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Original Article

Acupoint Catgut-embedding Therapy Attenuates Lipopolysaccharide-induced Inflammatory and Hypoglycaemic Responses and Mortality in Septic Mice

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Reducing mortality from septic shock and damage to multiple organs from systemic inflammation and endotoxic hypoglycaemia is an unmet medical need. In the current investigation, we evaluated the effects of acupoint catgut-embedding therapy (ACET) on lipopolysaccharide (LPS)-induced septic shock in mice. Four groups of male C57J/B6 mice were examined: (1) sham without LPS, (2) ACET without LPS, (3) sham with LPS, and (4) ACET with LPS. ACET was performed once a week for 2 weeks at the following acupoints: Shui Fen (Ren-9), Qihai (Ren-6), and bilateral Siman (Ki-14). During week

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3, mice received saline or LPS (15 mg/kg intraperitoneally). Biochemical parameters, protein and gene expression levels, and survival rates were analysed after saline or LPS administration. As a result, LPS-induced hypotension was unaffected, but the increases in lactic dehydrogenase, creatinine, and interleukin-17 levels were attenuated by ACET. Strikingly, ACET pretreatment completely prevented mortality in septic mice. At the molecular level, both LPS-induced IκB-α degradation and inflammation were attenuated. Prevention of LPS-induced hypoglycaemia was associated with the regulation of hypothalamic 5' AMP-activated protein kinase and hepatic phosphoenolpyruvate carboxykinase. The synergistic actions of ACET prevented mortality in septic mice. In conclusion, the current investigation revealed the important medicinal value of ACET for the reduction of mortality during sepsis.

Key words: Acupoint catgut-embedding therapy, lipopolysaccharide, sepsis, endotoxic hypoglycemia, inflammation, mortality.

Introduction

Sepsis is considered one of the oldest and most elusive syndromes in medicine [1]. During a microbial infection, an uncontrolled host response can lead to septic shock, multiple organ dysfunction syndrome (MODS), and death. In modern society, in-hospital mortality has approached 30% [2]. Therefore, determining an effective strategy for the intensive care management of sepsis remains a major challenge.

Sepsis caused by Gram-negative bacteria is thought to result from the production of endotoxin, the lipid A component of lipopolysaccharide (LPS). Through binding to Toll-like receptor (TLR) 4, LPS induces the expression of several inflammatory genes via acute NF-κB activation [2, 3]. Later, unregulated LPS induces the production of proinflammatory mediators, which in turn cause systemic inflammation [4]. Severe sepsis with hypotension then leads to septic shock and multiple organ failure [5]. Additionally, LPS can cause hypoglycaemia by modulating the hypothalamic AMP-activated protein kinase cascade to suppress both hepatic phosphoenolpyruvate carboxykinase (PEPCK) expression and hepatic gluconeogenesis via the vagus nerve [6]. Interventions to prevent the above-mentioned symptoms of sepsis are expected to reduce later mortality in septic patients [7-9].

In the field of alternative therapies, nutraceutical based approach by using substance like phycocyanobilin, glycine, and lipoic acid to demonstrate a preventive effects on cell and rodent models of sepsis [10]. On the other hand, the use of acupuncture as a sepsis intervention has been previously examined. For example, enhancing survival rates and re-establishing the migration ability of neutrophils in the cecal ligation and puncture (CLP) model by employing acupuncture have been reported [11]. In terms of the use of effective acupoints and their underlying meridians as an intervention for sepsis, electroacupuncture at the Zusanli (St-36) acupoint on the Stomach meridian has been shown to reduce serum tumor necrosis factor (TNF) levels in the CLP model [12]. Torres-Rosas and colleagues also demonstrated that sciatic nerve activation with electroacupuncture restricts systemic inflammation in mice with polymicrobial peritonitis [13]. Massage of the Tianshu (St-25) and Zusanli (St-36) acupoints on the Yangming Stomach meridian at foot and Stomach meridian, respectively, has been shown to attenuate gut-derived sepsis [14]. Acupuncture at the Yingu (Ki-10) acupoint on the Kidney meridian has also been shown to provide both anti-inflammatory and renoprotective effects on LPS-induced nephritis in rats [15].

Acupoint catgut-embedding therapy (ACET) is a trearemtment derived from acupuncture with an absorbable catgut suture implanted in an acupoint to extend the Ren meridian stimulation of the acupoint. It has been used to treat many disorders, such as obesity, gastrointestinal disease, epilepsy, asthma, and menopause, due to its ease of use, durability, and efficacy, as well as the long interval between each treatment [16]. In addition, Ren meridian is also regarded as a channel of life-force energy(qi) within human fine body during shock resuscitation. Recently, ACET has been shown to be an effective and superior treatment for weight loss compared to traditional acupuncture, such as electroacupuncture and auricular acupuncture, as well as pharmacotherapy and diet control [17]. In a randomized, double-blinded, placebo-controlled study, the anti-obesity effects elicited by stimulation of an acupoint combo including

Qihai (Ren-6), Shui Fen (Ren-9), bilateral Siman (Ki-14), bilateral Shuidao (St-28), and Zusanli (St-36) in abdominally obese women were demonstrated [18]. It was notes that the acupoints used for the clinical anti-obesity study were located on the Ren, Kidney, and stomach meridians.

Considering the initiation of obesity and insulin resistance was linked to metabolic and the gut microbiome, leaky gut, and endotoxemia theory [19, 20]; therefore, the present study aims to explore the preventive effects of ACET with the Ren-6, Ren-9, and Ki-14 acupoint combo in a mouse model of LPS-induced sepsis.

Methods

Animal handling and the ACET procedure

Male C57J/B6 mice (7 weeks of age) were purchased from BioLASCO (Taipei, Taiwan) and housed at 24 ± 1°C with 55–65% humidity and a 07:00–19:00 light-dark cycle. Both the animal care and experiments were approved by the Institutional Animal Care and Use Committee at the National Research Institution of Chinese Medicine (IACUC No: 106-370-4) under the Guide for the Care and Use of Laboratory Animals [U.S. National Institutes of Health (NIH), Maryland, USA].

At 8 weeks of age, mice either received weekly ACET treatments or a sham operation for 2 weeks. In brief, mice were anesthetized with isoflurane (4–5% to induce anaesthesia and 1–3% to maintain anaesthesia). To prepare the catgut-embedding needle set, a segment of 0.3-mm-long sterilized catgut was inserted into a disposable needle (22 G×1.25, 0.70mm×32mm) with the aid of an acupuncture needle (22 G×1.25, 0.35mm×40mm). Later, the prepared needle was perpendicularly inserted into taught skin. Finally, catgut was implanted into the abdominal muscle and fat by pushing the acupuncture needle 0.5–1mm into the skin. Catgut was implanted into four acupoints [Shui Fen (Ren-9), Qihai (Ren-6), and bilateral Siman (Ki-14)] in each mouse. Sham mice received random needle punctures under anaesthesia. (1) In terms of acupoints identification, we firstly located the umbilicus of mice at eighth-thirteen part of inferior margin of sternum to anus. And the acupoints could be identified according to the distance from the umbilicus.

Noninvasive blood pressure measurements

Prior to septic shock induction with LPS, mice received ACET treatment or sham operation were further divided into LPS or saline injection groups. The mean blood pressure, systolic blood pressure, diastolic blood pressure, and heart rate were measured in mice both before and 3 hours after LPS or control injection using a noninvasive tail cuff-based sphygmomanometer (BP-98A, Softron Biotechnology, Tokyo, Japan).

Pyruvate tolerance test

After an overnight fast, each mouse received an intraperitoneal injection of sodium pyruvate (2 g/kg body weight), and blood sugar levels were measured at 0, 30, 60, 90, and 120 min after injection.

Real-time RT-PCR

For measuring multiple gene expression in liver, mice were sacrificed at 6 h after LPS injection. Liver samples were lysed with the TRI-reagent for total RNA extraction. One microgram of total RNA was reverse-transcribed to generate templates. Twenty-five micrograms of cDNA were mixed with primer sets and the LightCycler® 480 SYBR Green I Master mix (Roche Life Science, Indianapolis, IN, USA) and placed in a LightCycler® 480 Multiwell plate. PCR was performed using the LightCycler

480 system using annealing conditions of 60°C for 10 sec. Primer sets were as follows:β-actin: forward 5'-CTAGAAGCACTTGCGGTGCAC-3' and reverse 5'-GAAATCGTGCGTGACATCAAA-3'; NOS-2 :forward 5'-GAGACAGGGAAGTCTGAAGCAC-3' and reverse 5'-CCAGCAGTAGTTGCTCCTCTTC-3'; IL-1β: forward 5'-TGGACCTTCCAGGATGAGGACA-3' and reverse 5'-GTTCATCTCGGAGCCTGTAGTG-3'; TNF-α: forward 5'-GGTGCCTATGTCTCAGCCTCTT-3' and reverse 5'-GCCATAGAACTGATGAGAGGGAG-3'; IL-6: forward 5'-TACCACTTCACAAGTCGGAGGC-3' and reverse 5'-CTGCAAGTGCATCATCGTTGTTC-3'; IL-10: forward 5'-CGGGAAGACAATAACTGCACCC-3' and reverse 5'- CGGTTAGCAGTATGTTGTCCAGC-3'; MCP-1: forward 5'-GCTACAAGAGGATCACCAGCAG-3' and reverse 5'-GTCTGGACCCATTCCTTCTTGG-3'; SOCS-1: forward 5'-AGTCGCCAACGGAACTGCTTCT-3' and reverse 5'- GTAGTGCTCCAGCAGCTCGAAA-3'; SOCS-2: forward 5'-GCGCGTCTGGCGAAAGCCCT-3' and reverse 5'- GAAAGTTCCTTCTGGAGCCTCTT-3'; and SOCS-3: forward 5'- GGACCAAGAACCTACGC ATCCA-3' and reverse 5'- CACCAGCTTGAGTACACAG TCG-3'.

Western blotting

Liver and hypothalamus tissue were collected at 30 min and 6 h after LPS injection, respectively. Liver or hypothalamic tissue was homogenized in ice-cold lysis buffer. After tissue debris was removed by centrifugation, equal amounts of protein (40 μg) were subjected to electrophoresis on sodium dodecyl sulphate-10% polyacrylamide gels. The gels were transferred to nitrocellulose membranes, and the blots were blocked with 5% (w/v) nonfat dry milk in Tris-buffered saline containing 0.1% (v/v) Tween 20 (TBST) for 1 hour and then incubated with primary antibodies, including IκB-α (SC-371, Santa Cruz), PEPCK (10004943, Cayman), AMPK (#2532, Cell signaling), and β-actin (MAB1501, Chemion), at 4°C overnight prior to incubation for 1 hour at room temperature with the corresponding secondary antibody. Finally, results were visualised after the development of the film with the aid of an enhanced chemiluminescence kit (Amersham Biosciences, Uppsala, Sweden). The intensity of the blots was quantified using ImageJ software (NIH).

Blood biochemistry analyses and the cytokine protein array

Serum analysis was carried out using blood samples collected by cardiac puncture 6 hours after LPS injection. Serum lactic dehydrogenase (LDH), glutamate oxaloacetate transaminase (GOT), glutamic-pyruvic transaminase (GPT), and creatinine (CRE) levels were measured by a FUJI DRI-CHEM 3000 analyser (Fujifilm, Tokyo, Japan). Using a mouse inflammation antibody array (Abcam), equal amounts of 2-fold-diluted serum samples from mice in the same group were pooled together, and the procedures provided by the manufacturer were followed. In brief, the blocked printed membrane was incubated with diluted sample for 2 hours at room temperature. The membrane was then washed and incubated with HRP-conjugated streptavidin antibody for chemiluminescence detection. Signals were visualised as dots on the film exposed to the membrane. Densitometric data were obtained using ImageJ software after the exposed film was scanned and digitally analysed.

Statistical analyses

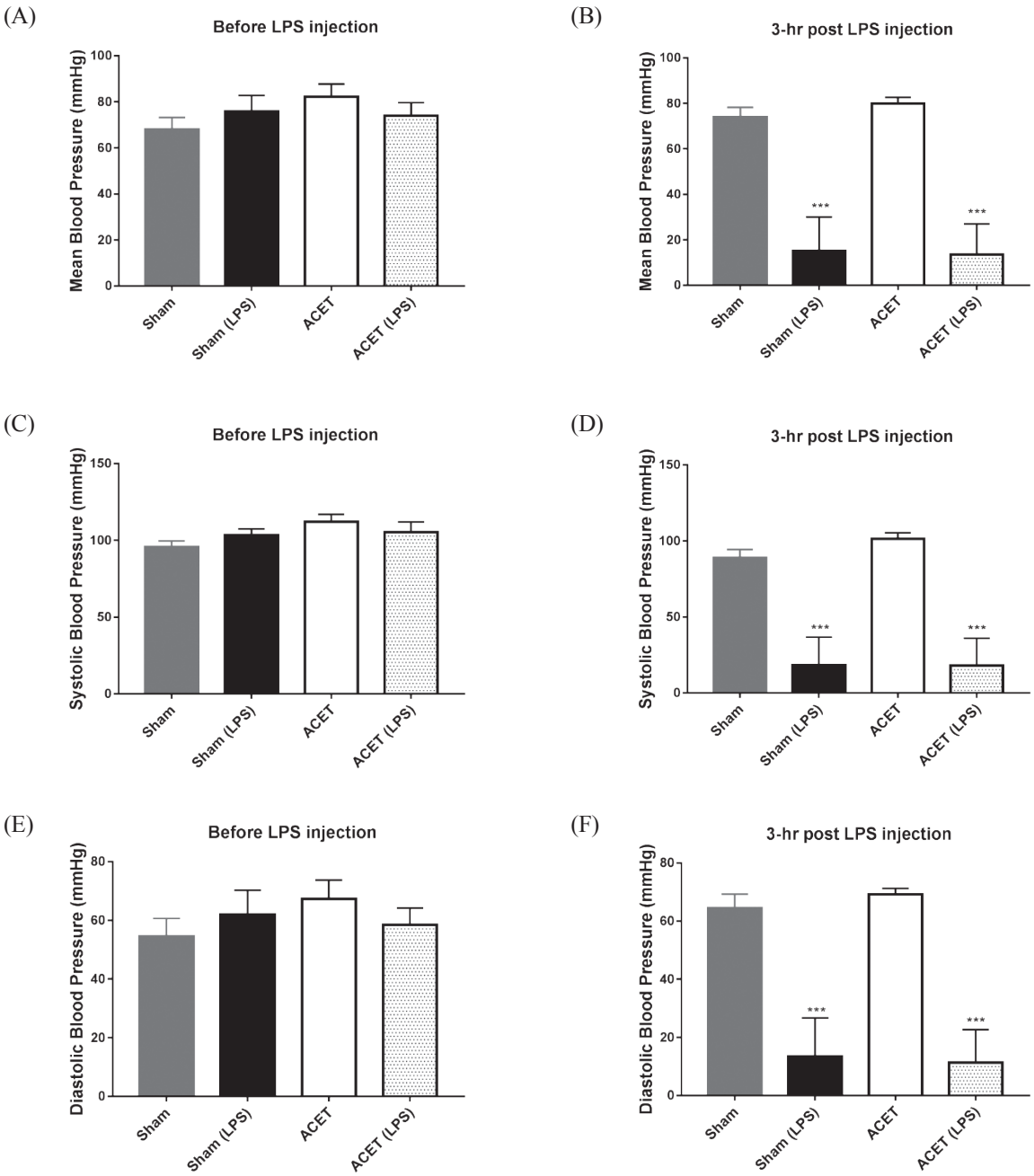
The significance of the various treatments was based on the Student's *t* test (Mann-Whitney test). Results are expressed as the mean ± SEM. Differences were considered significant if the $P<0.05$, 0.01, or

0.001.

Results

ACET attenuates LPS-induced organ damage

ACET was carried out once a week for 2 weeks. At the end of the therapy, mice were fasted overnight before receiving an intraperitoneal saline or LPS (15 mg/kg) injection. The effects of ACET on LPS-induced hypotension were then evaluated. As shown in Fig 1A-H, blood pressure and heart rate were measured before and after LPS injection. LPS administration significantly reduced mean blood pressure, systolic blood pressure, diastolic blood pressure, and heart rate. However, ACET did not affect these reductions.



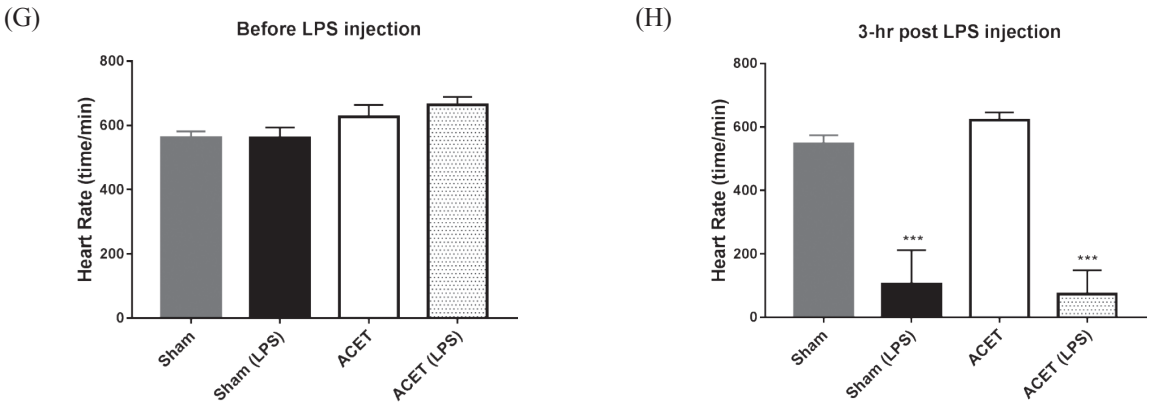


Fig 1. ACET has no effects on hypotension in septic mice. Blood pressure and heart rate were measured before and 3 hours after LPS injection. (A and B) Mean blood pressure. (C and D) Systolic blood pressure. (E and F) Diastolic blood pressure. (G and H) Heart rate. Data represent the mean ± SEM (n = 6). ***$P < 0.001$ when compared with the corresponding non-LPS-treated group.

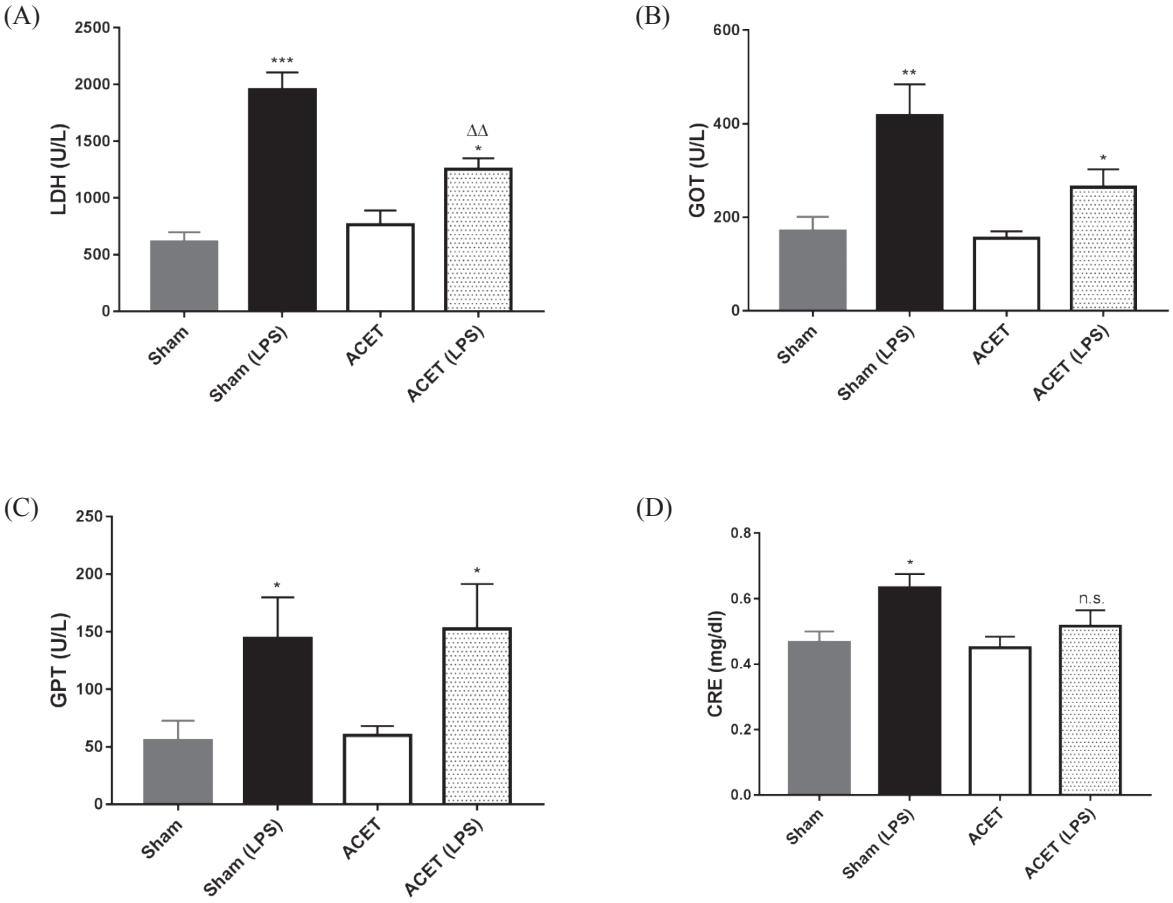


Fig 2. ACET improves blood biochemistry parameters in septic mice. Blood chemistry parameters including (A) lactate dehydrogenase (LDH), (B) glutamate oxaloacetate transaminase (GOT), (C) glutamate-pyruvate transaminase (GPT), and (D) creatinine (CRE) were analysed. Data represent the mean ± SEM (n = 6). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ when compared to the corresponding non-LPS-treated groups. $^{\Delta\Delta}P < 0.01$ when compared to the sham group injected with LPS.

The potential effects of ACET on LPS-induced organ damage were then evaluated. After 6 hours, LPS led to acute liver and kidney damage, as shown in Fig 2. Levels of LDH ($P < 0.001$), GOT ($P < 0.01$), GPT ($P < 0.05$), and CRE ($P < 0.05$) in LPS-treated sham mice (no catgut embedding) were significantly elevated. When ACET was applied, the extent of LPS-induced LDH ($P < 0.01$), GOT, and CRE levels became ameliorated (Fig 2A, B, and D). In contrast, LPS-induced GPT levels were unaffected by ACET (Fig 2C).

LPS-stimulated inducible nitric oxide synthase gene expression (via NF-κB signall- ing) is attenuated by ACET

To investigate the effects of ACET pretreatment on LPS-mediated sepsis, LPS-treated sham mice were found to have reduced IκB-α levels within 30 min (Fig 3A). In contrast, LPS was unable to cause the

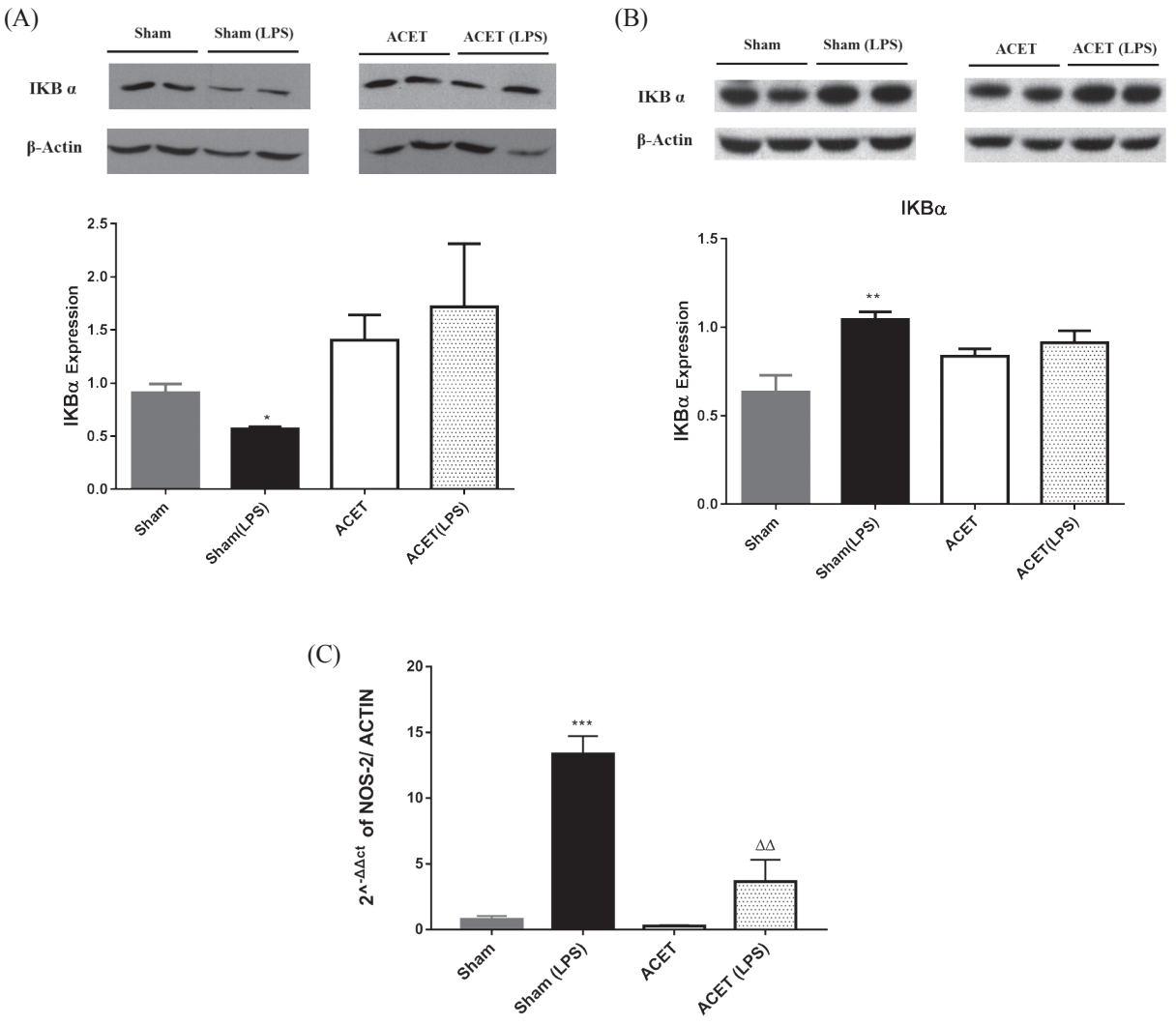
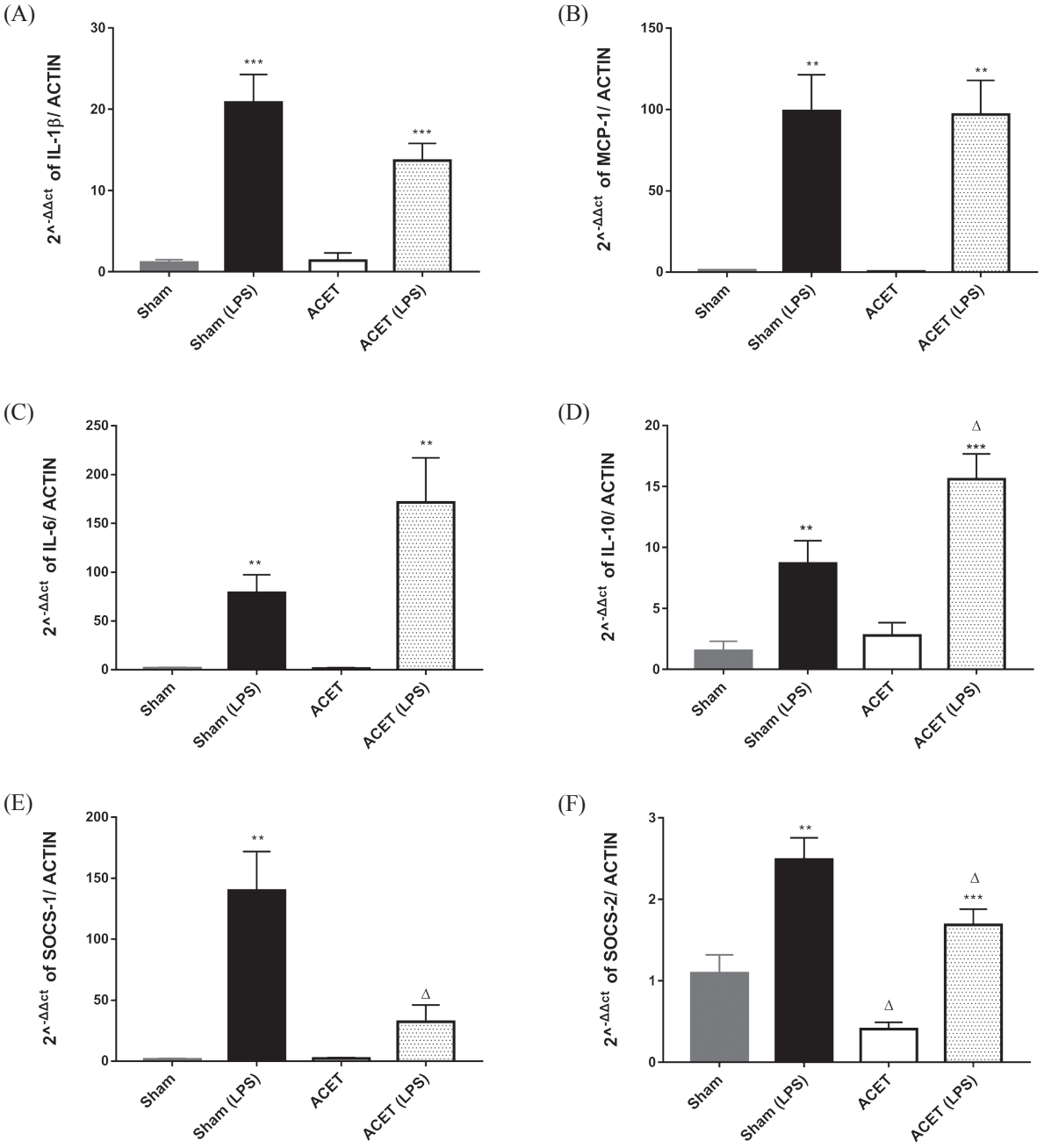


Fig 3. The effects of ACET on LPS-mediated NF-κB signalling activation and inducible nitric oxide (NOS-2) gene transcription. Hepatic IκB-α protein levels (A) 30 min or (B) 6 hours after LPS treatment. The western blots are representative of triplicate experiments. (C) Hepatic NOS-2 gene expression levels 6 hours after LPS treatment. Data represent the mean ± SEM (n = 6). ***$P < 0.001$ when compared with the corresponding non-LPS-treated group. $^{\Delta\Delta}P < 0.01$ when compared with the sham group injected with LPS.

degradation of IκB-α in ACET-treated mice under the same conditions. Six hours after LPS treatment, the increase in IκB-α protein levels was similar in both the sham and ACET groups (Fig 3B). Finally, LPS-stimulated NOS-2 expression increased approximately 17-fold in the sham group ($P < 0.001$) compared to control injection. In contrast, NOS-2 gene expression after LPS injection was significantly reduced in the ACET group compared with the sham group ($P < 0.01$).

ACET modulates LPS-induced liver inflammation

The effects of ACET pretreatment on LPS-induced inflammation were evaluated by measuring hepatic gene expression related to inflammation 6 hours after LPS injection. There were no significant differences in LPS-induced IL-1β, MCP-1, or IL-6 gene expression



(G)

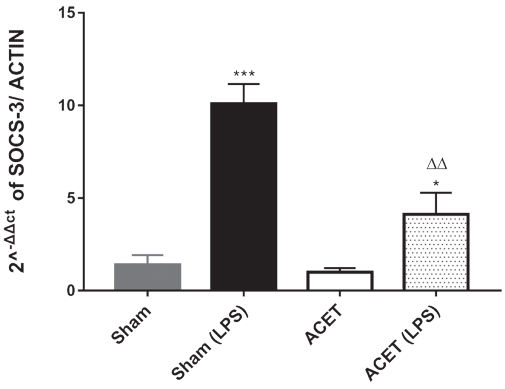


Fig 4. ACET modulates the levels of LPS-induced IL-10 and SOCS (1–3) gene expression in the liver. Quantitative PCR was performed to measure the mRNA expression levels of (A) IL-1β, (B) MCP-1, (C) IL-6, (D) IL-10, (E) SOCS-1, (F) SOCS-2, and (G) SOCS-3. Data were normalized to β-actin levels and presented as the mean ± SEM (n = 6). $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ when compared with compared to corresponding non-LPS-treated groups. $^{\Delta}P < 0.05$ and $^{\Delta\Delta}P < 0.01$ when compared with data from the same treatment (i.e., saline or LPS).

levels between the sham and ACET groups (Fig 4A–C). However, LPS-induced IL-10 expression was elevated compared with the sham and ACET groups (Fig 4D). In addition, whereas LPS-induced expression levels of SOCS1–3 were significantly attenuated in the ACET group (Fig 4E–G), there was no difference in SOCS-1 expression between the ACET group with or without LPS. Further, basal SOCS-2 expression was reduced in the ACET group compared with the sham group.

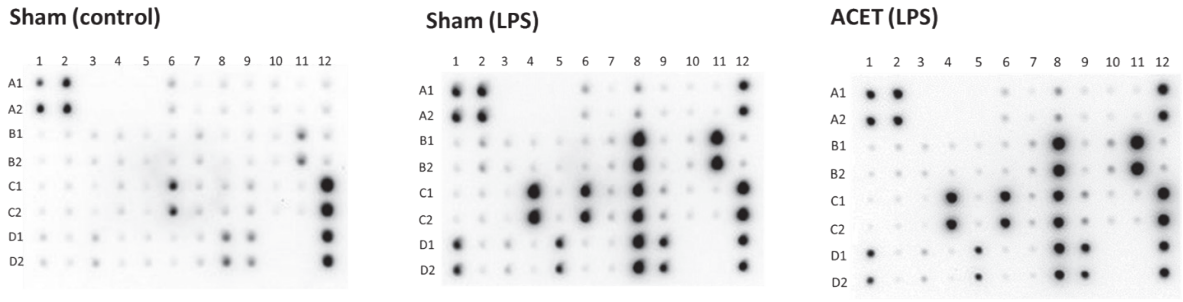
Effects of ACET on serum inflammatory cytokine profiles in septic mice

Potential effects of ACET on serum inflammatory cytokine levels in septic mice were compared by employing an antibody protein array, as shown in Fig 5A and B. The results were divided into the mild inflammatory response group (Fig 5C) and the strong inflammatory response group (Fig 5D). In the mild response group, IL-1α, IL-4, IL-17, I-TAC, LIX, and M-CSF were significantly induced by LPS treatment in mice not pre-treated with ACET. ACET caused a significant increase in IL-12 p70 levels and a decrease in IL-17 and I-TAC levels. On the other hand, in the

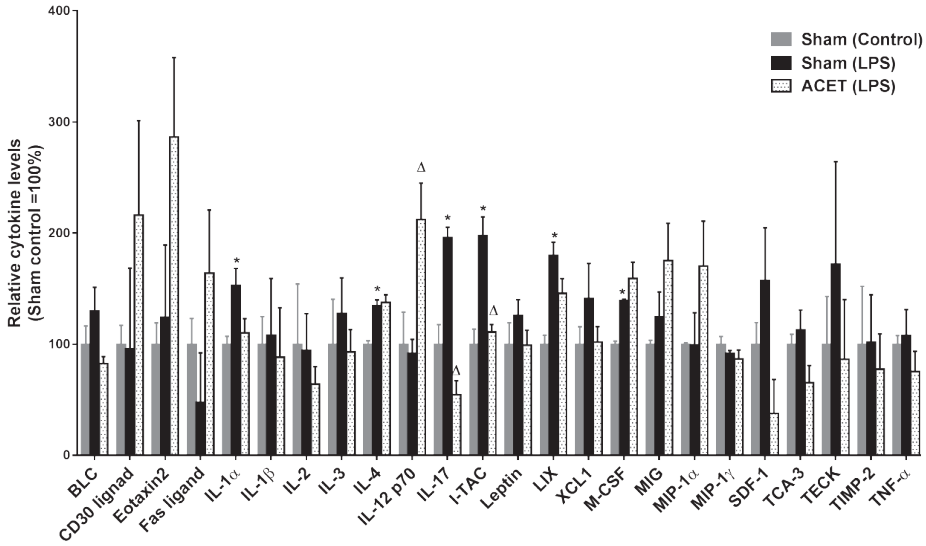
(A)

	1	2	3	4	5	6	7	8	9	10	11	12
A1	POS	POS	NEG	NEG	BLANK	BLC	CD30 ligand	Eotaxin1	Eotaxin2	Fas ligand	Fractal kine	G-CSF
A2												
B1	GM-CSF	IFN-Gamma	IL-1 alpha	IL-1 beta	IL-2	IL-3	IL-4	IL-6	IL-9	IL-10	IL-12 P40/70	IL-12 P70
B2												
C1	IL-13	IL-17	I-TAC	KC	Leptin	LIX	XCL1	MCP-1	M-CSF	MIG	MIP-1 alpha	MIP-1 Gamma
C2												
D1	RANTES	SDF-1	TCA-3	TECK	TIMP-1	TIMP-2	TNF-alpha	sTNFR1	sTNFRII	BLANK	BLANK	POS
D2												

(B)



(C)



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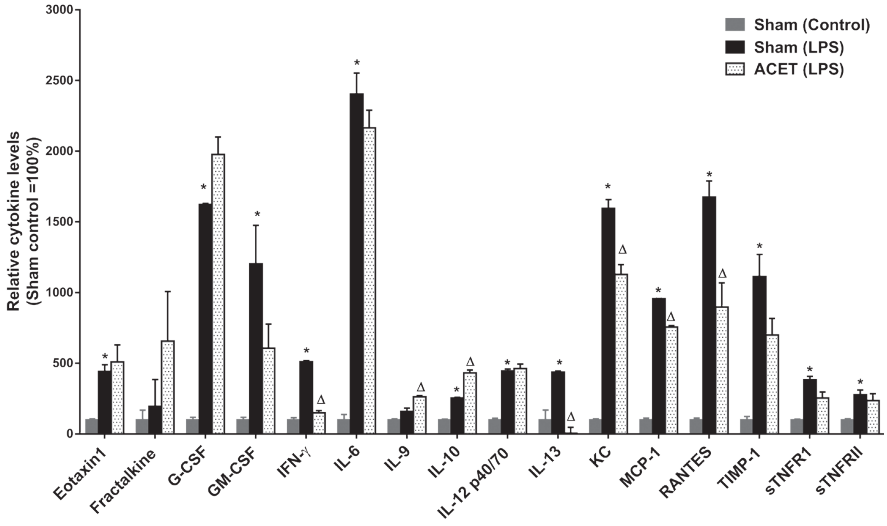


Fig 5. Effects of ACET on serum cytokine profiles in response to LPS treatment. (A) The cytokine profile of pooled serum in each group 6 hours after LPS treatment was analysed using a mouse inflammation antibody array kit with 40 inflammatory factors. (B) The images obtained by the antibody array. (C) The quantitative results of the inflammatory factors with a mild response to LPS treatment. (D) The quantitative results of inflammatory factors with a strong response to LPS treatment. Quantitative data are presented as the mean ± SEM (n = 2). $^{*}P < 0.05$ when compared between the sham (control) and sham (LPS) groups. $^{\Delta}P < 0.05$ when compared between the sham (LPS) and ACET (LPS) groups.

strong response group, LPS induced the expression of eotaxin 1, G-CSF, GM-CSF, IFN-γ, IL-6, IL-10, IL-12 p40/70, IL-13, KC, MCP-1, RANTES, TIMP-1, sTNFR1, and sTNFP11 up to 25-fold in the sham group. ACET resulted in a significant decrease in IFN-γ, IL-13, KC, MCP-1, and RANTES, and led to an increase in IL-9 and IL-10 protein levels.

ACET application restored LPS-modulated hypothalamic AMPK and hepatic PEPCK protein levels

In terms of hepatic PEPCK protein expression, hepatic PEPCK levels in the sham group after LPS treatment were clearly reduced, whereas other groups had similar protein levels after LPS treatment (Fig 6A). In addition, hypothalamic AMPK levels in the sham group after LPS treatment were also significantly reduced in contrast to the other three groups (Fig 6B).

ACET application prevents LPS-induced hypoglycaemia and mortality in septic mice

The effects of ACET on LPS-induced hypoglycaemia were examined. As shown in Fig 7A, fasting glucose levels in the sham group decreased 50% after LPS treatment ($P < 0.01$). In contrast, there were no differences in glucose levels between the ACET group before and after LPS treatment. As a result, the ACET group maintained fasting glucose levels after LPS treatment compared to a 50% reduction in glucose levels in the sham group after LPS treatment ($P < 0.001$). A pyruvate tolerance test was used to evaluate the ability of the liver to carry out gluconeogenesis. The average blood glucose levels in the ACET group at

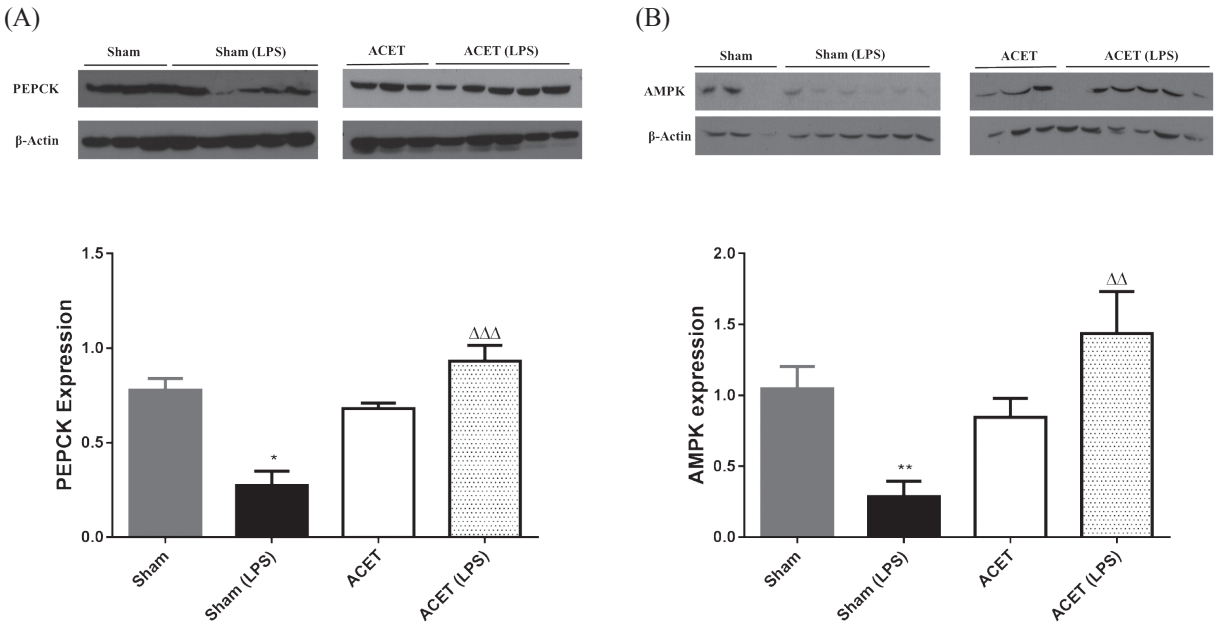
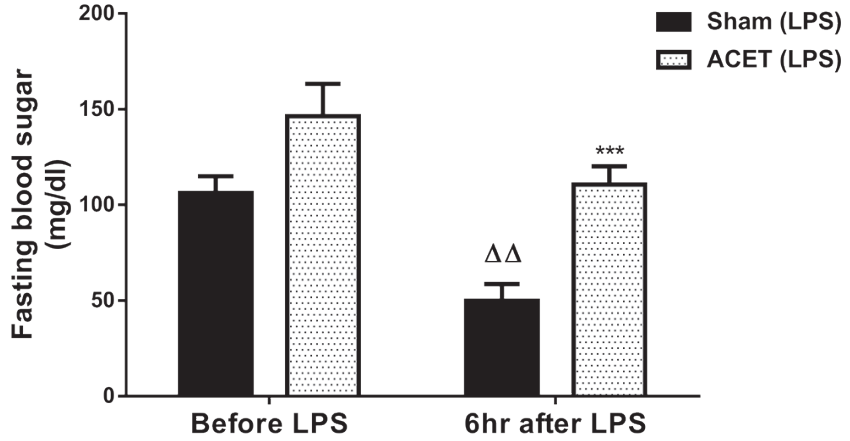


Fig 6. ACET reversed LPS-modulated hepatic PEPCK and hypothalamic AMPK protein levels in septic mice. A representative image of liver PEPCK (A) and hypothalamic AMPK (B) levels (n = 3 ～ 5). Data are presented as the mean ± SEM (n = 3 ～ 5). *$P < 0.05$ and **$P < 0.01$ when compared with the sham group with or without LPS. $^{\Delta\Delta}P < 0.05$ and $^{\Delta\Delta\Delta}P < 0.05$ when compared with or without ACET.

(A)



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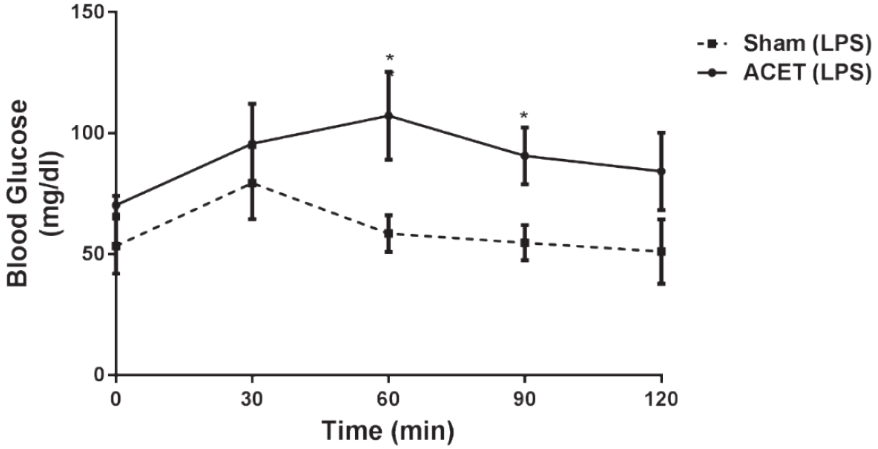

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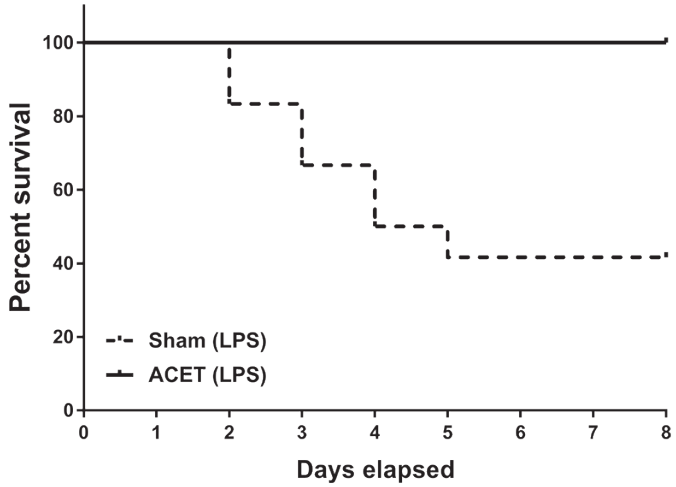

Fig 7. ACET attenuates hypoglycaemia and mortality in septic mice. (A) Fasting blood glucose levels of each group were measured before and after LPS injection. Data are presented as the mean ± SEM (n = 6). ***$P < 0.001$ when compared between the sham and ACET groups. $^{\Delta\Delta}P < 0.05$ when compared with the sham group. (B) The pyruvate tolerance test was performed, and changes in blood glucose levels over time are illustrated. Data are presented as the mean ± SEM (n = 6). *$P < 0.05$ when compared between the sham and ACET groups. A representative image of liver PEPCK. (C) The survival of septic mice was monitored for 8 days. Data represent the survival rate (%) of septic C57J/B6 mice, sham or treated with ACET (n = 12)

30 min ($P < 0.05$) and 60 min ($P < 0.05$) after pyruvate injection were significantly higher than those of the sham group, although the differences in the areas under the curve were not significant (Fig 7B).

Finally, when the survival rates of LPS-treated mice in the presence or absence of ACET were compared, 7 out of 12 mice (42% survival) in the LPS-treated sham group died within 2–5 days after LPS injection. In contrast, all 12 mice in the ACET group survived after LPS injection. The overall difference in survival rate between the LPS-treated sham and ACET groups was significant ($P = 0.0007$).

Discussion

LPS-induced sepsis appears to be dosage and species dependent [21]. The required dose of endotoxin to induce an acute inflammatory reaction is 2 ng/kg body weight in humans and 500 ng/kg in mice, whereas a dose of endotoxin greater than 20 mg/kg consistently results in 100% mortality in mice [22]. In the present investigation, we demonstrated that the application of ACET at four acupoints on the Ren and Kidney meridians could effectively prevent septic shock and subsequent mortality in a mouse model of sepsis (sepsis induced by the injection of 15 mg/kg purified LPS). After a 3-hour LPS induction, hypotension became evident in septic mice. An ACET intervention was unable to affect normal or LPS-reduced blood pressure. However, ACET could attenuate acute LPS-induced MODS. First, elevated levels of LDH and CRE were both reduced in septic mice treated with ACET. Elevated LDH levels are suggestive of acute liver disease, lung disease, heart failure, and tissue degradation [23]. In addition, serum CRE is a marker of renal health. Therefore, ACET pretreatment potentially prevents LPS-induced acute liver injury and nephritis [15]. Second, serum IL-17 levels are related to acute lung injury (ALI) in response to LPS. A reduction in IL-17 levels using antibodies has been shown to relieve ALI symptoms [24, 25]. Therefore, a significant reduction in IL-17 levels in septic mice after ACET suggests the attenuation of lung injury. Finally, chemokine levels of I-TAC, RANTES, MCP-1, and KC were all reduced in septic mice treated with ACET. Although the recruitment of leukocytes by chemokines is an important host defence mechanism, the overproduction of leukocyte antimicrobial factors could also lead to profound tissue injury, organ dysfunction, and organ failure. Collectively, the attenuation of LPS-induced organ damage could explain the reduction in mortality of septic mice treated with ACET.

We also observed certain inhibitory effects of ACET on proinflammatory responses in septic mice. In this study, we demonstrated that ACET could preserve the LPS-induced proteolytic degradation of IκB-α. IκB-α is degraded for NF-κB transactivation, although it also serves as a negative-feedback inhibitor of NF-κB activation [26]. In the present study, IκB-α levels were maintained in septic mice treated with ACET; downstream NOS-2 gene expression was also inhibited in septic mice treated with ACET. Therefore, all results indicate that ACET affects the transactivation of NF-κB, a pivotal player in the regulation of inflammatory cytokine gene expression [27]. The therapeutic benefits of ACET were also supported by studies showing that acupuncture produced anti-inflammatory effects on diseases such as endotoxemia, autoimmune diseases, and ischemia-reperfusion status via the inhibition of NF-κB signal transduction [28, 29].

In terms of anti-inflammatory cytokines, IL-10 has been recognized as a potent inhibitor of LPS-

induced proinflammatory cytokine gene expression, as well as monokine synthesis [30]. Furthermore, IL-4 is known to inhibit cytokine expression in activated human monocytes. In contrast, IL-6 plays a dual role in systemic inflammatory reactions. It is an important inducer of acute-phase reactions by acting on the liver and hypothalamic-pituitary-adrenal axis [31], but it also plays an anti-inflammatory role by inducing the activation of antiprotease inhibitors and corticosterone release to control the tissue inflammatory response [32]. In the present study, we observed increases in IL-10 and IL-6 levels, but not IL-4 levels, in ACET-treated septic mice.

The SOCS family is involved in the negative regulation of adaptive and innate immune responses during systemic inflammation; such regulation occurs via the inhibition of signal progression at the level of JAK/STAT activation and of LPS-induced NF-κB activation by the blockade of TLR signalling [33, 34]. In this study, ACET alone was unable to elicit SOCS expression. Instead, LPS stimulated SOCS-1, SOCS-2, and SOCS-3 mRNA expression, which was significantly decreased in septic mice treated with ACET. In the case of SOCS-2, ACET alone reduced its mRNA expression. We speculate that this effect might result from the attenuation of LPS signal transduction, such as by NF-κB signalling. Considering that SOCS-1 and SOCS-3 also act as negative regulators of insulin signalling [35], our current observations may explain the mechanism of action of the insulin-sensitizing effects of ACET in obese women [36].

Hypoglycaemia is an important sign of overwhelming sepsis and one of the major causes of septic shock [37]. A previous study reported that liver gluconeogenesis is impaired during endotoxemia due to the suppression of PEPCK expression [38]. This study demonstrated that 6 hours after LPS treatment, fasting blood glucose levels of ACET-treated mice were maintained, whereas LPS-injected sham mice experienced hypoglycaemia. These results imply that ACET can restore suppressed hepatic gluconeogenesis by increasing PEPCK expression after LPS induction and thus prevent further endotoxemic shock. Hypothalamic AMPK also plays an important role in the maintenance of glucose homeostasis. LPS has been reported to reduce hypothalamic AMPK phosphorylation but without effects on total hypothalamic AMPK levels [6]. However, in this study, we demonstrated that a higher dose of LPS could reduce total hypothalamic AMPK levels, suggesting a dose-dependent effect of LPS on hypothalamic AMPK regulation. ACET pretreatment could reverse such a reduction in hypothalamic AMPK levels, thus restoring hepatic PEPCK expression and preventing endotoxemic hypoglycaemia.

Finally, there were some limitations of this study. First, the therapeutic effects of ACET on sepsis remain to be evaluated. Second, the specificity of the acupoints used cannot be determined in this study. Additionally, whether there were any dose-dependent effects of ACET must be further explored.

Conclusion

Our preclinical study revealed the potential medicinal value of ACET to attenuate LPS induced-organ damages, inflammation, hypoglycaemia, and mortality.

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原始論文

穴位針灸埋線減緩脂多醣誘導之敗血症小鼠之發炎和低血糖反應及致死率

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減低敗血性休克死亡率及減緩因為系統性發炎及內毒素性低血糖所導致的多重器官損傷是醫療尚未滿足之需求。在這篇研究報告中，我們評估了穴位埋線針灸對於脂多糖所誘導的敗血性休克小鼠的影響。四組 C57J/B6 小鼠分組方式如下：(1) 無埋線＋無脂多糖 (2) 埋線＋無脂多糖 (3) 無埋線＋脂多糖 (4) 埋線＋脂多糖。穴位埋線每周一次共計兩次。穴位為：水分（Ren-9），氣海（Ren-6），四滿（Ki-14）。第三周起，小鼠接受腹腔注射食鹽水或是脂多糖（15mg/kg）。生化參數，蛋白及基因表現及存活率統計等實驗於注射後陸續分析。結果顯示，脂多糖誘導低血壓並不受到埋線影響。但是脂多糖注射所增加的乳酸脫氫酶，肌酸酐，介白素 -17 皆因埋線而改善。令人驚訝的，埋線預處理之小鼠在脂多糖注射後完全存活。在

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分子層次上，埋線降低了脂多糖誘導之 IκB-α 降解及發炎反應。埋線所減緩因脂多糖注射所導致的低血糖則與下視丘的單磷酸腺苷活化蛋白質激酶以及肝臟的磷酸烯醇丙酮酸羧激酶有關。這些協同作用避免了小鼠因脂多糖注射而死亡。因此總結來說，目前的研究結果提供了穴位埋線針灸對於降低因敗血症導致死亡的潛在醫學應用價值。

關鍵字：穴位埋線針灸、脂多糖、敗血症、內毒素性低血糖、發炎、致死率