cascades that result in the activation of NF-κB, which drives transcription of a range of important pro-inflammatory cytokines and chemokine genes (Sriskandan and Altmann, 2008). In fact, NF-κB can be rapidly activated by many pathogenic stimuli including TNF-α and IL-1. In addition, reactive oxygen species (ROS) have been shown to be involved in NF-κB activation (Schreck et al., 1991; Adcock et al., 1994). LPS-induced cardiac dysfunction may be in part the result of production of ROS mediated by inflammatory mediators such as TNF-α (Rudiger and Singer, 2007). In addition, the redox-sensitive gene heme oxygenase-1 (HO-1) can be activated by oxidative stress to induce HO-1 protein expression, resulting in cytoprotective

**Abbreviations:** LPS, lipopolysaccharide; ROS, reactive oxygen species; LVDP, left ventricular developed pressure; iNOS, inducible nitric oxide synthase; MCP, monocyte chemoattractant protein; HO, heme oxygenase.

\* Corresponding author. Tel.: +886 2 27361661x3199; fax: +886 2 27390450.

E-mail address: [sheujr@tmu.edu.tw](mailto:sheujr@tmu.edu.tw) (J.-R. Sheu).

effects in various diseases (Takahashi et al., 2004). It has been suggested that manipulation of the HO-1 pathway may represent a possible therapeutic strategy to counteract the oxidative stress of endotoxaemia and to limit myocardial deformation (Tamion et al., 2010).

Chemokines have been shown to participate in the pathogenesis of sepsis (Ramnath et al., 2008). Monocyte chemoattractant protein (MCP)-1, a prototype CC chemokine, is a potent chemoattractant and a regulatory mediator involved in a variety of inflammatory diseases (Luster, 1998). MCP-1 expression is regulated at the transcriptional level by stimulatory agents such as TNF- $\alpha$ , interferon (IFN)- $\gamma$ , platelet-derived growth factor and stress factors (Melgarejo et al., 2009). Recently, anti-MCP-1 treatment has been proposed to be of potential therapeutic value in the treatment of sepsis and endotoxaemia (Ramnath et al., 2008).

There is increasing evidence that apoptosis is also involved in sepsis-induced cardiovascular dysfunction (Ayala et al., 2008; Ward, 2008). Apoptosis is potentially triggered by cytokines, TNF- $\alpha$ , ROS and NO released by infiltrating polymorphonuclear leukocytes or macrophages (Zhao and Vinten-Johansen, 2002). Therapeutic strategies aimed at inhibition of apoptosis have resulted in improved cardiac function in animal models of sepsis (Fauvel et al., 2001; Nevière et al., 2001; Buerke et al., 2008).

Huang Qin (*Scutellaria baicalensis* Georgi) is a medicinal plant officially listed in the Chinese Pharmacopoeia. It is traditionally used for the treatment of various inflammatory diseases, hepatitis, tumours and diarrhoea. *Scutellaria baicalensis* Georgi contains four major flavones: wogonin, wogonoside, baicalein and baicalin, which make up about 1.3%, 3.55%, 5.41% and 10.11%, respectively, of the dry material (Li-Weber, 2009). Baicalein (5,6,7-trihydroxy-2-phenyl-4H-1-benzopyran-4-one) shows a variety of biological activities, including anti-thrombotic (Kimura et al., 1997), antiviral (Wu et al., 2001), anti-cancer (Zhang et al., 2003), anti-oxidant (Bochorakova et al., 2003) and anti-inflammatory (Shen et al., 2003) activities. In an *in vitro* study, baicalein suppressed the LPS-induced production of NO in RAW264.7 mouse macrophages (Wakabayashi, 1999). Recently, we reported that baicalein reduces plasma NO levels *in vivo* in septic rats, leading to improved vasoreactivity, blood pressure and survival rate (Cheng et al., 2007). However, it is uncertain whether the beneficial effect of baicalein on cardiac contractile function directly contributes to the prevention of circulatory failure. Therefore, the aim of this study was to evaluate the protective effect of baicalein on myocardial dysfunction caused by endotoxaemia in conscious rats and to explore the possible mechanisms.

## 2. Materials and methods

### 2.1. Animal preparation

Wistar-Kyoto rats (male, 280–300 g) were purchased from the National Laboratory Animal Breeding and Research Center of the National Science Council, Taiwan. Handling of the animals was in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised in 1985). This study was approved by the Institutional Animal Care and Use Committee of National Defense Medical Center, Taiwan. All animals were housed at an ambient temperature of  $23 \pm 18^\circ\text{C}$  and humidity of  $55 \pm 5\%$ . Rats were anaesthetized by intraperitoneal injection of sodium pentobarbital (40–50 mg/kg). The left carotid artery was cannulated and exteriorized to the back of the neck and connected to a pressure transducer (P231D, Statham, Oxnard, CA, USA) to measure phasic blood pressure, mean arterial blood pressure (MBP) and heart rate, which were displayed on a polygraph recorder (ML 785 PowerLab, AD instruments, Castle Hill, Australia). The right jugular vein was cannulated and exteriorized

to the back of the neck for the administration of drugs. After the catheters were fixed, rats were fasted overnight for recovery but allowed water *ad libitum*.

### 2.2. Experimental groups

The animals were randomly allocated into four groups ( $n=6$  in each group): (1) sham group (1 mL/kg normal saline given intravenously); (2) sham + Bai group (10 mg/kg baicalein given intravenously); (3) LPS group, *Escherichia coli* LPS (10 mg/kg, intravenous infusion over 10 min); (4) LPS + Bai group, *Escherichia coli* LPS 10 mg/kg plus baicalein (10 mg/kg, intravenously). The dose of baicalein used was based on our previous study on sepsis (Cheng et al., 2007). Bacterial LPS (*Escherichia coli* serotype 0127:B8, L3127) and baicalein were obtained from Sigma Chemical Company (St. Louis, MO, USA). The experiments were performed on pairs of conscious rats, a model that is likely to be clinically relevant (Mathiak et al., 2000) and avoids the interference of anaesthetics with cytokine release (Yang et al., 2007). After recording baseline haemodynamic variables, LPS was infused and baicalein or vehicle (0.3 mL dimethyl sulfoxide) infusion was started 30 min after LPS treatment. The changes in blood pressure and heart rate were monitored for 6 h in all animal groups. The state of conscious rats after LPS administration became gradually less active: they moved slowly and appeared immobile after 5–6 h. The blood glucose levels significantly increased at 1 h after LPS administration ( $\Delta 50 \pm 7.8$  mg/dL) compared with basal levels ( $102 \pm 3.1$  mg/dL). Hyperglycaemia was used as an indicator of successful induction of sepsis by LPS challenge. At the end of each experiment, the rats were euthanized by intraperitoneal administration of pentobarbital (60 mg/kg) with 5000 USP units of heparin added as an anti-coagulant.

### 2.3. Isolated heart preparation and left ventricular pressure recording

Hearts were isolated 6 h after LPS administration and perfused with a modified Krebs–Henseleit solution equilibrated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> at a constant flow of 7–9 mL/min and temperature of 37°C when being mounted on the Langendorff apparatus. The buffer contained 118.0 mM NaCl, 4.7 mM KCl, 1.8 mM CaCl<sub>2</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.2 mM MgSO<sub>4</sub>, 25.0 mM NaHCO<sub>3</sub> and 11.0 mM glucose. A 2 F high-fidelity micro-manometer catheter containing a pressure transducer (SPR-407, Millar Institute, Houston, TX, USA) was inserted into the left ventricle via the left atrium. The heart was paced at 300 beats/min and allowed to equilibrate for 15 min. Left ventricle contractility was continuously evaluated by the left ventricular developed pressure (LVDP) and the rates of contraction and relaxation (+dP/dt and -dP/dt) measured using a PowerLab/8SP analogue-to-digital converter (ADInstruments).

### 2.4. Western blot analysis

Six hours after LPS administration, the left ventricular myocardium was isolated, immediately frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  until processed. Detection of the proteins by Western blotting was performed as described previously (Chen et al., 2006). The primary antibody probes in this experiment were mouse monoclonal anti-inducible nitric oxide synthase (iNOS) (BD Biosciences, USA; 1:2000), anti-phospho-I $\kappa$ B $\alpha$  (Cell Signalling, USA; 1:1000) and anti-phospho-p65 (Epitomics, USA; 1:1000) and mouse polyclonal anti-MCP-1 (eBioscience, USA; 1:1000) and anti-HO-1 (Santa-Cruz, USA; 1:1000). To standardize densitometry measurements between individual samples, the ratios of iNOS, phospho-I $\kappa$ B $\alpha$ , phospho-p65, MCP-1 or HO-1 to  $\alpha$ -actin were calculated.

### 2.5. Superoxide anion production in myocardium 6 h after LPS administration

Superoxide anion production in the left ventricular myocardium was measured by modified lucigenin-enhanced chemiluminescence and was performed as described previously (Chen et al., 2006; Shih et al., 2008). Samples of left ventricle (3 × 3 mm) taken 6 h after LPS administration were used. Scintillation plates containing Krebs-HEPES buffer with lucigenin (1.25 mM) were placed into a microplate luminometer (Hidex, Microplate Luminometer, Finland). These results were expressed as counts per second (CPS) per milligram dry weight of myocardium.

### 2.6. Measurement of caspase-3 activity in cardiac tissue

Cardiac caspase-3 activity was determined using colorimetric assay kits (Assay Designs, MI, USA) according to the manufacturer's instructions. Results are expressed as units/μg protein.

### 2.7. Statistical analysis

The data are expressed as group means ± SEM. Statistical evaluation was performed with one-factor analysis of variance followed by the Newman-Keuls *post hoc* comparison test. A *P* value of less than 0.05 was deemed significant.

## 3. Results

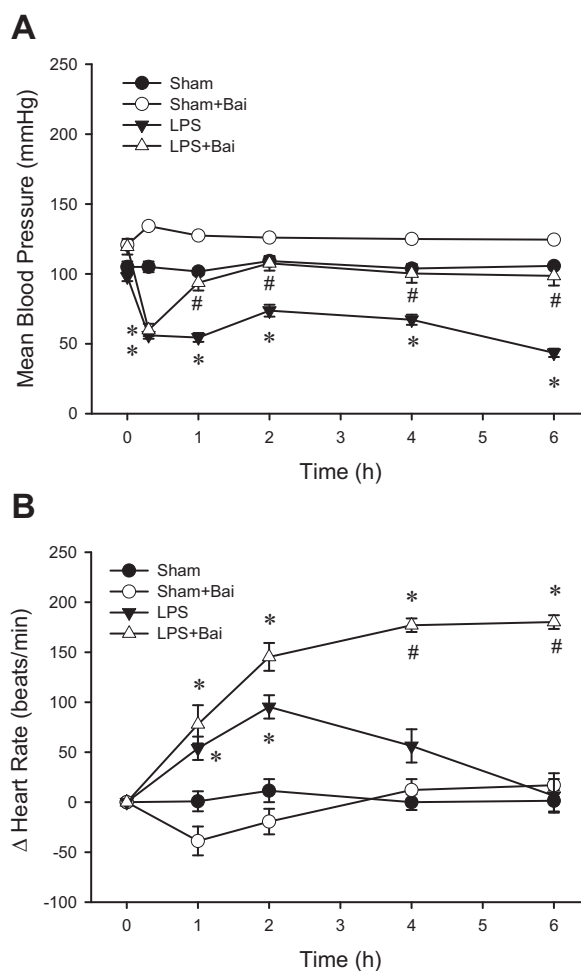
### 3.1. Effects of baicalein on haemodynamic changes in endotoxaemic rats

The haemodynamic data including MBP and heart rate are shown in Fig. 1. The basal MBP of rats did not differ significantly between the four groups. In the sham and sham + Bai groups, there was no significant change in MBP during the experimental period. In the LPS group, rats showed a marked fall in MBP 30 min after LPS administration, which lasted until 1 h after LPS and then progressively increased between 1 and 2 h, followed by an increasing rate of decrease in MBP for 2–6 h after LPS. Post-treatment with baicalein 30 min after LPS administration significantly attenuated the hypotension caused by LPS.

The basal heart rate did not differ significantly between the four groups. In the sham and sham + Bai groups, there was no significant change in heart rate during the experimental period. LPS administration caused a significant increase in heart rate during the first 2–3 h of the experimental period compared with the sham group, and then progressively decreased to the basal level. Post-treatment with baicalein 30 min after LPS administration also resulted in a profound elevation of heart rate, which was maintained at a significantly higher level than in the sham group until the end of the experiment (6 h after LPS).

### 3.2. Effects of baicalein on cardiac contractile dysfunction caused by LPS

The LVDP (Fig. 2A) and average  $\pm$ dP/dt (Fig. 2B and C) evaluated at 6 h after LPS administration were significantly decreased in the hearts of the LPS-treated groups compared with those of the sham group (*P* < 0.05). Post-treatment with baicalein resulted in the recovery of LVDP and  $\pm$ dP/dt compared with the LPS groups (*P* < 0.05). Baicalein alone (sham + Bai group) did not significantly affect these parameters of cardiac contractile function.



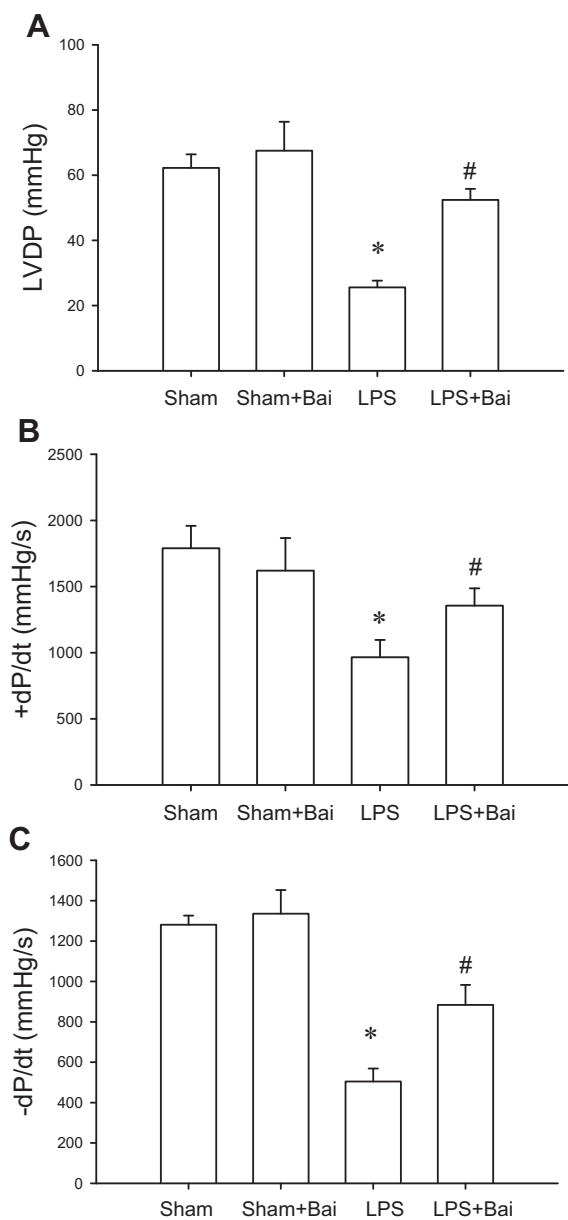
**Fig. 1.** Effects of post-treatment with baicalein on mean arterial blood pressure (A) and changes in heart rate (B) in conscious rats with sepsis induced by LPS injection. Baicalein (Bai) 10 mg/kg was given 30 min after LPS 10 mg/kg injection. Values are expressed as mean ± SEM. \**P* < 0.05 vs. the sham group; #*P* < 0.05 vs. the LPS group, *n* = 6.

### 3.3. Effects of baicalein on cardiac iNOS, MCP-1, phospho-IκBα, phospho-p65, and HO-1 protein expression

Six hours after administration of LPS, the levels of cardiac protein expression of iNOS (Fig. 3A and B), MCP-1 (Fig. 3C and D), phospho-IκBα (Fig. 4A and B) and phospho-p65 (Fig. 4C and D) were significantly elevated compared with those in the sham group (*P* < 0.05). Post-treatment with baicalein significantly reduced expression of these pro-inflammatory proteins compared with the LPS group (*P* < 0.05). However, the levels of iNOS and MCP-1 in the LPS + Bai group were significantly higher than those in the sham group (*P* < 0.05). By contrast, the level of HO-1 protein was markedly reduced 6 h after LPS administration compared with that of the sham group (*P* < 0.05), whereas post-treatment with baicalein significantly elevated the induction of HO-1 during endotoxaemia (*P* < 0.05) (Fig. 5A and B). Cardiac expression of iNOS, MCP-1, phospho-IκBα, phospho-p65 and HO-1 protein in the sham + Bai group did not differ from those of the sham group.

### 3.4. Effects of baicalein on superoxide anion production

The levels of superoxide anion production in left ventricular myocardium 6 h after LPS administration were significantly elevated compared with the sham group. Post-treatment with

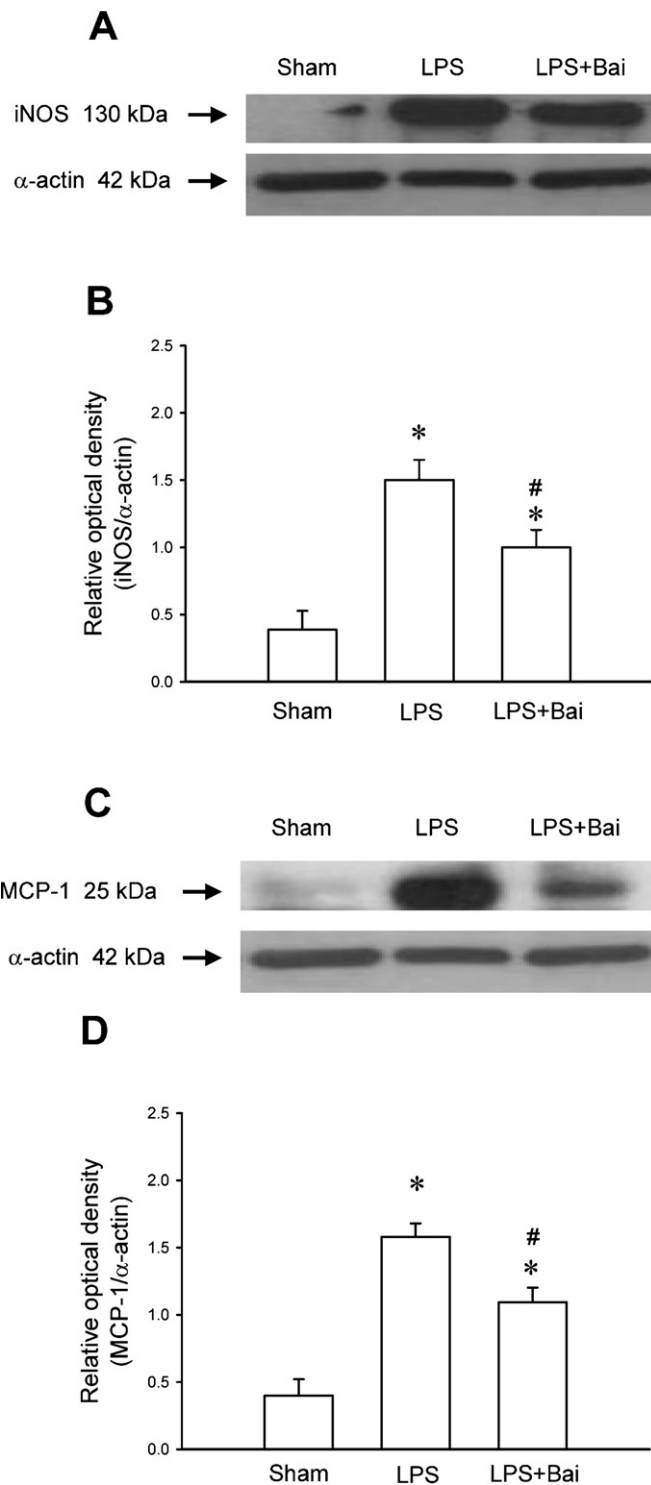


**Fig. 2.** Effects of post-treatment with baicalein on cardiac contractile dysfunction caused by LPS. A: left ventricular developed pressure (LVDP); B and C: +dP/dt and -dP/dt in hearts 6 h after being subjected to LPS administration. Baicalein (Bai) 10 mg/kg was given 30 min after LPS 10 mg/kg injection. Data are given as mean  $\pm$  SEM. \* $P < 0.05$  vs. the sham group, # $P < 0.05$  vs. the LPS group,  $n = 6$ .

baicalein significantly inhibited this increase in superoxide anion production compared with that of the LPS group (sham:  $23.5 \pm 3.9$ ; LPS:  $58.6 \pm 5.2$ ; LPS+Bai:  $35.4 \pm 5.1$  CPS/mg tissue weight,  $n = 6$ ) ( $P < 0.05$ ) (Fig. 5C). The level of superoxide anion in the sham+Bai group ( $19.5 \pm 2.8$  CPS/mg tissue weight,  $n = 6$ ) did not differ significantly from that of the sham group ( $P > 0.05$ ).

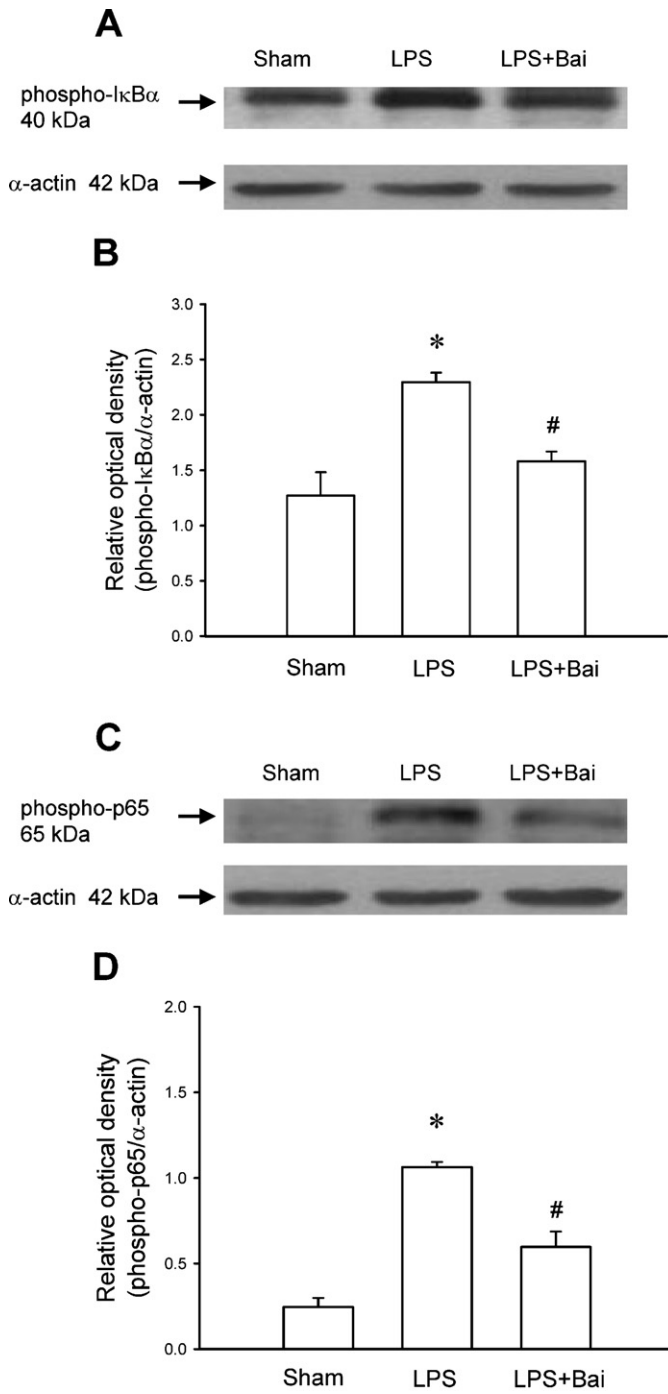
### 3.5. Effects of baicalein on cardiac caspase-3 activity

Six hours after administration of LPS, the caspase-3 activity in the LPS group was significantly higher than that of the sham group (sham:  $326.3 \pm 17.2$ ; LPS:  $546.5 \pm 16.0$  units/ $\mu$ g protein) ( $P < 0.05$ ) (Fig. 6). Post-treatment with baicalein (LPS+Bai:  $418.4 \pm 23.7$  units/ $\mu$ g protein) significantly reduced the induction of caspase-3 activity by LPS ( $P < 0.05$ ), but it remained



**Fig. 3.** Effects of post-treatment with baicalein on (A, B) iNOS and (C, D) MCP-1 protein expression in left ventricular myocardium of rats 6 h after being subjected to LPS administration. Representative Western blots are shown. Baicalein (Bai) 10 mg/kg was given 30 min after LPS 10 mg/kg injection. Data are given as mean  $\pm$  SEM. \* $P < 0.05$  vs. the sham group, # $P < 0.05$  vs. the LPS group,  $n = 6$ .

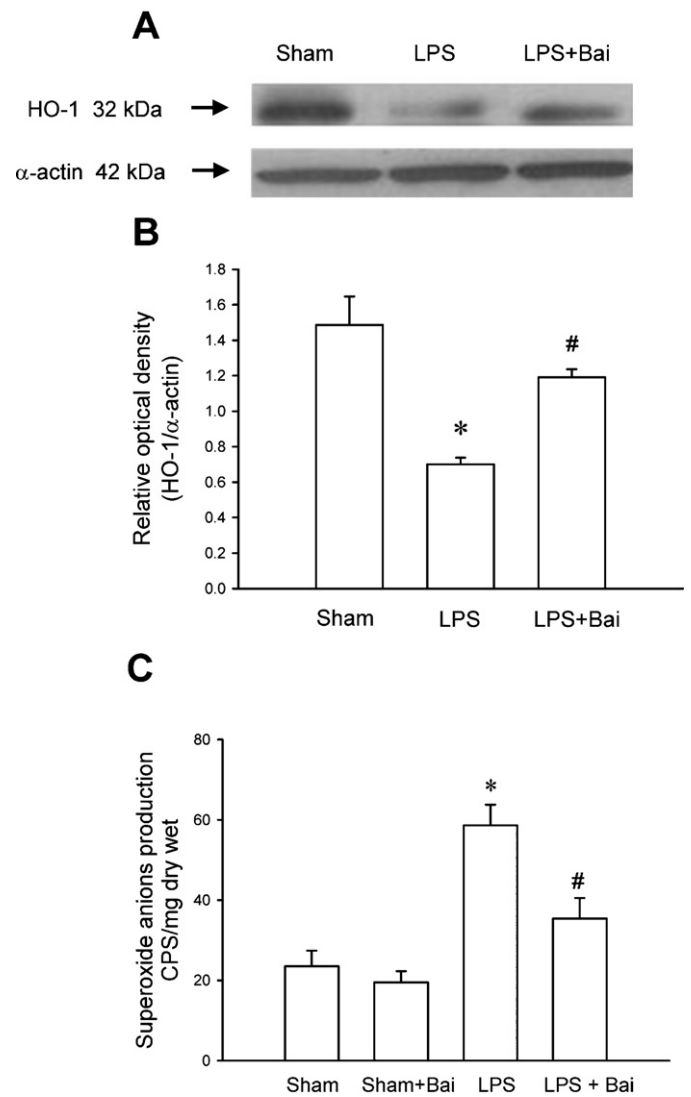
significantly higher than that of the sham group ( $P < 0.05$ ). The level of caspase-3 activity in the sham+Bai group ( $345.4 \pm 20.6$  units/ $\mu$ g protein) did not differ significantly from that of the sham group. This result indicated that post-treatment with baicalein may attenuate myocardial apoptosis induced by endotoxaemia.



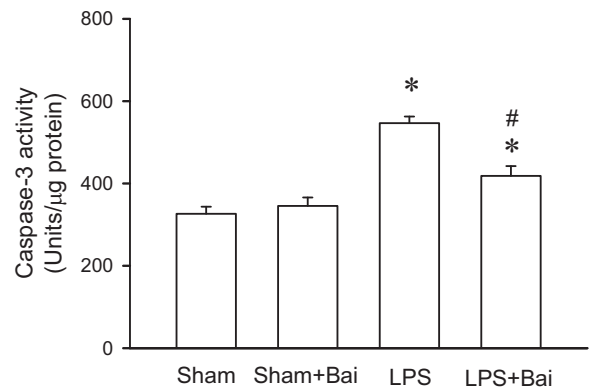
**Fig. 4.** Effects of post-treatment with baicalein on (A, B) phospho-IκBα and (C, D) phospho-p65 protein expression in left ventricular myocardium of rats 6 h after being subjected to LPS administration. Representative Western blots are shown. Baicalein (Bai) 10 mg/kg was given 30 min after LPS 10 mg/kg injection. Data are given as mean ± SEM. \**P* < 0.05 vs. the sham group, #*P* < 0.05 vs. the LPS group, *n* = 6.

#### 4. Discussion

In a previous study, we showed that baicalein improves circulatory failure and the survival rate in septic rats (Cheng et al., 2007). Here, we further investigated the cardioprotective effect of baicalein during endotoxaemia, which may directly contribute to prevention of circulatory failure. Baicalein improved cardiac contractile function and prevented occurrence of septic shock 6 h after administration of LPS, accompanied by sustained tachycardia. An anti-inflammatory effect is involved in cardioprotection



**Fig. 5.** Effects of post-treatment with baicalein on (A, B) heme oxygenase-1 (HO-1) protein expression and (C) superoxide anion production in left ventricular myocardium of rats 6 h after being subjected to LPS administration. Representative Western blots are shown. Baicalein (Bai) 10 mg/kg was given 30 min after LPS 10 mg/kg injection. Data are given as mean ± SEM. CPS: counts per second, \**P* < 0.05 vs. the sham group, #*P* < 0.05 vs. the LPS group, *n* = 6.



**Fig. 6.** The effect of post-treatment with baicalein on caspase-3 activity in left ventricular myocardium of rats 6 h after being subjected to LPS administration. Baicalein (Bai) 10 mg/kg was given 30 min after LPS 10 mg/kg injection. Data are given as mean ± SEM. \**P* < 0.05 vs. the sham group, #*P* < 0.05 vs. the LPS group, *n* = 6.

by baicalein, evidenced by attenuation of cardiac iNOS and MCP-1 protein expression and suppression of cellular NF- $\kappa$ B activation. Induction of cardiac HO-1 production and the anti-apoptotic effects of baicalein may also contribute to prevention of myocardial depression. However, LPS challenge caused complex and serious inflammatory responses. Rats were treated with baicalein 30 min after LPS challenge (i.e., post-treatment) to evaluate the therapeutic effect: the inflammatory responses had been initiated before baicalein treatment. Therefore, it was difficult to reverse totally the LPS-induced effects, but partial reversal was achieved.

In sepsis, heart rate and cardiac output are increased, seemingly to compensate for a general vasodilatation and to maintain blood pressure (Bradley et al., 1945). In this study, baicalein improved cardiac contractile function and maintained blood pressure at a high level in septic rats, accompanied by a lasting tachycardia. An increase in heart rate can be a compensatory effect to maintain blood pressure and cardiac output to improve perfusion to organs and prevent multiple organ failure at late-stage sepsis. Baicalein alone (sham + Bai group) did not elicit this increase in heart rate. This indicates that baicalein preserved cardiac function, thus maintaining circulatory function to the late phase of endotoxaemia. However, the possibility that a sustained rise in heart rate is a potential side effect of baicalein in sepsis cannot be ruled out.

Sepsis leads to the expression of iNOS, which produces high levels of NO, in the myocardium (Preiser et al., 2001; Khadour et al., 2002). This is responsible for direct effects on vascular tone, depression of mitochondrial respiration and further release of pro-inflammatory cytokines, leading to myocardial depression (Rudiger and Singer, 2007). NO reacts with superoxide anion to generate a cytotoxic product, peroxynitrite (Pacher et al., 2007), which also contributes to myocardial dysfunction. In our previous study, baicalein suppressed iNOS expression in aorta and plasma levels of NO metabolites in sepsis (Cheng et al., 2007). In the present study, we also showed that baicalein suppressed iNOS induction by LPS in cardiac tissues (Fig. 3A and B), which contributed to improvement of the LPS-induced myocardial dysfunction by baicalein.

The innate immune response is activated in sepsis (O'Brien et al., 2007). A systemic response to infection brought about by various inflammatory mediators, such as cytokines and chemokines, leads to the infiltration of specific leukocyte populations including neutrophils and monocytes into host tissues. MCP-1, a prototype CC chemokine, is a potent chemoattractant and a regulatory mediator involved in a variety of inflammatory diseases (Luster, 1998). MCP-1 expression is regulated at the transcriptional level by stimulatory agents such as TNF- $\alpha$ , IFN- $\gamma$ , platelet-derived growth factor and stress factors (Melgarejo et al., 2009). Recently, anti-MCP-1 treatment has been proposed to be of potential therapeutic value in the treatment of sepsis and endotoxaemia (Ramnath et al., 2008). In the present study, we demonstrated for the first time that baicalein suppresses MCP-1 expression (Fig. 3C and D), which could reduce the influx of macrophages into tissues and alleviate the inflammatory responses during sepsis.

NF- $\kappa$ B is clearly one of the most important regulators of pro-inflammatory gene expression. Triggering through toll-like receptors by bacterial ligands, e.g., LPS, initiates signalling cascades that result in the activation of NF- $\kappa$ B, which drives transcription of a range of important pro-inflammatory cytokines and chemokine genes, such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 and iNOS. In fact, NF- $\kappa$ B can be rapidly activated by many pathogenic stimuli, including TNF- $\alpha$  and IL-1 (Xie et al., 1994; Sriskandan and Altmann, 2008). Post-treatment with baicalein attenuated the LPS-induced NF- $\kappa$ B activation in myocardium evidenced by suppression of phospho-I $\kappa$ B $\alpha$  and phospho-p65 levels. This contributed to the reduction of iNOS and MCP-1 expression, leading to amelioration of cardiac inflammation.

In addition, ROS have been shown to be involved in NF- $\kappa$ B activation (Schreck et al., 1991; Adcock et al., 1994). Anti-oxidants have been reported to possess beneficial effects in sepsis (Berger and Chioloro, 2007). LPS-induced cardiac dysfunction may be in part the result of ROS production induced by inflammatory mediators such as TNF- $\alpha$  (Rudiger and Singer, 2007). Elevated oxidative stress can induce HO-1 protein expression, which can produce protective effects in various diseases (Takahashi et al., 2004). The porphyrin ring of haem can be broken by HO-1 to yield equimolar amounts of biliverdin IX $\alpha$ , free iron (Fe<sup>2+</sup>) and carbon monoxide (CO). Iron, an oxidant, is directly sequestered and inactivated by co-induced ferritin (Harrison and Arosio, 1996). Biliverdin IX $\alpha$  is rapidly converted by biliverdin reductase to bilirubin IX $\alpha$ , which has been reported to be an anti-oxidant (Stocker et al., 1987). CO can suppress inflammatory responses and apoptosis (Otterbein et al., 2000). Beneficial effects of the HO-1/CO system in patients with severe sepsis/septic shock have recently been reported (Takaki et al., 2010). It has been suggested that manipulation of the HO-1 pathway may represent a future therapeutic strategy to counteract oxidative stress in endotoxaemia (Tamion et al., 2010). In the present study, baicalein was able to induce cardiac HO-1 expression at the late stages of sepsis, which may attenuate free radical formation and contribute to its anti-inflammatory effect.

Inhibition of apoptosis in animal models of sepsis has resulted in improved cardiac function (Fauvel et al., 2001; Nevière et al., 2001; Buerke et al., 2008). The cysteine proteases comprising the caspase family have been considered one of the major executioners of programmed cell death or apoptosis (Yaginuma et al., 2001). Caspase-3 is involved in a wide variety of functional responses in ventricular myocytes including a negative inotropic response (Laugwitz et al., 2001). Caspase-3 activation directly targets the three main components of the myofilament machinery, namely,  $\alpha$ -actin,  $\alpha$ -actinin and troponin T, and induces the breakdown of myofibrillar proteins, leading to a decrease in ATPase activity and force development (Communal et al., 2002). Apoptosis is potentially triggered by cytokines, TNF- $\alpha$ , ROS and NO released by infiltrating polymorphonuclear leukocytes or macrophages (Zhao and Vinten-Johansen, 2002). Baicalein reduces plasma levels of TNF- $\alpha$  (Cheng et al., 2007), attenuates superoxide anion formation and suppresses iNOS and MCP-1 expression in cardiac tissue, which would all lead to a decrease in LPS-induced apoptosis of cardiomyocytes.

## 5. Conclusions

Baicalein, an active flavone from *Scutellaria baicalensis* Georgi, improves cardiac contractile function by means of its anti-inflammatory effects. Its anti-oxidant and anti-apoptotic effects may also contribute to the cardioprotective effects of baicalein.

## Acknowledgements

This work was supported by research grants from the National Science Council (NSC 98-2815-C-016-010-B), Ministry of National Defense (DOD97-08-03), and Armed Forces Tao-Yuan General Hospital (AFTYGH-9804), Taiwan.

## References

- Adcock, I.M., Brown, C.R., Kwon, O., Barnes, P.J., 1994. Oxidative stress induces NF kappa B DNA binding and inducible NOS mRNA in human epithelial cells. *Biochemical and Biophysical Research Communications* 199, 1518–1524.
- Ayala, A., Peri, M., Venet, F., Lomas-Neira, J., Swan, R., Chung, C.S., 2008. Apoptosis in sepsis: mechanisms, clinical impact and potential therapeutic targets. *Current Pharmaceutical Design* 14, 1853–1859.
- Berger, M.M., Chioloro, R.L., 2007. Antioxidant supplementation in sepsis and systemic inflammatory response syndrome. *Critical Care Medicine* 35, S584–S590.

- Bochorakova, H., Paulova, H., Slanina, J., Musil, P., Taborska, E., 2003. Main flavonoids in the root of *Scutellaria baicalensis* cultivated in Europe and their comparative antiradical properties. *Phytotherapy Research* 17, 640–644.
- Bradley, S.E., Chasis, H., Goldring, W., Smith, H.W., 1945. Hemodynamic alterations in normotensive and hypertensive subjects during the pyrogenic reaction. *Journal of Clinical Investigation* 24, 749–758.
- Buerke, U., Carter, J.M., Schlitt, A., Russ, M., Schmidt, H., Sibelius, U., Grandel, U., Grimminger, F., Seeger, W., Mueller-Werdan, U., Werdan, K., Buerke, M., 2008. Apoptosis contributes to septic cardiomyopathy and is improved by simvastatin therapy. *Shock* 29, 497–503.
- Chen, S.Y., Hsiao, G., Hwang, H.R., Cheng, P.Y., Lee, Y.M., 2006. Tetramethylpyrazine induces heme oxygenase-1 expression and attenuates myocardial ischemia/reperfusion injury in rats. *Journal of Biomedical Science* 13, 731–740.
- Cheng, P.Y., Lee, Y.M., Wu, Y.S., Chang, T.W., Jin, J.S., Yen, M.H., 2007. Protective effect of baicalin against endotoxic shock in rats in vivo and in vitro. *Biochemical Pharmacology* 73, 793–804.
- Communal, C., Sumandea, M., de Tombe, P., Narula, J., Solaro, R.J., Hajjar, R.J., 2002. Functional consequences of caspase activation in cardiac myocytes. *Proceedings of the National Academy of Sciences of the United States of America* 99, 6252–6256.
- Fauvel, H., Marchetti, P., Chopin, C., Formstecher, P., Nevière, R., 2001. Differential effects of caspase inhibitors on endotoxin-induced myocardial dysfunction and heart apoptosis. *American Journal of Physiology: Heart and Circulatory Physiology* 280, H1608–1614.
- Harrison, P.M., Arosio, P., 1996. The ferritins: molecular properties, iron storage function and cellular regulation. *Biochimica et Biophysica Acta* 1275, 161–203.
- Khadour, F.H., Panas, D., Ferdinandy, P., Schulz, C., Csont, T., Lalu, M.M., Wildhirt, S.M., Schulz, R., 2002. Enhanced NO and superoxide generation in dysfunctional hearts from endotoxemic rats. *American Journal of Physiology: Heart and Circulatory Physiology* 283, H1108–H1115.
- Kimura, Y., Yokoi, K., Matsushita, N., Okuda, H., 1997. Effects of flavonoids isolated from *Scutellariae radix* on the production of tissue-type plasminogen activator and plasminogen activator inhibitor-1 induced by thrombin and thrombin receptor agonist peptide in cultured human umbilical vein endothelial cells. *Journal of Pharmacy and Pharmacology* 49, 816–822.
- Laugwitz, K.L., Moretti, A., Weig, H.J., Gillitzer, A., Pinkernell, K., Ott, T., Pragst, I., Städele, C., Seyfarth, M., Schömig, A., Ungerer, M., 2001. Blocking caspase-activated apoptosis improves contractility in failing myocardium. *Human Gene Therapy* 12, 2051–2063.
- Li-Weber, M., 2009. New therapeutic aspects of flavones: the anticancer properties of *Scutellaria* and its main active constituents wogonin, baicalin and baicalin. *Cancer Treatment Reviews* 35, 57–68.
- Luster, A.D., 1998. Chemokines-chemotactic cytokines that mediate inflammation. *New England Journal of Medicine* 338, 436–445.
- Mathiak, G., Szewczyk, D., Abdullah, F., Ovadia, P., Feuerstein, G., Rabinovici, R., 2000. An improved clinically relevant sepsis model in the conscious rat. *Critical Care Medicine* 28, 1947–1952.
- Melgarejo, E., Medina, M.A., Sanchez-Jimenez, F., Urdiales, J.L., 2009. Monocyte chemoattractant protein-1: a key mediator in inflammatory processes. *International Journal of Biochemistry and Cell Biology* 41, 998–1001.
- Merx, M.W., Weber, C., 2007. Sepsis and the heart. *Circulation* 116, 793–802.
- Nevière, R., Fauvel, H., Chopin, C., Formstecher, P., Marchetti, P., 2001. Caspase inhibition prevents cardiac dysfunction and heart apoptosis in a rat model of sepsis. *American Journal of Respiratory and Critical Care Medicine* 163, 218–225.
- O'Brien Jr., J.M., Ali, N.A., Abernethy, S.K., Abraham, E., 2007. Sepsis. *American Journal of Medicine* 120, 1012–1022.
- Otterbein, L.E., Bach, F.H., Alam, J., Soares, M., Tao, L.H., Wysk, M., Davis, R.J., Flavell, R.A., Choi, A.M., 2000. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nature Medicine* 6, 422–428.
- Pacher, P., Beckman, J.S., Liaudet, L., 2007. Nitric oxide and peroxynitrite in health and disease. *Physiological Reviews* 87, 315–424.
- Preiser, J.C., Zhang, H., Vray, B., Hrabak, A., Vincent, J.L., 2001. Time course of inducible nitric oxide synthase activity following endotoxin administration in dogs. *Nitric Oxide* 5, 208–211.
- Ramnath, R.D., Ng, S.W., Guglielmotti, A., Bhatia, M., 2008. Role of MCP-1 in endotoxemia and sepsis. *International Immunopharmacology* 8, 810–818.
- Rudiger, A., Singer, M., 2007. Mechanisms of sepsis-induced cardiac dysfunction. *Critical Care Medicine* 35, 1599–1608.
- Schreck, R., Rieber, P., Baeuerle, P.A., 1991. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *EMBO Journal* 10, 2247–2258.
- Shen, Y.C., Chiou, W.F., Chou, Y.C., Chen, C.F., 2003. Mechanisms in mediating the anti-inflammatory effects of baicalin and baicalein in human leukocytes. *European Journal of Pharmacology* 465, 171–181.
- Shih, C.C., Chen, S.J., Chen, A., Wu, J.Y., Liaw, W.J., Wu, C.C., 2008. Therapeutic effects of hypertonic saline on peritonitis-induced septic shock with multiple organ dysfunction syndrome in rats. *Critical Care Medicine* 36, 1864–1872.
- Sriskandan, S., Altmann, D.M., 2008. The immunology of sepsis. *Journal of Pathology* 214, 211–223.
- Stocker, R., Yamamoto, Y., McDonagh, A.F., Glazer, A.N., Ames, B.N., 1987. Bilirubin is an antioxidant of possible physiological importance. *Science* 235, 1043–1046.
- Takahashi, T., Morita, K., Akagi, R., Sassa, S., 2004. Heme Oxygenase-1: a novel therapeutic target in oxidative tissue injuries. *Current Medicinal Chemistry* 11, 1545–1561.
- Takaki, S., Takeyama, N., Kajita, Y., Yabuki, T., Noguchi, H., Miki, Y., Inoue, Y., Nakagawa, T., Noguchi, H., 2010. Beneficial effects of the heme oxygenase-1/carbon monoxide system in patients with severe sepsis/septic shock. *Intensive Care Medicine* 36, 42–48.
- Tamion, F., Bauer, F., Richard, V., Laude, K., Renet, S., Slama, M., Thuillez, C., 2010. Myocardial dysfunction in early state endotoxemia role of heme-oxygenase-1. *Journal of Surgical Research* 158, 94–103.
- Wakabayashi, I., 1999. Inhibitory effects of baicalin and wogonin on lipopolysaccharide-induced nitric oxide production in macrophages. *Pharmacology and Toxicology* 84, 288–291.
- Ward, P.A., 2008. Sepsis, apoptosis and complement. *Biochemical Pharmacology* 76, 1383–1388.
- Wu, J.A., Attele, A.S., Zhang, L., Yuan, C.S., 2001. Anti-HIV activity of medicinal herbs: usage and potential development. *American Journal of Chinese Medicine* 29, 69–81.
- Xie, Q.W., Kashiwabara, Y., Nathan, C., 1994. Role of transcription factor NF- $\kappa$ B/Rel in induction of nitric oxide synthase. *Journal of Biological Chemistry* 269, 4705–4708.
- Yaginuma, H., Sato, N., Homma, S., Oppenheim, R.W., 2001. Roles of caspases in the programmed cell death of motoneurons in vivo. *Archives of Histology and Cytology* 64, 461–474.
- Yang, F.L., Li, C.H., Hsu, B.G., Tsai, N.M., Lin, S.Z., Harn, H.J., Chen, H.I., Liao, K.W., Lee, R.P., 2007. The reduction of tumor necrosis factor- $\alpha$  release and tissue damage by pentobarbital in the experimental endotoxemia model. *Shock* 28, 309–316.
- Zanotti-Cavazzoni, S.L., Hollenberg, S.M., 2009. Cardiac dysfunction in severe sepsis and septic shock. *Current Opinion in Critical Care* 15, 392–397.
- Zhang, D.Y., Wu, J., Ye, F., Xue, L., Jiang, S., Yi, J., Zhang, W., Wei, H., Sung, M., Wang, W., Li, X., 2003. Inhibition of cancer cell proliferation and prostaglandin E<sub>2</sub> synthesis by *Scutellaria baicalensis*. *Cancer Research* 63, 4037–4043.
- Zhao, Z.Q., Vinten-Johansen, J., 2002. Myocardial apoptosis and ischemic preconditioning. *Cardiovascular Research* 55, 438–455.