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# Platonin mitigates vascular hyporeactivity of thoracic aorta in septic rats

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ABSTRACT

*Background:* Vascular hyporeactivity contributes to hemodynamic alterations and circulatory failure in severe sepsis. Among the identified mechanisms, inflammation and oxidative stress are the most crucial ones in mediating the development of vascular hyporeactivity induced by sepsis. Platonin, a photosensitive dye and an antioxidant, possesses potent antiinflammation effects. We elucidated whether platonin could mitigate vascular hyporeactivity of thoracic aorta in septic rats.

*Material and methods:* Adult male Sprague–Dawley rats were randomized to receive sham operation (Sham), Sham plus platonin (100 μg/kg), cecal ligation and puncture (CLP), or CLP plus platonin (10, 50, or 100 μg/kg) and designated as the Sham, P, CLP, CLP + P(10), CLP + P(50), and CLP + P(100) group, respectively ($n = 6$ in each group). After maintaining for 12 hours, surviving rats were euthanized and thoracic aorta was isolated and vascular reactivity of aortic rings was determined.

*Results:* Vascular reactivity induced by vasoconstrictors phenylephrine and angiotensin II of the Sham and the P groups ($n = 6$ in both groups) were similar, whereas vascular reactivity of the CLP group ($n = 5$) were significantly lower than those of the Sham group (both $P < 0.001$). Of note, vascular reactivity induced by phenylephrine and angiotensin II of the CLP + P(10) group ($n = 5$) and the CLP group were not significantly different. In contrast, vascular reactivity induced by phenylephrine and angiotensin II of the CLP + P(50) and the CLP + P(100) groups ($n = 6$ in both groups) were significantly higher than those of the CLP group (phenylephrine: $P = 0.024$ and 0.017; angiotensin II: $P = 0.031$ and 0.036).

*Conclusion:* Platonin could mitigate vascular hyporeactivity of thoracic aorta in septic rats.



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## Introduction

Platonin, a photosensitive dye, possesses potent antioxidation and antiinflammation effects.[1] Clinical and experimental data have confirmed the beneficial effects of platonin against conditions associated with oxidative stress and/or inflammation.[2-6] For instance, platonin can improve clinical symptoms, mitigate inflammation severity, and maintain the remission state in cases with juvenile rheumatoid arthritis.[2] Experimental data also confirmed that platonin can improve circulatory failure, attenuate organ injuries, and decrease mortality in septic rats.[3,4] In addition, the injuries imposed by hemorrhage or ischemia-reperfusion can also be attenuated by platonin.[5,6]

As mentioned previously, platonin improves circulatory failure in sepsis.[3] Vascular hyporeactivity contributes to hemodynamic alterations and circulatory failure in sepsis.[7] Inflammation and oxidative stress are two crucial mechanisms in mediating the development of vascular hyporeactivity induced by sepsis.[8] In line with this notion, we thus speculated that platonin may very likely mitigate vascular hyporeactivity induced by sepsis.

To elucidate further, we thus conducted this rodent study with the hypothesis that platonin could mitigate thoracic aorta hyporeactivity in septic rats. This study employed a widely used cecal ligation and puncture (CLP) polymicrobial sepsis model[9] to facilitate investigation. Vascular reactivity was measured using an isolated thoracic aortic ring platform, as previously reported.[10] In addition, the levels of inflammation and oxidation of the isolated thoracic aorta were also measured.

## Methods

The animal study protocol was approved by the Animal Use and Care Committee of Taipei Tzu Chi Hospital, Taipei, Taiwan (105-IACUC-013). Care and handling of the animals were in accordance with the National Institutes of Health guidelines. A total of 36 adult male Sprague–Dawley rats (BioLASCO, Taipei, Taiwan; 200-250 gm) were used in this study.

### *Sepsis model*

As aforementioned, this study employed the CLP polymicrobial sepsis model to facilitate investigation. CLP was performed according to previous protocols.[9] In brief, all rats were anesthetized with a ketamine/xylazine mixture (110/10 mg/kg body weight, ipf) and one 1-cm transverse laparotomy was performed at the right lower quarter of the abdominal wall sterilely. Then, the cecum was ligated, and two 0.5-cm blade incisions were made followed by abdominal wall closure. To control the effects of operational procedures, another set of rats received laparotomy, cecum identification, and wound closure, but not CLP (i.e. sham operation). To alleviate would pain, subcutaneous injection of 0.25% bupivacaine (AstraZeneca, Taipei, Taiwan) was performed before wound closure. After recovery, all rats were closely monitored for 12 hours without restraint.

### *Experimental protocol*

Rats were randomized to receive sham operation (Sham), Sham plus platonin (100 μg/kg, Kankohsha Co, Osaka, Japan; dissolved in a mixture of 0.5 mL normal saline), CLP, or CLP plus platonin (10, 50, or 100 μg/kg) and designated as the Sham, P, CLP, CLP + P(10), CLP + P(50), and CLP + P(100) group, respectively ($n = 6$ in each group). Platonin was injected via tail vein immediately after CLP or at the comparable time point in the P group. The dosages of platonin were determined according to a previous report.[3] To control for the effects of vehicle, rats of the Sham and the CLP groups also received injection of 0.5-mL normal saline via tail vein. At 12 hours after intervention, all surviving rats were euthanized with decapitation.

### In vitro *assessment of vascular reactivity*

After euthanization, thoracotomy was performed and the descending thoracic aorta was isolated and the connective tissues were cleaned. The distal portion of the descending aorta was snap frozen in liquid nitrogen and stored at −80°C for subsequent analysis. The proximal portion of the descending aorta was cut into ring-like segments (3-4 mm) and then mounted in tissue bath chambers (Radnoti Four Unit Tissue Organ Bath System, ADInstruments Inc. Colorado Springs, CO) containing Krebs solution (NaCl 115.3 mM, KCl 4.9 mM, $CaCl_2$. $2H_2O$ 1.46 mM, $MgSO_4$ 1.2 mM, $KH_2PO_4$ 1.2 mM, $NaHCO_3$ 25 mM, d-glucose 11.1 mM; pH 7.4)[11] and maintained at 37°C with continuously bubbled with a gas mixture of 95% $O_2$/5% $CO_2$. All chemicals used for Krebs solution were obtained from Sigma-Aldrich (St. Louis, MO). Reactivity of the isolated vascular rings was measured and the tension was recorded using isometric force transducers (MLT0420, ADInstruments) connected to an acquisition system (PowerLab 16/35, ADInstruments) coupled to a recording software (LabChart Data Analysis Software, ADInstruments).

The aortic rings were stabilized under a resting tension of 1.0 g for 60 minutes before measurement. Then the aortic rings were exposed to one of the vasoconstrictors, phenylephrine (1 nM to 100 μM; Sigma-Aldrich) or angiotensin II (1 nM to 100 μM; Sigma-Aldrich), to induce contraction and the contraction obtained was recorded. Then the vasodilator acetylcholine (1 mM; Sigma-Aldrich) was added to induce relaxation. To ensure the integrity of endothelium, only those aortic ring preparations that acetylcholine could induce at least 80% relaxation in vasoconstrictor-induced contraction were used in this study. Each preparation was exposed to only one vasoconstrictor (either phenylephrine or angiotensin II). With the completion of each cycle of contraction and relaxation, the preparations were washed with Krebs solution and were stabilized for 30 min before initiation of the next cycle of contraction and relaxation. Cumulative concentration response curves to vasoconstrictors were then constructed for vascular reactivity analysis.

### *Inflammatory mediator prostaglandin $E_2$ measurement*

Snap frozen thoracic aorta tissues were processed as previously reported.[12] Concentration of prostaglandin $E_2$ ($PGE_2$) of the supernatant of thoracic aorta was assayed using