Resveratrol alleviates endotoxemia-associated adrenal insufficiency by suppressing oxidative/nitrative stress

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Abstract. We have recently demonstrated that endotoxin causes oxidative stress and overproduction of nitric oxide in adrenal glands, thereby leading to adrenocortical insufficiency. The aim of this study is to investigate the effects of resveratrol, a natural plant polyphenol with anti-oxidant and anti-nitrative properties, on endotoxemia-associated adrenocortical insufficiency. Resveratrol was administered immediately before injection of lipopolysaccharide (LPS). Twenty four hours later, the adrenocorticotropic hormone (ACTH) stimulation tests were performed to measure the plasma corticosterone level and the adrenal gland tissues were collected for histopathologic examination, and determination of malondialdehyde (MDA), total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, catalase (CAT) activity, inducible nitric oxide synthase (iNOS) expression, nitric oxide (NO) and peroxynitrite production. Treatment with resveratrol significantly inhibited endotoxemia-induced iNOS expression, NO production, and peroxynitrite formation and also attenuated LPS-induced oxidative stress in the adrenal gland, as evidenced by the decrease of pro-oxidant biomarker (MDA), and the increases of anti-oxidant biomarkers (T-AOC, CAT and SOD activity). H&E staining demonstrated that administration of LPS resulted in increased into the adrenal gland. H&E-stained sections of adrenal glands demonstrated signs of leukocyte infiltration and hemorrhage during endotoxemia, which were significantly improved by resveratrol treatment. In addition, resveratrol reversed the LPS-induced downregulation of ACTH receptor and silent information regulator 1 (SIRT1) in adrenal gland, as well as adrenocortical hyporesponsiveness to ACTH. Resveratrol exerts protective effects against endotoxemia-associated adrenocortical insufficiency by suppressing oxidative/nitrative stress. These findings support the potential for resveratrol as a possible pharmacological agent to improve adrenocortical insufficiency resulting from oxidative/nitrative damage.

Key words: Resveratrol, Endotoxemia, Adrenocortical insufficiency, Oxidative stress, Nitrative stress

REVERSIBLE adrenal insufficiency has frequently been diagnosed in critically ill patients with sepsis who have either low basal cortisol levels or adrenocortical hyporesponsiveness to adrenocorticotropic hormone (ACTH) stimulation [1, 2], which is always associated with a high mortality rate [3, 4]. According to the latest international guidelines for the management of severe sepsis, corticosteroid supplementation is recommended only to a sub-group of septic shock patients who have responded

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poorly to fluid resuscitation and vasopressor agents [4, 5]. Additionally, the adverse effects of corticotherapy on insulin resistance, protein catabolism, and immunosuppression also limit the use of corticosteroid supplementation therapy [5]. Thus, it seems to be important to seek out alternative pharmacological therapies for management of adrenal insufficiency during sepsis.

In recent years, accumulating evidence indicates that oxidative stress resulting from an imbalance between pro-oxidants and anti-oxidants plays an important role in the pathogenesis of adrenal insufficiency [6, 7]. As a result, overproduction of reactive oxygen species (ROS) can oxidize thiols, lipids, proteins and nucleic acids, consequently leading to oxidative injury in adrenal glands. In addition, superoxide (\(O_2^-\)) and nitric oxide (NO) are known to rapidly react with each other to form peroxynitrite (ONOO\(^-\)), nitrogen dioxide, and other toxic reactive nitrogen species (RNS) [8]. These NO-derived reactive nitrogen intermediates may cause pathologic tissue injury by nitrative protein modification (nitrative stress) [8].

Resveratrol (3,5,4’-trihydroxystilbene), a natural nonflavonoid polyphenol found in grapes and red wine, has exhibited antioxidant and antiproliferative activities via a silent information regulator 1 (SIRT1)-dependent pathway [9-11]. Our previous study has demonstrated that resveratrol ameliorates endotoxemia-associated acute lung injury through reducing oxidative/nitrative stress in lung tissues [12]. Thus, the present study aimed to investigate the effect of resveratrol on adrenal insufficiency in a model of endotoxemia; and then explored whether resveratrol affected oxidative/nitrative stress in adrenal glands in this model.

## Materials and Methods

### Animals and ethics statement

Male ICR mouse (25-30 g, 8 weeks old) were obtained from Shanghai SLAC Laboratory Animal Co (Shanghai, China) and housed at controlled room temperature with free access to food and water under a natural day/night cycle. All animal protocols were approved by the Ethical Committee of Experimental Animals of Second Military Medical University, and confirmed to the principal in the Guide for the Care and Use of Laboratory Animals published by the United States National Institute of Health (NIH publication No. 85-23, revised 1996).

### Drug treatment

Mice were randomly divided into the following groups and each group had 7 mice: (1) Control group; (2) Endotoxemia group; (3) Endotoxemia with resveratrol treatment group. Purified LPS extracted from the membrane of Escherichia coli O111:B4 (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in sterile pyrogen-free saline and injected into the peritoneal cavity at a dose of 5 mg/kg. Resveratrol (Sigma-Aldrich, St. Louis, MO, USA), dissolved in saline, was injected into peritoneal cavity immediately before injection of LPS at the indicated doses. The control group received an equivalent volume of saline.

### Histopathological analysis of adrenal glands

Mice were sacrificed by overdose chloralhydrate 24 h after drug treatment. The adrenal glands were fixed in 4% paraformaldehyde for histopathologic analysis. HE staining is used to observe tissue histopathological change, especially, inflammatory cell infiltration and hemorrhage. The samples were dehydrated and embedded in paraffin. Sections (4 mm thickness) were cut and stained with hematoxylin and eosin (HE staining). Oil Red O staining is commonly used to identify lipid deposits [13]. To determine adrenal glands lipid accumulation, frozen sections (4 mm) of adrenal glands were stained with 0.5% Oil Red O (Sigma-Aldrich) for 10min, washed, and counter stained with Mayer’s hematoxylin (Sigma-Aldrich) for 45s. The sections were visualized under a BX51 light microscopy (Olympus, Tokyo, Japan), and digital images were captured and documented.

### ACTH stimulation tests

The plasma corticosterone response to exogenous ACTH (Sigma-Aldrich) was determined as described previously [14]. Dexamethasone (Sigma-Aldrich) dissolved in saline, was injected i.p. at a dose of 5 μg/g body weight at 1800 h the night before and at 0800 h during the morning of the procedure. Two hours later, mice were anesthetized intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg). ACTH (30 μg/kg) was infused via a femoral arterial catheter. Arterial samples of 50 μL were obtained immediately before ACTH administration as well as at 15 min, 60 min, and 120 min after ACTH administration. Each sample was replaced with an equal volume of saline containing heparin (100 units/mL).
Measurement of corticosterone

Mouse plasma were collected and stored at -80°C for corticosterone measurement. We used an EIA kit (Cayman) to measure corticosterone levels according to the manufacturer’s instruction. This kit was characterized by a broad working range of 11.5–10,000 pg/mL. The specificity was 100% for corticosterone, 1.01% for progesterone, 0.25% for aldosterone, 0.18% for cortisol, 0.18% for testosterone, 0.02% for cortisone, and 0.01% for DHEA, DHEA-S and pregnenolone. Intra-assay and inter-assay variation ranged from 8.4% to 13.5% and from 3.5% to 7.5%, respectively.

NO production measurement

Total NO production by adrenal glands was determined by measuring the concentration of nitrite, a stable metabolite of NO in vitro, with a modified Griess (sigma-Aldrich) reaction method [15]. The absorbance was measured at 550 nm using a Bio-Rad (Hercules, CA) microplate reader and nitrite concentration was assessed by reference to the sodium nitrite standard curve.

Western blot analysis

Mouse adrenal glands were lysed with cold RIPA lysis buffer (Beyotime). Protein load was 30 μg/lane in 10% SDS-PAGE subsequently transferred to nitrocellulose membranes. The bolts were blocked with 5% skim milk powder in 0.1% Tris-buffered saline/Tween 20 (TBST) for 2 h and incubated with antibodies against iNOS (Santa Cruz), SIRT1 (Santa Cruz) or ACTH receptor (Santa Cruz) overnight at 4°C at a dilution of 1:1,000. Then, the membrane was incubated with a secondary horseradish peroxidase-conjugated antibody for 1 h at room temperature. Immunoreactive proteins were visualized using the enhanced chemiluminescence western blotting detection system (Santa Cruz). The chemiluminiscent signal from the membranes was quantified by a GeneGnome HR scanner using GeneTools software (SynGene). To control sampling errors, the ratio of band intensities to the β-actin was obtained to quantify the relative protein expression level.

Malondialdehyde level determination

Levels of malondialdehyde (MDA), as an index of membrane lipid peroxidation, were determined as previously described [16]. Adrenal glands were homogenized (100 mg/mL) in 10 vol of 1.15% KCl solution containing 0.85% NaCl and then centrifuged at 1,500 g for 15 min. 200 μL of the supernatant were then added to a reaction mixture consisting of 1.5 mL 0.8% thiobarbituric acid, 200 μL 8.1% sodium dodecyl sulfate, 1.5 mL 20% acetic acid (adjusted to pH 3.5 with NaOH) and 600 μL distilled H₂O. The mixture was then heated at 95°C for 40 min. After cooling to room temperature, the samples were cleared by centrifugation (10,000×g, 10 min) and their absorbance measured at 532 nm, using 1,1,3,3-tetramethoxypropane as an external standard. The level of lipid peroxides was expressed as nmol MDA/mg protein (Bradford assay).

Measurement of total antioxidant capacity and activities of superoxide dismutase and catalase

Adrenal glands (50 mg) were homogenized in cold saline. Homogenates were then centrifuged at 1,000 g for 10 min. The total antioxidant capacity (T-AOC), superoxide dismutase (SOD) and catalase (CAT) activity in adrenal glands tissue were determined according to the kit manufacturer’s instructions (Winching, Nanjing, China) as described previously [12]. The mean intra-assay coefficients of variation for T-AOC, SOD and CAT were 3.2%, 1.7% and 1.9%, respectively. The mean inter-assay coefficients of variation for T-AOC, SOD and CAT were 6.83%, 3.52% and 4.94%, respectively.

Quantitation of nitrotyrosine content

Adrenal glands (50 mg) were homogenized in cold saline. Nitrotyrosine content, a footprint of in vivo ONOO⁻ formation and an index of nitrative stress, was determined using a nitrotyrosine ELISA kit (Cell Sciences, Canton, MA) according to the kit manufacturer’s instructions [16].

Statistical analysis

Data were expressed as means ± SEM. Statistical significance in experiments comparing only two groups was determined by two-tailed Student’s t-test. The significance of the difference in mean values among more than two groups was evaluated by one-way analysis of variance (ANOVA), followed by post hoc analysis using Student-Newman-Keuls test. The significance of the difference in values of ACTH stimulation tests was evaluated by ANOVA followed by post hoc, pairwise analysis using Bennett’s test. All statistical analyses were done with SPSS 16.0 (SPSS, Inc.). A p-value of less than 0.05 was considered significant.

Measurement of corticosterone

Mouse plasma were collected and stored at -80°C for corticosterone measurement. We used an EIA kit (Cayman) to measure corticosterone levels according to the manufacturer’s instruction. This kit was characterized by a broad working range of 11.5–10,000 pg/mL. The specificity was 100% for corticosterone, 1.01% for progesterone, 0.25% for aldosterone, 0.18% for cortisol, 0.18% for testosterone, 0.02% for cortisone, and 0.01% for DHEA, DHEA-S and pregnenolone. Intra-assay and inter-assay variation ranged from 8.4% to 13.5% and from 3.5% to 7.5%, respectively.
Results

Resveratrol inhibits LPS-induced iNOS expression and NO production in adrenal gland

We have previously shown that the increases in adrenal iNOS expression and NO production contribute to LPS-induced adrenal insufficiency [15]. In the present study, it was found that treatment of resveratrol significantly attenuated the elevated iNOS expression in adrenal glands of endotoxemic mice in a dose-dependent manner (Fig. 1A). Resveratrol at the dose of 0.3 mg/kg had the most potent inhibitory effect on LPS-induced iNOS expression, meanwhile significantly suppressed LPS-induced NO production in the adrenal gland (Fig. 1B).

Effect of resveratrol on LPS-induced peroxynitrite production in adrenal gland

It has been shown that many of the cytotoxic effects attributed to NO are mediated by peroxynitrite, a product from the reaction of NO with superoxide anion [8]. As shown in Fig. 2, endotoxemia led to increased production of nitrotyrosine, a biomarker of peroxynitrite formation ($p<0.01$), whereas resveratrol treatment (0.3 mg/kg) significantly attenuated the endotoxemia-induced increase of nitrotyrosine formation in the adrenal gland ($p<0.01$).

Resveratrol ameliorates LPS-induced oxidative stress in adrenal gland

Oxidative stress is often defined as an imbalance of pro-oxidants and antioxidants [17]. In the present study, the effect of resveratrol on oxidative status in adrenal glands of endotoxemic mice was first investigated by determining levels of pro-oxidant biomarkers MDA (a major product of lipid peroxidation). As shown in Fig. 3A, a significant increase in MDA level was observed in endotoxemic mice compared with saline-treated control animals ($p<0.01$). Administration of resvera-
RSV alleviates adrenal insufficiency

Adrenal gland obtained from endotoxemic mice became markedly enlarged and hyperemic (Fig. 4B) compared with saline-treated mice (Fig. 4A). H&E-stained sections of adrenal glands demonstrated that administration of LPS resulted in increased leukocyte infiltration into the adrenal gland. In addition, sign of hemorrhage was present predominantly in the zona fasciculate containing the glucocorticoid-producing cells (Fig. 4E). These features were significantly alleviated in endotoxemic mice treated with resveratrol (Fig. 4F).

We also observed whether resveratrol treatment affected the neutral lipid (i.e., cholesterol ester) content in the adrenal cortex during endotoxemia. As shown in Fig. 5, Oil Red O staining revealed that the neutral lipid content of the adrenal cortex was clearly decreased in LPS-treated mice (Fig. 5B) compared with saline-treated control mice (Fig. 5A), particularly in the zona fasciculate. Resveratrol treatment could reverse LPS-induced reduction of neutral lipid content (Fig. 5C).

**Histological changes of adrenal gland tissue sections**

As shown in Fig. 4, gross anatomy showed that the adrenal gland obtained from endotoxemic mice became markedly enlarged and hyperemic (Fig. 4B) compared with saline-treated mice (Fig. 4A). H&E-stained sections of adrenal glands demonstrated that administration of LPS resulted in increased leukocyte infiltration into the adrenal gland. In addition, sign of hemorrhage was present predominantly in the zona fasciculate containing the glucocorticoid-producing cells (Fig. 4E). These features were significantly alleviated in endotoxemic mice treated with resveratrol (Fig. 4F).

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![Fig. 3](image)

Resveratrol attenuate LPS-induced oxidative stress in adrenal gland. Mice were injected with LPS (5 mg/kg, ip). Resveratrol at a dose of 0.3 mg/kg was administered immediately before injection of LPS. Twenty four hours later, Adrenal gland MDA level (A), SOD activity (B), CAT activity (C) and T-AOC level (D) were determined as described in “Material and Method”. Data are means ± S.E.M. of 7 mice for each group. * p < 0.05 vs Control, # p < 0.05 vs Endotoxemia; ** p < 0.01 vs Control, ## p < 0.01 vs Endotoxemia.
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Resveratrol improves LPS-induced adrenal insufficiency

Our previous studies have shown that LPS treatment leads to downregulation of ACTH receptor in rat fasciculata-reticularis cells [18]. In the present study, we first evaluated the effect of resveratrol on ACTH receptor expression in adrenal gland during endotoxemia. As shown in Fig. 6A, protein expression of ACTH receptor was clearly decreased in adrenal glands obtained from LPS-treated mice compared with saline-treated control mice. Resveratrol treatment could reverse LPS-induced downregulation of ACTH receptor. We then examined the effect of resveratrol on LPS-induced adrenocortical hyporesponsiveness by using exogenous ACTH stimulation test. It was found that intraperitoneal administration of LPS resulted in a

Fig. 4  Histological changes of adrenal gland in endotoxemia mice. Mice were injected with LPS (5 mg/kg, ip). Resveratrol at a dose of 0.3 mg/kg was administered immediately before injection of LPS. Twenty four hours later, the adrenal gland was removed for gross anatomy observation and histopathologic examination using hematoxylin and eosin staining. Representative images from 7 animals per group were shown. A and D, Control; B and E, Endotoxemia; C and F, Endotoxemia with resveratrol treatment. Scale bar of gross anatomy, 1mm. Original magnification, ×200, bar = 50 μm.

Fig. 5  Lipid depletion in adrenal gland of mice. Mice were injected with LPS (5 mg/kg, ip). Resveratrol at a dose of 0.3 mg/kg was administered immediately before injection of LPS. Twenty four hours later, the adrenal glands were collected. Representative images from 7 animals per group were shown. B shown neutral lipid clearly decreased in LPS-treated mice compared with (A) saline-treated control mice, particularly in the zona fasciculate containing the glucocorticoid-producing cells. LPS-induced decrease of adrenal neutral lipids was significantly attenuated by resveratrol treatment (C). Original magnification ×200, bar = 50 μm.
RSV alleviates adrenal insufficiency significantly improved (Fig. 6B). The suppressed adrenocortical responsiveness in endotoxemic mice, especially at 60 min and 120 min after ACTH stimulation, was reversed towards the normal range after resveratrol treatment (Fig. 6C). Taken together, our results suggest that resveratrol improves LPS-induced adrenal insufficiency.

Fig. 6 Resveratrol improves LPS-induced adrenal insufficiency. Mice were injected with LPS (5 mg/kg, ip). Resveratrol at a dose of 0.3 mg/kg was administered immediately before injection of LPS. Twenty four hours later, protein level of ACTH receptor in adrenal gland (A) was determined by Western blot (A). ACTH stimulation tests were performed as described in “Material and Methods”, and corticosterone levels were measured in serum collected before or 15 min, 60 min and 120 min after exogenous ACTH stimulation (B and C). The results indicated LPS increases the baseline level of corticosterone (B) and resveratrol can return to near normal levels. C indicated increases in corticosterone from baseline in response to ACTH in groups for each time after ACTH stimulation, respectively. Data were expressed as mean ± SEM. ** p < 0.01 vs Control. ### p < 0.01 vs LPS.
Effects of LPS and resveratrol on SIRT1 expression in adrenal gland

Previous study has demonstrated that resveratrol exerts antioxidant effects via upregulation of SIRT1 expression [19]. Therefore, we examined whether resveratrol affected SIRT1 expression in adrenal glands during endotoxemia. As shown in Fig. 7, it was found that endotoxemia led to significant decrease in SIRT1 expression in adrenal glands (p<0.01 vs control), which was reversed by resveratrol treatment at the dose of 0.3 mg/kg (p<0.01 vs endotoxemia).

Discussion

The present study demonstrates that LPS treatment for 24 h significantly impairs corticosterone response to exogenous ACTH stimulation. Intraperitoneal administration of resveratrol at a dose of 0.3 mg/kg reversed the LPS-induced adrenocortical hyporesponsiveness to ACTH. This finding was in agreement with previous in vitro study, which reported that resveratrol enhanced ACTH-stimulated corticosterone secretion in primary isolated adrenocortical cells [20]. However, these data conflicted with Supornsilchai et al’s study [21] which demonstrated that rats consuming a resveratrol-containing diet at a dose of 0.84 g/kg/d for 12 weeks showed significant decreases in plasma corticos-
toxemia-associated adrenal insufficiency. These data suggest that the protective effects of resveratrol against endotoxemia-associated adrenocortical hyporesponsiveness may be, at least partly, due to its potent antioxidant property.

What is interesting is antioxidative effect is not specific to resveratrol. Previous studies have shown that other kind of antioxidants also exert protective effects on adrenocortical steroidogenesis during oxidative stress. For example, we have proved that administration of hydrogen sulfide (H$_2$S) inhibits LPS-induced ROS production in adrenal glands and attenuates adrenocortical insufficiency during endotoxemia [15]. Abidi et al. show that antioxidant N-acetylcysteine can completely blocked the 4-hydroxy-2-nonenal (HNE)-induced loss of steroidogenic response in Y1-B51 cells, a highly responsive mouse adrenocortical cell line [28]. Moreover, Astort et al. demonstrate that antioxidant, α-tocopherol treatment can abolish high glucose-induced oxidative stress, thus improving steroid production in adrenal cells [29]. Whether N-acetylcysteine and α-tocopherol are able to improve endotoxemia-associated adrenal insufficiency merits further investigation.

Nitric oxide has been implicated in the pathophysiology of organ dysfunction during endotoxemia [27, 30, 31]. In the normal condition, NO is produced mainly by the constitutive isoforms of NO synthase (neuronal NO synthase, nNOS, and endothelial NO synthase, eNOS), whereas its production during inflammation is mainly due to the inducible NO synthase (iNOS) [31-33]. Excessive levels of NO produced from iNOS leads to a reaction of NO with superoxide to create ONOO$^-$ . ONOO$^-$ formation represents one of the major mechanisms of inflammation-associated organ injury, including substantial oxidative/nitrative stress and potential destruction of host cellular constituents [8]. Previous studies have shown that systemic LPS treatment resulted in a significant increase in iNOS expression and NO production in adrenal glands [2, 15]. In addition, the specific iNOS inhibitor completely reversed the LPS-induced adrenocortical hyporesponsiveness to ACTH, suggesting that NO produced via iNOS inhibit adrenocortical steroidogenesis during endotoxemia [15].

The effect of resveratrol on iNOS expression and activity may depend on the type of tissue and pathological context. It has been shown that resveratrol treatment increases myocardial expression of both iNOS and eNOS in a rat model of myocardial infarction [34]. Resveratrol also restores the reduced retinal iNOS expression in a rat model of oxygen-induced retinopathy [35]. Moreover, resveratrol increase iNOS activity in primary cultured rat vascular smooth muscle cells [36]. Although the above-mentioned literatures have reported that resveratrol treatment leads to enhanced expression or activity of iNOS, there are plenty of studies demonstrating that resveratrol decreases iNOS expression and NO production induced by various pro-inflammatory stimuli such as LPS [12] and interferon-γ [37]. Consistent with most of the literature in this area, we found that resveratrol significantly suppressed LPS-induced iNOS expression, NO production, and nitrotyrosine formation in the adrenal gland. These data suggest that the inhibition of iNOS-related nitrative stress may attribute to the protective effects of resveratrol against endotoxemia-associated adrenal insufficiency.

Endothelial dysfunction, edema, hyperemia and hemorrhage are key characteristics associated with severe inflammation-associated injuries in a variety of organs including lung [38], liver [39], kidney [39] and brain [40]. The adrenal gland is a highly vascularized organ. Polito et al. have reported that patients with sepsis show signs of adrenal hemorrhage [41]. Moreover, accumulating data indicate that endothelial dysfunction and adrenal hemorrhage contribute to inflammation-related adrenal insufficiency [41, 42]. We have previously reported that resveratrol treatment significantly alleviates mechanical stretch-induced lung endothelial injury [43]. In the present study, we found resveratrol significantly attenuated the adrenal edema after LPS injection. These findings let us suggest that protection of the adrenal vasculature might also contribute to the beneficial effects of resveratrol on the adrenal cortex function during endotoxemia. More experiments should be performed in the future to explore the mechanisms involved in the protective effect of resveratrol on adrenal vascular function.

SIRT1 has been known to mediate numerous functions of resveratrol [10, 11]. Previous study has demonstrated that resveratrol exerts antioxidative effects via upregulation of SIRT1 expression [19]. The present study found that LPS administration led to a significant decrease in SIRT1 expression in adrenal glands, which was almost entirely reversed by resveratrol treatment. These data suggest that SIRT1 pathway may be involved in the protective effect of resveratrol on LPS-induced adrenal insufficiency. Notably, the mecha-
nisms underlying LPS-induced adrenal insufficiency remain largely unknown. Our previous study has demonstrated that LPS treatment leads to downregulation of ACTH receptor in rat fasciculata-reticularis cells, which seems to be an important underlying mechanism of LPS-induced adrenocortical hyporesponsiveness to ACTH [18]. In the present study, we found that LPS-induced downregulation of ACTH receptor in adrenal glands was reversed by resveratrol treatment. The stimulatory effect of resveratrol on ACTH receptor might, at least partly, explain the beneficial effect of resveratrol on LPS-induced adrenal insufficiency. It is of interest to explore whether SIRT1 signaling pathway contributes to the stimulatory effect of resveratrol on adrenocortical ACTH receptor expression during endotoxemia, which merits future investigation.

In conclusion, the present study has demonstrated for the first time that resveratrol exerts protective effects against endotoxemia-associated adrenocortical insufficiency by suppressing oxidative/nitrative stress. These findings support the potential for resveratrol as a possible pharmacological agent to improve adrenocortical insufficiency resulting from oxidative/nitrative damage.

Conclusions

Resveratrol exerts protective effects against endotoxemia-associated adrenocortical insufficiency by suppressing oxidative/nitrative stress. These findings support the potential for resveratrol as a possible pharmacological agent to improve adrenocortical insufficiency resulting from oxidative/nitrative damage.

Disclosure of Interest

Authors do not have any conflict of interests.

Author Contributions

All experiments were performed at the Department of Physiology and The Key Laboratory of Molecular Neurobiology of Ministry of Education, Second Military Medical University. Xin Ni, and Xiao-Yan Zhu designed the study. Guo-Li Duan and Chang-Nan Wang performed the experiments. Guo-Li Duan and Qin Yu analysed and interpreted the data. Yu-Jian Liu and Xiao-Lu Tang provided supervision and technical and material support. Guo-Li Duan and Xiao-Yan Zhu wrote the draft of the manuscript. All authors critically reviewed the manuscript and approved the final version for publication.

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References

1. Neary N, Nieman L (2010) Adrenal insufficiency: etiology, diagnosis and treatment. Curr Opin Endocrinol Diabetes Obes 17: 217-223.
2. Tsai MH, Peng YS, Chen YC, Liu NJ, Ho YP, et al. (2006) Adrenal insufficiency in patients with cirrhosis, severe sepsis and septic shock. Hepatology 43: 673-681.
3. Lipiner-Friedman D, Sprung CL, Laterre PF, Weiss Y, Goodman SV, et al. (2007) Adrenal function in patients with sepsis, severe sepsis and septic shock. Crit Care Med 35: 1012-1018.
4. Marik PE, Pastores SM, Annane D, Meduri GU, Sprung CL, et al. (2008) Recommendations for the diagnosis and management of corticosteroid insufficiency in critically ill adult patients: consensus statements from an international task force by the American College of Critical Care Medicine. Crit Care Med 36: 1937-1949.
5. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, et al. (2013) Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med 41: 580-637.
6. Malikova J, Fluck CE (2014) Novel insight into etiology, diagnosis and management of primary adrenal insufficiency. Horm Res Paediatr 82: 145-157.
7. Prasad R, Kowalczyk JC, Meimaridou E, Storr HL, Metherell LA (2014) Oxidative stress and adrenocortical insufficiency. J Endocrinol 221: R63-73.
8. Roberts RA, Laskin DL, Smith CV, Robertson FM, Allen EM, et al. (2009) Nitrative and oxidative stress in toxicology and disease. Toxicol Sci 112: 4-16.
9. King RE, Kent KD, Bomser JA (2005) Resveratrol reduces oxidation and proliferation of human reti-
izational pigment epithelial cells via extracellular signal-regulated kinase inhibition. *Chem Biol Interact* 151: 143-149.

10. Knutson MD, Leeuwenburgh C (2008) Resveratrol and novel potent activators of SIRT1: effects on aging and age-related diseases. *Nutr Rev* 66: 591-596.

11. Kuno A, Tanno M, Horio Y (2015) The effects of resveratrol and SIRT1 activation on dystrophic cardiomyopathy. *Ann N Y Acad Sci* 1348: 46-54.

12. Zhang HX, Duan GL, Wang CN, Zhang YQ, Zhu XY, et al. (2014) Protective effect of resveratrol against endotoxemia-induced lung injury involves the reduction of oxidative/nitrite stress. *Pulm Pharmacol Ther* 27: 150-155.

13. Jeon BT, Jeong EA, Shin HJ, Lee Y, Lee DH, et al. (2012) Resveratrol attenuates obesity-associated peripheral and central inflammation and improves memory deficit in mice fed a high-fat diet. *Diabetes* 61: 1444-1454.

14. Wang CN, Liu YJ, Duan GL, Zhao W, Li XH, et al. (2014) CBS and CSE are critical for maintenance of mitochondrial function and glucocorticoid production in adrenal cortex. *Antioxid Redox Signal* 21: 2192-2207.

15. Wang CN, Duan GL, Liu YJ, Yu Q, Tang XL, et al. (2015) Overproduction of nitric oxide by endothelial cells and macrophages contributes to mitochondrial oxidative stress in adrenocortical cells and adrenal insufficiency during endotoxemia. *Free Radic Biol Med* 83: 31-40.

16. Yang T, Mao YF, Liu SQ, Hou J, Cai ZY, et al. (2010) Protective effects of the free radical scavenger edaravone on acute pancreatitis-associated lung injury. *Eur J Pharmacol* 630: 152-157.

17. Jones DP (2006) Redefining oxidative stress. *Antioxid Redox Signal* 8: 1865-1879.

18. Liu S, Zhu X, Liu Y, Wang C, Wang S, et al. (2011) Endotoxin tolerance of adrenal gland: attenuation of corticosterone production in response to lipopolysaccharide and adrenocorticotropic hormone. *Crit Care Med* 39: 518-526.

19. Yun JM, Chien A, Jialal I, Devaraj S (2012) Resveratrol up-regulates SIRT1 and inhibits cellular oxidative stress in the diabetic milieu: mechanistic insights. *J Nutr Biochem* 23: 699-705.

20. Ziolekowska A, Belloni AS, Nussdorfer GG, Nowak M, Malendowicz LK (2006) Endocrine disruptors and rat adrenocortical function: studies on freshly dispersed and cultured cells. *Int J Mol Med* 18: 1165-1168.

21. Supornsilchai V, Svechnikov K, Seidlova-Wuttke D, Wuttke W, Soder O (2005) Phytoestrogen resveratrol suppresses steroidogenesis by rat adrenocortical cells by inhibiting cytochrome P450 c21-hydroxylase. *Horm Res* 64: 280-286.

22. Chandra AK, Ghosh R, Chatterjee A, Sarkar M (2007) Amelioration of vanadium-induced testicular toxicity and adrenocortical hyperactivity by vitamin E acetate in rats. *Mol Cell Biochem* 306: 189-200.

23. Liu S, Zhang XP, Han NN, Lv S, Xiong JY (2015) Pretreatment with low dose etomidate prevents etomidate-induced rat adrenal insufficiency by regulating oxidative stress-related MAPKs and apoptosis. *Environ Toxicol Pharmacol* 39: 1212-1220.

24. Floreani M, Napoli E, Quintieri L, Palatini P (2003) Oral administration of trans-resveratrol to guinea pigs increases cardiac DT-diaphorase and catalase activities, and protects isolated atri from menadione toxicity. *Life Sci* 72: 2741-2750.

25. Moridi H, Karimi J, Sheikh N, Goodarzi MT, Saidijam M, et al. (2015) Resveratrol-Dependent Down-regulation of Receptor for Advanced Glycation End-products and Oxidative Stress in Kidney of Rats With Diabetes. *Int J Endocrinol Metab* 13: e23542.

26. Palsamy P, Subramanian S (2011) Resveratrol protects diabetic kidney by attenuating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via Nrf2-Keap1 signaling. *Biochim Biophys Acta* 1812: 719-731.

27. Gutierrez-Perez A, Cortes-Rojo C, Noriega-Cisneros R, Calderon-Cortes E, Manzo-Avalos S, et al. (2011) Protective effects of resveratrol on calcium-induced oxidative stress in rat heart mitochondria. *J Bioenerg Biomembr* 43: 101-107.

28. Abidi P, Zhang H, Zaidi SM, Shen WJ, Leers-Sucheta S, et al. (2008) Oxidative stress-induced inhibition of adrenal steroidogenesis requires participation of p38 mitogen-activated protein kinase signaling pathway. *J Endocrinol* 198: 193-207.

29. Astort F, Repetto EM, Martinez Calejman C, Cipelli JM, Sanchez R, et al. (2009) High glucose-induced changes in steroid production in adrenal cells. *Diabetes Metab Res Rev* 25: 477-486.

30. Baumgarten G, Kneuermann P, Schuhmacher G, Vrevolgry V, von Rappard J, et al. (2006) Toll-like receptor 4, nitric oxide, and myocardial depression in endotoxemia. *Shock* 25: 43-49.

31. Cohen RI, Hassell AM, Marzouk K, Marini C, Liu SF, et al. (2001) Renal effects of nitric oxide in endotoxia. *Am J Respir Crit Care Med* 164: 1890-1895.

32. Ishikawa K, Calzavacca P, Bellomo R, Bailey M, May CN (2012) Effect of selective inhibition of renal inducible nitric oxide synthase on renal blood flow and function in experimental hyperdynamic sepsis. *Crit Care Med* 40: 2368-2375.

33. La Mura V, Pasarin M, Rodriguez-Vilarrupla A, Garcia-Pagan JC, Bosch J, et al. (2014) Liver sinusoidal endothelial dysfunction after LPS administration: a role for inducible-nitric oxide synthase. *J Hepatol* 61: 1321-1327.

34. Fukuda S, Kaga S, Zhan L, Bagchi D, Das DK, et al. (2006) Resveratrol ameliorates myocardial damage by inducing vascular endothelial growth factor-angiogen-
sis and tyrosine kinase receptor Flk-1. Cell Biochem Biophys 44: 43-49.

35. Kim WT, Suh ES (2010) Retinal protective effects of resveratrol via modulation of nitric oxide synthase on oxygen-induced retinopathy. Korean J Ophthalmol 24: 108-118.

36. Ekshyyan VP, Hebert VY, Khandelwal A, Dugas TR (2007) Resveratrol inhibits rat aortic vascular smooth muscle cell proliferation via estrogen receptor dependent nitric oxide production. J Cardiovasc Pharmacol 50: 83-93.

37. Chung EY, Kim BH, Hong JT, Lee CK, Ahn B, et al. (2011) Resveratrol down-regulates interferon-gamma-inducible inflammatory genes in macrophages: molecular mechanism via decreased STAT-1 activation. J Nutr Biochem 22: 902-909.

38. Mannam P, Zhang X, Shan P, Zhang Y, Shinn AS, et al. (2013) Endothelial MKK3 is a critical mediator of lethal murine endotoxemia and acute lung injury. J Immunol 190: 1264-1275.

39. La Mura V, Pasarin M, Meireles CZ, Miquel R, Rodriguez-Vilarrupla A, et al. (2013) Effects of simvastatin administration on rodents with lipopolysaccharide-induced liver microvascular dysfunction. Hepatology 57: 1172-1181.

40. Hughes CG, Morandi A, Girard TD, Riedel B, Thompson JL, et al. (2013) Association between endothelial dysfunction and acute brain dysfunction during critical illness. Anesthesiology 118: 631-639.

41. Polito A, Lorin de la Grandmaison G, Mansart A, Louise E, Lefebvre H, et al. (2010) Human and experimental septic shock are characterized by depletion of lipid droplets in the adrenals. Intensive Care Med 36: 1852-1858.

42. Jahangir-Hekmat M, Taylor HC, Levin H, Wilbur M, Llerena LA (2004) Adrenal insufficiency attributable to adrenal hemorrhage: long-term follow-up with reference to glucocorticoid and mineralocorticoid function and replacement. Endocr Pract 10: 55-61.

43. Dong WW, Liu YJ, Lv Z, Mao YF, Wang YW, et al. (2015) Lung endothelial barrier protection by resveratrol involves inhibition of HMGB1 release and HMGB1-induced mitochondrial oxidative damage via an Nrf2-dependent mechanism. Free Radic Biol Med 88: 404-416.