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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

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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.

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.

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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-α, interferon (IFN)-γ, 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-α, 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 ± 18 °C and humidity of 55 ± 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 (P23ID, 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 (Δ 50 ± 7.8 mg/dL) compared with basal levels (102 ± 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_2 and 5% CO_2 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 $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$ 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 °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-IκBα (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-IκBα, phospho-p65, MCP-1 or HO-1 to α-actin were calculated.