

## Full-length article

## Effects of neutral sulfate berberine on LPS-induced cardiomyocyte TNF- $\alpha$ secretion, abnormal calcium cycling, and cardiac dysfunction in rats<sup>1</sup>

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### Key words

lipopolysaccharide; berberine; cardiomyocytes; tumor necrosis factor; intracellular calcium; heart dysfunction

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### Abstract

**Aim:** To evaluate the effect of neutral sulfate berberine on cardiac function, tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) release, and intracellular calcium concentration ( $[Ca^{2+}]_i$ ) in cardiomyocytes exposed to lipopolysaccharide (LPS). **Methods:** Primary cultured rat cardiomyocytes were prepared from ventricles of 3–4-day old Sprague-Dawley rats. TNF- $\alpha$  concentrations in cell-conditioned media were measured by using a Quantikine enzyme-linked immunosorbent assay kit, and cardiomyocyte  $[Ca^{2+}]_i$  was measured by using Fura-2/AM. The isolated rat hearts were perfused in the Langendorff mode. **Results:** LPS at doses of 1, 5, 10, and 20  $\mu\text{g/mL}$  markedly stimulated TNF- $\alpha$  secretion from cardiomyocytes, and neutral sulfate berberine inhibited LPS-induced TNF- $\alpha$  production. Intracellular calcium concentration was significantly decreased after LPS stimulation for 1 h, and increased 2 h after LPS treatment. Pretreatment with neutral sulfate berberine reversed the LPS-induced  $[Ca^{2+}]_i$  alterations, although neutral sulfate berberine did not inhibit a rapid increase in cardiomyocyte  $[Ca^{2+}]_i$  induced by LPS. Perfusion of isolated hearts with LPS (100  $\mu\text{g/mL}$ ) for 20 min resulted in significantly impaired cardiac performance at 120 min after LPS challenge: the maximal rate of left ventricular pressure rise and fall ( $\pm dp/dt_{\text{max}}$ ) decreased compared with the control. In contrast,  $\pm dp/dt_{\text{max}}$  at 120 min in hearts perfused with neutral sulfate berberine (1  $\mu\text{mol/L}$ ) for 10 min followed by 20 min LPS (100  $\mu\text{g/mL}$ ) was greater than the corresponding value in the LPS group. **Conclusion:** Neutral sulfate berberine inhibits LPS-stimulated myocardial TNF- $\alpha$  production, impairs calcium cycling, and improves LPS-induced contractile dysfunction in intact heart.

### Introduction

Myocardial depression is a common complication in patients with septic shock, and 40% of patients with sepsis develop cardiac dysfunction characterized by biventricular dilatation, decreased ejection fraction, reduced response to fluid resuscitation, and altered cardiac index<sup>[1]</sup>. Parker *et al* reported that these cardiovascular abnormalities were reversible in surviving patients, but not in non-survivors, demonstrating that myocardial depression significantly contributed to the mortality of patients with septic shock<sup>[2]</sup>. It is generally believed that endotoxin, a lipopolysaccharide (LPS) component of the outer membrane of Gram-negative bacteria,

was a major mediator of the cardiovascular dysfunction during sepsis. Administration of LPS to healthy humans could induce myocardial dysfunction, a phenomenon that has been simulated in experimental animal models<sup>[3,4]</sup>. Evidently, it was very important to develop a potential therapeutic agent for LPS-induced heart failure.

Berberine, an isoquinoline derivative alkaloid, is a major component of *Rhizoma coptidis* (Huanglian), a herb used in traditional Chinese medicine. Berberine has been found to have multiple pharmacological activities, including anti-diarrheal, anti-tumor and anti-inflammatory effects, and vasorelaxant and antiproliferative properties<sup>[5–7]</sup>. Some studies have demonstrated that berberine has wide cardiovascu-

lar effects. Chiou *et al* reported that berberine induced endothelium-independent vascular relaxation via blocking the release of  $\text{Ca}^{2+}$  from internal stores<sup>[8]</sup>. Recently, the efficacy and safety of berberine for the treatment of chronic heart failure was assessed, and it was demonstrated that treatment with berberine increased left ventricular ejection fraction and exercise capacity, decreased the frequency and complexity of ventricular premature complexes, improved quality of life and decreased mortality in patients with congestive heart failure<sup>[9]</sup>. However, it is still unclear whether berberine has a prophylactic or therapeutic effect on LPS-induced myocardial dysfunction.

Although the mechanism of LPS-induced myocardial dysfunction is not completely understood, it has recently been established that upregulation of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) levels and abnormal calcium cycling in cardiomyocytes were involved in LPS-induced myocardial dysfunction<sup>[10]</sup>. Therefore, in the present study we examined the effect of neutral sulfate berberine on TNF- $\alpha$  release from and intracellular calcium concentration ( $[\text{Ca}^{2+}]_i$ ) in cardiomyocytes exposed to LPS. In order to verify the direct action of neutral sulfate berberine on LPS-induced cardiac dysfunction, changes in cardiac function of isolated intact rat hearts exposed to LPS and/or neutral sulfate berberine were also determined.

## Materials and methods

**Cell culture preparation** Neonatal Sprague-Dawley rats were purchased from the Guangdong Province Center for Laboratory Animals. All animals received humane care. Primary cultured rat cardiomyocytes were prepared from the ventricles of 3–4-day-old Sprague-Dawley rats according to the published procedure<sup>[11]</sup>. Briefly, the neonatal rats were killed and their hearts were excised. The ventricles were minced and digested in a 0.125% trypsin solution [0.2 g/L ethylenediamine tetraacetic acid (EDTA),  $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free Hanks' buffered salt solution]. Then the cells were collected and resuspended in Dulbecco's modified Eagles' medium (DMEM)-F12 (Gibco-BRL) supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100  $\mu\text{g}/\text{mL}$  streptomycin. After 2-h incubation at 37 °C in 5% carbon dioxide and 95% oxygen in a humidified incubator (Thermo Forma, USA), most of the contaminating fibroblasts were attached to the culture flask, and the cardiomyocytes were prepared from the unattached cells. Cardiomyocyte viability was examined using trypan blue exclusion, and the cell suspension was adjusted to a density of  $5 \times 10^5$  cells/mL and cultured for 48 h, during which time the attached cardiomyocytes began to contract

spontaneously. The cells were used in the experiments on d 4 of culture.

**Measurement of TNF- $\alpha$**  Neonatal cardiomyocytes plated at a density of  $5 \times 10^5$  cells/mL were incubated with LPS (O55: B5, Sigma) at concentrations of 1, 5, 10, and 20  $\mu\text{g}/\text{mL}$  for 6 h. In another experiment, the neonatal rat cardiomyocytes were treated with 10  $\mu\text{g}/\text{mL}$  LPS alone or in combination with 1, 2, or 4  $\mu\text{mol}/\text{L}$  neutral sulfate berberine (Sigma) for 6 h. TNF- $\alpha$  concentrations in cell-conditioned media were measured by using a Quantikine enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, USA) for rat TNF- $\alpha$ , according to the manufacturer's instructions.

**Fura-2 acetoxymethyl ester loading and measurement of  $[\text{Ca}^{2+}]_i$**  Neonatal rat cardiomyocytes ( $5 \times 10^5$  cells/mL) were loaded with Fura-2/acetoxymethyl (AM) ester (Sigma) at a final concentration of 5  $\mu\text{mol}/\text{L}$  for 30 min in the dark, then the cells were washed with Hanks' solution 3 times to remove the extracellular Fura-2/AM. Based on methods described previously<sup>[12]</sup>, Fura-2 fluorescence was monitored with a Shimadzu RF-5000 fluorescence spectrophotometer (Japan) with excitation at 340 and 380 nm and emission at 510 nm, which was performed in a water-jacketed cuvette at 37 °C. Basal and agent-induced changes in the excitation ratio of Fura-2 (340/380 nm) were measured for approximately 10 min after the addition of 50  $\mu\text{mol}/\text{mL}$  KCl, 5  $\mu\text{g}/\text{mL}$  isoproterenol, 200  $\mu\text{g}/\text{mL}$  LPS and 1 or 2 nmol/mL neutral sulfate berberine. Maximum ( $R_{\text{max}}$ ) and minimum ( $R_{\text{min}}$ ) fluorescence values were determined by adding 50  $\mu\text{L}$  of Triton X-100 (10%) and 100  $\mu\text{mol}/\text{L}$  ethyleneglycol-*bis*( $\beta$ -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), respectively. The cardiomyocyte  $[\text{Ca}^{2+}]_i$  was then calculated. In some experiments, the neonatal rat cardiomyocytes were treated with 200  $\mu\text{g}/\text{mL}$  LPS and/or 2 nmol/mL neutral sulfate berberine for 1–2 h, after which the cardiomyocyte  $[\text{Ca}^{2+}]_i$  was examined using Fura-2/AM.

**Perfused heart preparation** Based on a method described by Stamm *et al*<sup>[10]</sup>, isolated rat hearts were perfused in Langendorff mode. In brief, male Sprague-Dawley rats weighing 200 $\pm$ 20 g, obtained from the Guangdong Province Center for Laboratory Animals were anticoagulated with heparin (200 U, iv) and anesthetized with intraperitoneal administration of sodium pentobarbital (35 mg/kg). The hearts were rapidly excised and arrested in chilled normal saline. After the aorta was cannulated, hearts were perfused retrogradely on a Langendorff apparatus with filtered Krebs-Henseleit buffer at 37 °C and a constant pressure of 80  $\text{cmH}_2\text{O}$ . The Krebs-Henseleit solution was composed of (mmol/L): NaCl 118, KCl 4.7,  $\text{KH}_2\text{PO}_4$  1.2,  $\text{NaHCO}_3$  25,  $\text{CaCl}_2$  2.5,  $\text{MgSO}_4$  1.2, and glucose 11. The solution was

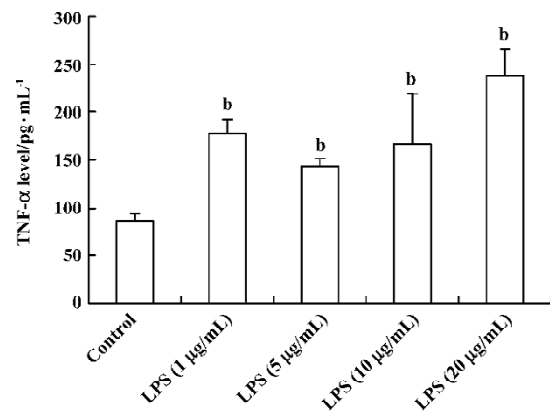
equilibrated with a 5% CO<sub>2</sub> and 95% O<sub>2</sub> gas mixture (pH 7.35–7.45). A small latex balloon filled with fluid was inserted in the left ventricle and connected to a pressure transducer, then the maximal rate of left ventricular pressure rise and fall ( $\pm dp/dt_{max}$ ) and heart rate were measured by using a Biolab-410 physiological function system (China) throughout the experiment. The hearts were divided into 4 groups: the hearts in the control group were perfused with Krebs-Henseleit buffer; the hearts in the LPS group were perfused with 100  $\mu\text{g}/\text{mL}$  LPS for 20 min followed by Krebs-Henseleit buffer; in the neutral sulfate berberine plus LPS group, the hearts were infused with 1  $\mu\text{mol}/\text{L}$  berberine for 10 min followed by 100  $\mu\text{g}/\text{mL}$  LPS for 20 min, and Krebs-Henseleit buffer to the end of the experiment; and the hearts in the berberine group were perfused with 1  $\mu\text{mol}/\text{L}$  berberine for 10 min followed by Krebs-Henseleit buffer to the end of the experiment.

**Statistical analysis** Results are expressed as mean $\pm$ SD. Statistical analysis was performed using the SPSS software package (version 8.0). Paired *t*-tests, Student's *t*-test and one-way ANOVA followed by the Student-Newman-Keuls test were used to determine statistical significance. The difference between means was considered statistically significant when *P* was less than 0.05.

## Results

**Influence of LPS and neutral sulfate berberine on TNF- $\alpha$  secretion in neonatal rat cardiomyocytes** LPS stimulated TNF- $\alpha$  release from cardiomyocytes. In the presence of 10% fetal bovine serum, untreated cardiomyocytes produced a basal level of 86.6 $\pm$ 7.3 pg/mL of TNF- $\alpha$ . Treatment of cardiomyocytes for 6 h with LPS at concentrations of 1, 5, 10, and 20  $\mu\text{g}/\text{mL}$  all caused a significant increase in the amount of TNF- $\alpha$  released (Figure 1). In contrast, berberine inhibited LPS-induced TNF- $\alpha$  release from neonatal rat cardiomyocytes in a dose-dependent manner. Berberine at a concentration of 4 nmol/mL almost completely prevented the increase in TNF- $\alpha$  release in cardiomyocytes exposed to 10  $\mu\text{g}/\text{mL}$  LPS (Table 1).

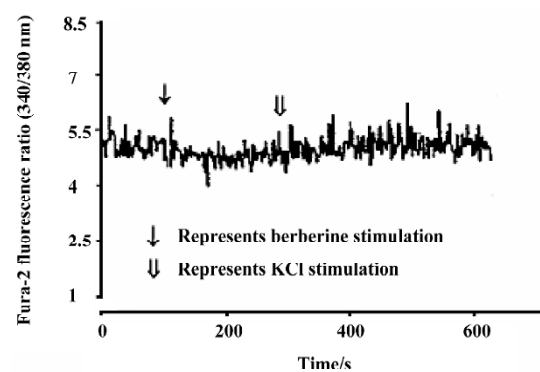
**Effects of neutral sulfate berberine on [Ca<sup>2+</sup>]<sub>i</sub> changes in neonatal rat cardiomyocytes exposed to KCl, isoproterenol, and LPS** Addition of 50 mmol/L KCl caused an increase in the [Ca<sup>2+</sup>]<sub>i</sub> of cardiomyocytes from 70.61 $\pm$ 4.26 nmol/L to 159.63 $\pm$ 11.88 nmol/L (*n*=6, *P*<0.01), and this increase was completely suppressed by pretreatment of the cardiomyocytes with 2  $\mu\text{mol}/\text{L}$  berberine (75.26 $\pm$ 6.06 nmol/L; Figure 2). As shown in Table 2, pretreatment of cardiomyocytes with 2  $\mu\text{mol}/\text{L}$  berberine for 10 min did not suppress the isoproterenol-evoked increase in [Ca<sup>2+</sup>]<sub>i</sub> of cardio-



**Figure 1.** TNF- $\alpha$  secretion from cardiomyocytes stimulated with LPS at different doses. *n*=6. Mean $\pm$ SD. <sup>b</sup>*P*<0.05 vs control.

**Table 1.** Effect of neutral sulfate berberine on TNF- $\alpha$  secretion from cardiomyocytes treated with LPS. *n*=8. Mean $\pm$ SD. <sup>b</sup>*P*<0.05 vs control. <sup>c</sup>*P*<0.05 vs LPS group.

Group	TNF- $\alpha$ (pg/mL)
Control	81.0 $\pm$ 21.9
LPS (10 $\mu\text{g}/\text{mL}$ )	125.5 $\pm$ 21.5 <sup>b</sup>
LPS (10 $\mu\text{g}/\text{mL}$ )+berberine (1 $\mu\text{mol}/\text{L}$ )	112.0 $\pm$ 13.8 <sup>b</sup>
LPS (10 $\mu\text{g}/\text{mL}$ )+berberine (2 $\mu\text{mol}/\text{L}$ )	103.8 $\pm$ 8.0 <sup>c</sup>
LPS (10 $\mu\text{g}/\text{mL}$ )+berberine (4 $\mu\text{mol}/\text{L}$ )	89.7 $\pm$ 18.5 <sup>c</sup>
Berberine (4 nmol/mL)	68.3 $\pm$ 26.4

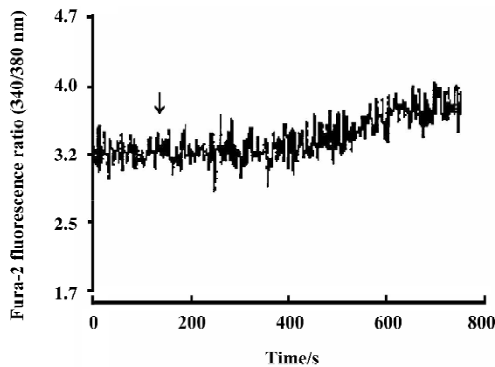


**Figure 2.** Changes in the 340/380 nm ratio of Fura-2 fluorescence intensity in Fura-2-loaded cardiomyocytes after neutral sulfate berberine (2  $\mu\text{mol}/\text{L}$ ) and KCl (50 mmol/L) stimulation.

myocytes. LPS at a concentration of 200  $\mu\text{g}/\text{mL}$  evoked a rapid increase in [Ca<sup>2+</sup>]<sub>i</sub> of cardiomyocytes 10 min after stimulation (Figure 3). Berberine treatment alone for 10 min stimulated an increase in the [Ca<sup>2+</sup>]<sub>i</sub> of cardiomyocytes, but did not inhibit the LPS-induced rapid elevation of the [Ca<sup>2+</sup>]<sub>i</sub> of

**Table 2.** Effects of neutral sulfate berberine (2 μmol/L) pretreatment for 10 min on isoproterenol (ISO, 5 μg/mL)-induced intracellular free calcium concentration changes in cardiomyocytes. Mean±SD. <sup>b</sup>*P*<0.05 vs control. <sup>c</sup>*P*<0.05 vs before ISO stimulation.

Group	<i>n</i>	[Ca <sup>2+</sup> ] <sub>i</sub> (nmol/L)	
		Before ISO stimulation	After ISO stimulation
Control	3	54.78±2.40	83.48±3.04 <sup>c</sup>
Berberine	6	99.95±16.42 <sup>b</sup>	148.02±11.04 <sup>c</sup>



**Figure 3.** Changes in the 340/380 nm ratio of Fura-2 fluorescence intensity in Fura-2-loaded cardiomyocytes after LPS (200 μg/mL) stimulation. ↓ Represents LPS (200 μg/mL) stimulation. Abscissa represents time (s); ordinate represents Fura-2 fluorescence intensity ratio (340/380 nm).

cardiomyocytes (Table 3). However, [Ca<sup>2+</sup>]<sub>i</sub> in cardiomyocytes decreased markedly when the cells were treated with LPS (200 μg/mL) for 1 h, and became significantly elevated 2 h after LPS exposure. Both of these effects were reversed by 2 μmol/L berberine. Berberine treatment for 1 and 2 h produced an elevation in [Ca<sup>2+</sup>]<sub>i</sub> of cardiomyocytes (Table 4, 5).

**Effects of neutral sulfate berberine and LPS on heart rate and ±dp/dt<sub>max</sub> in isolated rat hearts** Infusion with 100 μg/mL LPS for 20 min significantly decreased the heart rate from 256±56 beats/min to 140±104 beats/min at 120 min in isolated rat hearts (*P*<0.05), whereas this effect was not found in control hearts. Infusion with 1 μmol/L berberine for 10 min also reduced the heart rate from 289±35 beats/min to 194±38 beats/min at 120 min (*P*<0.05). Moreover, berberine did not antagonize the LPS-induced drop in heart rate (from 253±56 beats/min to 160 ± 49 beats/min at 120 min). As shown in Tables 6 and 7, perfusion of isolated heart with 100 μg/mL LPS for 20 min resulted in significantly impaired cardiac performance at 120 min after LPS challenge, and the maximal rate of left ventricular pressure rise and fall (±dp/dt<sub>max</sub>) decreased compared with the controls. In contrast, ±dp/dt<sub>max</sub> at 120

**Table 3.** Effects of neutral sulfate berberine (1 μmol/L) treatment for 10 min on LPS (200 μg/mL)-induced intracellular free calcium concentration changes in cardiomyocytes. Cardiomyocytes pretreated with berberine for 10 min were stimulated with LPS. Data represent intracellular free calcium concentrations in cardiomyocytes at 10 min post LPS treatment. Mean±SD. <sup>b</sup>*P*<0.05 vs before LPS (200 μg/mL) stimulation. <sup>c</sup>*P*<0.05 vs control.

Group	<i>n</i>	[Ca <sup>2+</sup> ] <sub>i</sub> (nmol/L)	
		Before LPS stimulation	After LPS stimulation
Control	8	83.1±12.2	200.3±20.6 <sup>b</sup>
Berberine (1 μmol/L)	7	201.7±86.1	244.4±102.8 <sup>b</sup>
Berberine (2 μmol/L)	6	245.1±23.6 <sup>c</sup>	353.2±104.6 <sup>b</sup>

**Table 4.** Changes in intracellular free calcium concentration in cardiomyocytes 1 h after neutral sulfate berberine (2 μmol/L) and LPS (200 μg/mL) treatment. Mean±SD. <sup>c</sup>*P*<0.01 vs control group. <sup>f</sup>*P*<0.01 vs LPS group. <sup>i</sup>*P*<0.01 vs berberine+LPS group.

Group	<i>n</i>	[Ca <sup>2+</sup> ] <sub>i</sub> (nmol/L)
Control	7	75.06±12.80
LPS	7	43.21±7.55 <sup>c</sup>
Berberine+LPS	4	73.76±2.92
Berberine	4	95.74±5.60 <sup>c,f,i</sup>

**Table 5.** Changes in intracellular free calcium concentration in cardiomyocytes 2 h after neutral sulfate berberine (2 μmol/L) and LPS (200 μg/mL) treatment. Mean±SD. <sup>b</sup>*P*<0.05 vs control. <sup>e</sup>*P*<0.05 vs LPS group.

Group	<i>n</i>	[Ca <sup>2+</sup> ] <sub>i</sub> (nmol/L)
Control	6	78.60±27.44
LPS	7	198.60±17.71 <sup>b</sup>
Berberine+LPS	4	139.12±14.90 <sup>e</sup>
Berberine	4	168.15±9.53 <sup>b</sup>

min of hearts perfused with 1 μmol/L berberine for 10 min, followed by 100 μg/mL LPS for 20 min was higher than that for hearts in the LPS group.

**Discussion**

The mechanism of LPS-induced cardiodepression remains controversial. Recently, some evidence has demonstrated that TNF-α contributes to LPS-induced cardiac

**Table 6.** Effects of neutral sulfate berberine (1 μmol/L) and LPS (100 μg/mL) on +dp/dt<sub>max</sub> in isolated rat hearts. Mean±SD. <sup>b</sup>P<0.05 vs control. <sup>c</sup>P<0.05 vs LPS group.

Group	n	+dp/dt <sub>max</sub> (mmHg/s)			
		0 min	40 min	90 min	120 min
Control	4	2067±242	1746±395	1675±213	1582±213
LPS	8	2111±359	1263±722	1140±428	845±340 <sup>b</sup>
Berberine+LPS	8	1767±432	1505±739	1551±489	1418±556 <sup>c</sup>

**Table 7.** Effects of neutral sulfate berberine (1 μmol/L) and LPS (100 μg/mL) on -dp/dt<sub>max</sub> in isolated rat hearts. Mean±SD. <sup>b</sup>P<0.05 vs control. <sup>c</sup>P<0.05 vs LPS group.

Group	n	-dp/dt <sub>max</sub> (mmHg/s)			
		0 min	40 min	90 min	120 min
Control	4	1522±117	1303±168	1173±364	1107±421
LPS	8	1792±423	957±621	777±264	551±192 <sup>b</sup>
Berberine+LPS	8	1426±380	1074±684	952±358	908±314 <sup>c</sup>

dysfunction, although other mediators have been involved in this process<sup>[10,13]</sup>. Kapadia *et al* reported that during endotoxemia, TNF-α gene and protein expression increased in feline myocardium<sup>[14]</sup>. Furthermore, LPS has been found to induce the secretion of TNF-α from cultured adult rat cardiomyocytes via the CD14 signal pathway, LPS has also been shown to trigger apoptosis in rat cardiomyocytes, which can be blocked by pretreatment with the soluble TNF-α fragment, indicating that the cardiodepressant action of LPS may be in part due to TNF-α-induced apoptosis, which decreases the number of working myocardial cells<sup>[15]</sup>. Inhibition of myocardial NF-κB activation in transgenic mice constitutively overexpressing a nondegradable I-κBα in cardiomyocytes can inhibit cardiac TNF-α synthesis and prevent cardiac dysfunction induced by LPS<sup>[4]</sup>. In the present study, we confirmed that LPS stimulated TNF-α secretion from neonatal rat cardiomyocytes and, importantly, we also found that neutral sulfate berberine dose-dependently inhibited TNF-α release from cardiomyocytes exposed to LPS, although the mechanism by which neutral sulfate berberine suppresses LPS-stimulated TNF-α release from cardiomyocytes remains to be further investigated.

There is some evidence that abnormal calcium cycling in cardiomyocytes may be related to LPS-induced cardiac depression, although reports from different laboratories are inconsistent<sup>[16-18]</sup>. Stamm and colleagues demonstrated that recirculating perfusion of isolated rat heart with LPS for 30

min significantly impaired myocardial contractility, which was associated with lower [Ca<sup>2+</sup>]<sub>i</sub> levels and attenuated systolic increases in [Ca<sup>2+</sup>]<sub>i</sub>. They found that these LPS effects were not only mimicked by rat TNF-α but also blocked by anti-TNF-α antibody, suggesting a direct relationship between LPS-induced TNF-α release, abnormal calcium cycling and reduced contractility in intact hearts<sup>[10]</sup>. Thompson *et al* reported that 18-h exposure to LPS stimulated myocardial calcium overload and that dantrolene inhibited calcium overload and improved LPS-induced cardiac dysfunction<sup>[19]</sup>. Therefore, decreases in [Ca<sup>2+</sup>]<sub>i</sub> in the early stage and calcium overload in the later stage after LPS challenge played important roles in LPS-induced myocardial depression. In the present study, we observed that neutral sulfate berberine blocked a KCl-evoked increase in [Ca<sup>2+</sup>]<sub>i</sub>, although neutral sulfate berberine itself induced a rapid rise in [Ca<sup>2+</sup>]<sub>i</sub> in cardiomyocytes. However, neutral sulfate berberine could not inhibit isoproterenol- and LPS-evoked rapid increases in cardiomyocyte [Ca<sup>2+</sup>]<sub>i</sub>. These data indicated that LPS-stimulated rapid increases in [Ca<sup>2+</sup>]<sub>i</sub> might involve multiple mechanisms, including calcium release from the sarcoplasmic reticulum. However, [Ca<sup>2+</sup>]<sub>i</sub> in cardiomyocytes decreased markedly when the cells were treated with LPS for 1 h, and elevated significantly 2 h after LPS exposure, both of which were reversed by neutral sulfate berberine.

In summary, we observed that neutral sulfate berberine not only inhibited TNF-α release, but also prevented abnor-

mal calcium cycling in cardiomyocytes, suggesting that neutral sulfate berberine may improve LPS-induced cardiac dysfunction. To further test this hypothesis, we also examined the effect of neutral sulfate berberine on LPS-induced myocardial depression in isolated intact rat hearts. We found that neutral sulfate berberine itself had a negative effect on frequency in isolated whole rat hearts, and did not reverse the LPS-induced negative impact on frequency, but prevented LPS-induced systolic and diastolic ventricular dysfunction. These findings demonstrated that neutral sulfate berberine inhibited LPS-stimulated TNF- $\alpha$  release and normalized intracellular calcium levels in cardiomyocytes exposed to LPS, and attenuated LPS-induced myocardial depression. Neutral sulfate berberine may be a potential therapeutic agent for the treatment of LPS-induced heart dysfunction, and its *in vivo* effect on heart function during endotoxemia deserves to be further investigated.

## References

- 1 Parrillo JE, Parker MM, Natanson C, Suffredini AF, Danner RL, Cunnion RE, *et al*. Septic shock in humans. Advances in the understanding of pathogenesis, cardiovascular dysfunction, and therapy. *Ann Intern Med* 1990; 113: 227–42.
- 2 Parker MM, McCarthy KE, Ognibene FP, Parrillo JE. Right ventricular dysfunction and dilatation, similar to left ventricular changes, characterize the cardiac depression of septic shock in humans. *Chest* 1990; 97: 126–31.
- 3 Suffredini AF, Fromm RE, Parker MM, Brenner M, Kovacs JA, Wesley RA, *et al*. The cardiovascular response of normal humans to the administration of endotoxin. *N Engl J Med* 1989; 321: 280–7.
- 4 Haudek SB, Spencer E, Bryant DD, White DJ, Maass D, Horton JW, *et al*. Overexpression of cardiac I- $\kappa$ B $\alpha$  prevents endotoxin-induced myocardial dysfunction. *Am J Physiol Heart Circ Physiol* 2001; 280: H962–8.
- 5 Zhang MF, Shen YQ. The antidiarrheal and antiinflammatory effects of berberine. *Acta Pharmacol Sin* 1989; 10: 174–6.
- 6 Ko WH, Yao XQ, Lau CW, Law WI, Chen ZY, Kwok W, *et al*. Vasorelaxant and antiproliferative effects of berberine. *Eur J Pharmacol* 2000; 399: 187–96.
- 7 Kuo CL, Chi CW, Liu TY. The anti-inflammatory potential of berberine *in vitro* and *in vivo*. *Cancer Lett* 2004; 203: 127–37.
- 8 Chiou WF, Chen J, Chen CF. Relaxation of corpus cavernosum and raised intracavernous pressure by berberine in rabbit. *Br J Pharmacol* 1998; 125: 1677–84.
- 9 Zeng XH, Zeng XJ, Li YY. Efficacy and safety of berberine for congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol* 2003; 92: 173–6.
- 10 Stamm C, Cowan DB, Friehs I, Noria S, del Nido PJ, McGwan FX Jr. Rapid endotoxin-induced alterations in myocardial calcium handling: obligatory role of cardiac TNF- $\alpha$ . *Anesthesiology* 2001; 95: 1396–405.
- 11 Liao XD, Tang AH, Chen Q, Jin HJ, Wu CH, Chen LY, *et al*. Role of Ca<sup>2+</sup> signaling in initiation of stretch-induced apoptosis in neonatal heart cells. *Biochem Biophys Res Commun* 2003; 310: 405–11.
- 12 Liu PG, Xu YJ, Hopfner RL, Gopalakrishnan V. Phosphatidic acid increases inositol-1,4,5-trisphosphate and [Ca<sup>2+</sup>]<sub>i</sub> levels in neonatal rat cardiomyocytes. *Biochim Biophys Acta* 1999; 1440: 89–99.
- 13 Kumar A, Thota V, Dee L, Olson J, Uretz E, Parrillo JE. Tumor necrosis factor-alpha and interleukin 1-beta are responsible for *in vitro* myocardial cell depression induced by human septic shock serum. *J Exp Med* 1996; 183: 949–58.
- 14 Kapadia S, Lee J, Torre-Amione G, Birdsall HH, Ma TS, Mann DL. Tumor necrosis factor- $\alpha$  gene and protein expression in adult feline myocardium after endotoxin administration. *J Clin Invest* 1995; 96: 1042–52.
- 15 Comstock KL, Krown KA, Page MT, Martin D, Ho P, Pedraza M, *et al*. LPS-induced TNF- $\alpha$  release from and apoptosis in rat cardiomyocytes: obligatory role for CD14 in mediating the LPS response. *J Mol Cell Cardiol* 1998; 30: 2761–75.
- 16 Rigby SL, Hofmann PA, Zhong J, Adams HR, Rubin LJ. Endotoxemia-induced myocardial dysfunction is not associated with changes in myofilament Ca<sup>2+</sup> responsiveness. *Am J Physiol* 1998; 274: H580–90.
- 17 Tavernier B, Mebazaa A, Mateo P, Sys S, Ventura-Clapier R, Veksler V. Phosphorylation-dependent alteration in myofilament Ca<sup>2+</sup> sensitivity but normal mitochondrial function in septic heart. *Am J Respir Crit Care Med* 2001; 163: 362–7.
- 18 Ziolo MT, Katoh H, Bers DM. Expression of inducible nitric oxide synthase depresses beta-adrenergic-stimulated calcium release from the sarcoplasmic reticulum in intact ventricular myocytes. *Circulation* 2001; 104: 2961–6.
- 19 Thompson M, Kliever A, Maass D, Becker L, White DJ, Bryant D, *et al*. Increased cardiomyocyte intracellular calcium during endotoxin-induced cardiac dysfunction in guinea pigs. *Pediatr Res* 2000; 47: 669–76.