Polydatin: A New Therapeutic Agent against Multiorgan Dysfunction ^{revised 2015.04.20}

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29 **Author contributions**

30 Zhenhua Zeng and Zhongqing Chen carried out the molecular genetic studies, drafted the

31 manuscript and participated in the design of the study. Tao Li carried out the immunoassays.

32 Junli Zhang and Youguang Gao participated in the animal model study. Siqi Xu and Shumin

33 Cai and performed the Histopathological test and statistical analysis. Ke-seng Zhao conceived

34 of the study, and participated in its design and coordination and helped to draft the

35 manuscript. All authors read and approved the final manuscript.

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Polydatin: A new therapeutic agent against multiorgan dysfunction

Abstract

Background: Polydatin (PD), a monocrystalline and polyphenolic drug isolated from a traditional Chinese herb (*Polygonum cuspidatum*) is protective against mitochondrial dysfunction and has been approved for clinical trials in the treatment of shock. However, whether the administration of PD has a therapeutic effect on multiple organ dysfunction syndrome (MODS) requires investigation.

Material and methods: MODS was induced in Sprague–Dawley rats via hemorrhage and ligation and puncture of cecum (CLP)-induced sepsis. The rats were divided into three groups: MODS+PD, MODS+NS (normal saline), and a control group (no treatment). Survival time, blood biochemical indexes, and histopathological changes in various organs were evaluated; serum oxidative stress (advanced oxidative protein products [AOPPs]) and pro-inflammatory cytokines (tumor necrosis factor- α , interleukin (IL)-1 β , and IL-6) were assayed using ELISA. Apoptosis-related protein expression (Bcl-2 and Bax) was assayed by immunohistochemical and western blotting methods, while caspase-3 activity was assayed by spectrophotometry.

Results: PD improved organ function, prolonged survival time, and reduced MODS incidence and serum levels of AOPPs and pro-inflammatory cytokines. It also decreased Bax levels and caspase-3 activity and increased Bcl-2 levels in the kidney and liver.

Conclusions: PD may serve as a potential therapeutic for MODS, as it suppresses oxidative stress, inhibits inflammatory response, attenuates apoptosis, and protects against mitochondrial dysfunction.

Keywords: Apoptosis; inflammation; multiorgan dysfunction syndrome; oxidative stress; polydatin

1. Background

Polydatin (PD; 3,4,5-trihydroxystibene-3-monoglucoside) is a monocrystalline drug (Schema 1, right) isolated from the traditional Chinese medical herb *Polygonum cuspidatum*. We previously showed that PD restored microcirculation and normalized blood pressure by restoring arterial smooth muscle reactivity following severe shock (1-6). PD is now approved by the Sino Food and Drug Administration for clinical trials, which have entered stage II. We recently demonstrated that PD protects arterial smooth muscle cells and neuronal cells against mitochondrial dysfunction after severe ischemia-reperfusion injury in hemorrhagic shock rats (7-9). In addition, numerous pharmacological investigations of PD have mainly focused on anti-oxidation (10, 11), anti-inflammatory (12-16) and multiple organ protection properties (17). As levels of oxidative stress increase, an overactive inflammatory response, organ dysfunction and mitochondrial damage are common phenomenon in various organs following severe shock. These are closely related to the pathogenesis of MODS. Therefore, we hypothesize that PD could ameliorate multiple organ dysfunction through multiple therapeutic targets. To confirm our hypothesis, we investigated the therapeutic effects and molecular mechanisms of PD in rats with MODS induced by a “two hit” (combination of traumatic hemorrhagic shock and ligation and puncture of cecum [CLP]) (18).

2. Methods

2.1 Reagents and Antibodies

PD was supplied by Neptunus Co. (Shenzhen, Guangdong, China). Its purity is over 99.95%. Antibodies against Bcl-2 and Bax were obtained from Epitomics (Burlingame, CA, USA), while the Caspase 3 Activity Assay Kit and horseradish peroxidase-conjugated secondary antibodies were obtained from Beyotime Biotech (Beijing, China). ELISA kits for inflammatory cytokines (tumor necrosis factor- α [TNF- α], interleukin-1 β [IL-1 β], and

interleukin-6 [IL-6]) and advanced oxidative protein products (AOPPs) were obtained from Dakewe Biotech Company (Shenzhen, Guangdong, China). All other chemicals were from Sigma (St. Louis, MO, USA).

2.2 Animals and MODS Model

This study was conducted in strict adherence with the recommendations of the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health and was approved by the Committee on Ethics in Animal Experiments of the University of Southern Medical, China. Female Sprague-Dawley rats weighing 180–220 g were anesthetized with a mixture of 13.3% urethane and 0.5% intramuscularly chloralose α (0.65 mL/100 g body weight). A PE-50 cannula was placed in right femoral artery for mean arterial blood pressure (MAP) measurement using PowerLAB registration equipment (AD Instruments, Sydney, Australia). Another cannula was placed in the ipsilateral femoral vein for blood withdrawal and drug and blood administration. Then, traumatic hemorrhagic shock was initiated as the “first hit”. Blood was withdrawn into a syringe containing a diluted heparin solution (125 units/mL, 0.1 mL/1 mL blood volume) within 10 min until the MAP stabilized to 45–50 mmHg, and this MAP was maintained for 60 min. The shed blood was then preserved at room temperature without special treatment and was reinfused within 10 min. Two hours later, CLP was performed as the “second hit”, as described previously (19). Briefly, a 2-cm midline abdominal incision was performed. The cecum was exposed, ligated just distal to the ileocecal valve to avoid intestinal obstruction, and punctured twice with an 18-gauge needle. The bowel was then squeezed slightly to allow a small amount of fecal matter to flow from the holes and then returned to the abdominal cavity. The abdomen was closed in layers with sutures. Sham-operated animals underwent the same procedure with the exception that the cecum was neither ligated nor punctured. The individual who performed the CLP was blinded

to the final therapy. In total, 96 rats were divided into two main groups. One group (48 rats) was used to observe the survival time, and the other (48 rats) was used to study the mechanism of action of PD in MODS treatment. The rats in these groups were further divided into three groups. (1) rats in the control (sham) group were anesthetized and operated on without any other treatment (n = 16); (2) Those in the MODS+NS group were subjected to hemorrhage and CLP as described above, followed by administration of 0.3 mL normal saline (NS) every 6 h for 18 h (the first instance was immediately after CLP; a total of four injections were administered, n = 16); and (3) rats in the PD group were subjected to hemorrhage and CLP as described above and were then administered different doses of PD (15, 30, 45, and 60 mg/kg body weight) every 6 h for 18 h (four injections in all, n = 16 in each PD group). On the basis of our previous experiments (20), PD was dissolved in warm NS (0.3 mL) and administered intravenously.

2.3 Survival Study

After induction of MODS and initial drug administration, 48 rats (8 from each subgroup) were returned to individual cages, and their survival was monitored once an hour until 48 h. To minimize suffering, an i.p. injection of pentobarbital sodium (30 mg/kg) was performed intermittently in conscious animals. All animals had access to food and water *ad libitum*. Apnea for >1 min was considered to indicate the death of the animal. In addition, the remaining animals that survived over the 48-h study period were euthanized by cervical dislocation.

2.4 Blood Biochemical Tests, Arterial Blood Gas Analysis, and MODS Incidence

Another 48 rats (8 from each subgroup) were sacrificed at 24 h after CLP (they were all alive before they were sacrificed) and 0.5 mL arterial blood and 1.5 mL venous blood were

collected for serum analyses, oxidative stress determination, apoptosis assays, and histology. Venous blood samples were centrifuged for serum separation. One part of each serum sample was frozen at -80°C for a subsequent oxidative stress assay. The other part was assayed for biochemical variables by using an Automatic Biochemical Analyzer (Olympus AU5400, Tokyo, Japan). The assayed variables included markers of liver, kidney, heart, and lung function. Organ dysfunction was established on the basis of the standardized criteria proposed in 1995 by Marshall et al., and MODS was defined as dysfunction of two or more organs (18).

The optimal PD dose as determined from the measured variables was used in subsequent blood analyses and oxidative stress determinations, apoptosis assays, and histology.

2.5 Serum Oxidative Stress Assay

As a novel oxidative stress biomarker, advanced oxidation protein products (AOPPs) were detected in the plasma of patients with chronic uremia. It was suggested that AOPP measurements include highly oxidized proteins, especially albumin. Recent data in turn appear to indicate that oxidized fibrinogen is the key molecule that is responsible for the AOPP reaction in human plasma. As fibrinogen is an acute-phase reactant, it is evident that the antioxidant capacity of plasma is enhanced during each episode of inflammatory response (21). Thus, the AOPPs were selected as an oxidative stress index. The AOPPs Assay Kit was used for direct quantitative measurement of AOPPs in the serum samples. All procedures were performed in accordance with the protocol recommended by the manufacturer.

2.6 Measurement of Serum Levels of Inflammatory Cytokines

The frozen serum samples were analyzed to determine the concentrations of $\text{TNF-}\alpha$, $\text{IL-1}\beta$, and IL-6 using commercially available ELISA kits according to the manufacturer's

recommendations.

2.7 Histopathological Analysis and Pathological Scores

Kidney, liver, and lung tissue sections were prepared for hematoxylin-eosin (HE) staining and scored blindly under light microscopy (Imager Z2, Carlzeiss, Jena, Germany). Quantitative scoring standards based on standards for histopathology scores were used (22). The pathological scores of each tissue ranged from 0 to 3, where normal findings were evaluated as grade 0. More serious tissue damage conferred the higher scores.

2.8 Immunohistochemistry

The expression of Bcl-2 and Bax in the tissues was visualized using the immunohistochemical Envision method (Dako, Copenhagen, Denmark) with rabbit polyclonal anti-rat antibodies. The working concentrations of the anti-Bcl-2 and anti-Bax antibodies were 1:1000 and 1:1000, respectively.

2.9 Western Blot Analysis

Kidney and liver tissue samples were prepared for western blot analysis performed according to the standard method. GAPDH was used as the reference gene for normalization.

2.10 Caspase-3 Activity Analysis

The Caspase 3 Activity Assay Kit was used to measure caspase-3 activity in kidney and liver tissues. Tissue homogenates were dissolved in lysate buffer at different concentrations for protein determination. The change in absorbance due to cleavage of the colorless substrate specific for caspase-3 (Ac-DEVDp-nitroaniline) was determined at 405 nm by spectrophotometry.

2.11 Statistical Analysis

The median survival time was analyzed using Kaplan-Meier plots and compared using the log-rank test. Other results are expressed as means $\pm$ standard deviation values, and statistical analysis was performed using one-way analysis of variance followed by Tukey's multiple comparison test using the SPSS software (SPSS, Inc., Chicago, IL, USA). Values were considered significant at $P < 0.05$.

3. Results

3.1 PD Prolonged Survival Time and Reduced MODS Incidence

All animals in the control group survived for the entire 48 h observation period. The median survival times for rats administered different doses of PD were longer than those of rats in the MODS+NS group, and a dose-response relationship was noted; a PD dose of 45 mg/kg body weight showed the best therapeutic effect. In this latter group, the median survival time was markedly longer than that in the MODS+NS group (48 h vs. 29 h, $P < 0.01$). Further, 7 of the 8 rats in this group survived for 48 h, while none of the 8 rats in the MODS+NS group survived for 48 h ($P < 0.01$; Fig. 1). The values for all measured blood biochemical parameters (blood urea nitrogen [BUN], creatinine [Cr], total bilirubin, lactate dehydrogenase, alanine aminotransferase [ALT], and aspartate aminotransferase [AST]) were significantly higher in the MODS+NS group than the control group ($P < 0.05$ for all). On the basis of previously proposed MODS criteria (23), values that exceeded normal values by two-fold or more were selected for indices of organ dysfunction. In the MODS+NS group, the serum levels of ALT and AST were 4.1 and 7.2 times higher than those in the control group, respectively, and the BUN and Cr levels were 1.7 and 3.3 times higher than those in the control, respectively. Collectively, these indices showed the existence of liver and renal

dysfunction. However, although the respiratory function in the MODS+NS group was decreased, it was not at the level of respiratory dysfunction (pO₂, 78.5 mmHg) (Table 1). Therefore, MODS with two-organ dysfunction was observed. The incidence of MODS in the group that received 45 mg/kg body weight PD group was 1/8 and was considerably lower than the incidence of 6/8 observed in the MODS+NS group (Table 2). Thus, 45 mg/kg body weight (a total of four injections) PD was used in subsequent experiments.

3.2 Therapeutic Effects of PD on MODS

PD Improved Kidney, Liver, and Lung Histopathological Changes in MODS Rats

Significant pathological changes were found in the kidney, liver, and lungs of rats in the MODS+NS group. Proximal tubular epithelial cells in the kidney showed cloudy swelling and degeneration with a narrowed lumen and protein deposition. Some regions of the proximal tubular epithelium were absent (Fig. 2 II). Several hepatic central veins were narrowed with sinusoidal dilatation and congestion, the hepatocyte cytoplasm was cloudy and swollen, and many hepatocytes had ballooned or were degenerated (Fig. 2 V). In the lung tissue, pulmonary interstitial edema with alveolar collapse was observed, and numerous inflammatory cells had infiltrated the interstitium (Fig. 2 VII). PD alleviated these pathological changes although slight swelling of the renal tubular epithelial cells and liver cells persisted (Fig. 2 III, VI). The pathological scores of the kidneys, livers, and lungs in the MODS+PD group were 45%, 50%, and 40% lower in the PD group, respectively ($P < 0.01$, $P < 0.05$, and $P < 0.05$, respectively) (Fig. 3).

3.3 PD Reduced Serum Levels of AOPPs in MODS Rats

Compared to the control rats, the MODS rats had significantly higher serum levels of AOPPs ($P < 0.05$). The AOPP level was $44.55 \pm 10.83 \mu\text{M}$ in the MODS+NS group, and it reduced

by 24% in the MODS+PD group ($33.66 \pm 7.70 \mu\text{M}$, $P < 0.05$; Fig. 4).

3.4 PD Reduced Proapoptotic Protein Expression and Activity in MODS Rats

Immunohistochemical and western blot analysis showed that Bax expression in the kidney and liver of MODS+NS rats was considerably higher than that in the control rats. However, Bax expression in the kidney and liver of MODS+PD rats was lower than that in the MODS+NS rats ($P < 0.05$) (Fig. 5A-B). Bcl-2 expression in the kidneys and livers of MODS+NS rats was considerably lower than that in the control group. However, it was significantly higher in the MODS+PD group than the MODS+NS group ($P < 0.05$) (Fig. 5C-D).

Using ELISA, caspase-3 activity was assayed in kidney and liver homogenates 24 h after MODS induction. Compared to the control group, caspase-3 activity was higher in the MODS+NS group and significantly lower in the MODS+PD group ($P < 0.01$ vs. MODS+NS group) (Fig. 6).

3.5 PD Reduced Serum Levels of Pro-inflammatory Cytokines in MODS Rats

Serum levels of the inflammatory cytokines IL-1, IL-6, and TNF- α were assayed using ELISA 24 h after MODS induction. Compared to the control group, the MODS+NS group showed significantly higher levels of all three cytokines. In contrast, the levels were slightly lowered in the MODS+PD group (Fig. 7).

4. Discussion

Trauma and hemorrhage can cause SIRS, which can be especially lethal if it is further complicated by a second pathology, such as sepsis. The concept of a two-hit insult to explain the development of MODS in trauma patients has gained popularity in recent years (24). The

initial injury acts as the first hit, which primes the immune system while rendering the host susceptible to subsequent infections (18). In our study, we used a severe but nonlethal level of blood loss with no resuscitation as the first hit. Hemorrhagic shock led to microcirculatory disorders, general hypoxia and ischemia-reperfusion injury. This was followed by CLP to induce polymicrobial sepsis, which led to peritonitis, sepsis and a systemic inflammatory response syndrome. This “two hit” rat model of MODS does not mimic all of the features of MODS that are present clinically, but could help us to explore the pathogenesis and therapeutic methods available for MODS. Finally, all these conditions collectively led to a MODS model including renal and liver dysfunction.

Using this model, we showed that PD treatment significantly prolonged survival time and reduced the incidence of MODS, showing dose-dependent efficacy. In the group that received 45 mg/kg body weight PD, the 48 h survival rate was 7/8, which was substantially higher than the 0/8 rate observed in the MODS+NS group; further, the incidence of MODS was 1/8, which was substantially lower than the incidence of 6/8 observed in the MODS+NS group. Collectively, the results indicate that PD may be an effective drug for MODS treatment.

In the next stage of the study, the mechanism of action of PD against MODS was investigated. First, we found that blood AOPP levels were increased in MODS rats, possibly because the release of reactive oxygen species during hypoxia and the ischemia-reperfusion process. These levels then reduced after PD treatment, probably because of the antioxidant properties of PD attributable to its molecular structure, which includes a conjugated double bond and several —OH groups (28-30). It has been demonstrated that the 4'-hydroxyl in resveratrol is the most reactive in scavenging free radicals because the phenoxyl radical can delocalize the unpaired electron on the entire molecule (31) (Schema 1). Based on the same theory, substitution of this hydroxyl in resveratrol by a glycoside group in PD increases the radical's steric hindrance and prevents its reaction with another molecule of polydatin (also

named piceid) (31).

Second, we found that the blood levels of pro-inflammatory cytokines (namely, TNF- α , IL-1, and IL-6) were enhanced in MODS rats because of sepsis and SIRS caused by CLP. However, they decreased with PD treatment, possibly because of the anti-inflammatory actions of PD brought about by the decrease in NF- κ B expression (7, 32, 33).

Third, we found that the Bax/Bcl-2 ratio and caspase-3 activity in the kidney and liver were increased in MODS rats. The increase in the Bax/Bcl-2 ratio reflected the release of cytochrome c from the mitochondria into the cytosol, which led to caspase-3 activation with cell apoptosis and histopathologic changes in the kidney and liver. PD treatment attenuated pro-apoptotic protein expression and reduced the pathological injury.

It has been reported that the dose of 10 mg/kg body weight of PD had a half-life of 4 h (25, 26). Furthermore, resveratrol, an analogue of PD (with a similar half-life elimination rate) given at 6, 12, and 18 h post-CLP treatment (sepsis model) significantly improved the survival rate of mice with sepsis (27), and had a better therapeutic effect than a single dose. Thus, three doses of PD were administered in our study with intervals of 6 h.

Our study had some limitations. First of all, the survival time was observed over only 48 h, as all the animals in the shock + NS group died within 48 h; there were significant differences between the PD groups and the shock group over this time period, and rats were sacrificed at this stage to prevent further suffering. Furthermore, as a consequence of the large workload in this study, we only analyzed the effects of PD on the functions of three organs (kidney, liver and lung). Other important organs, including the heart, peripheral vascular tissue, gastrointestinal tract, brain and coagulation system, should be investigated in future studies to explore the effects of PD more precisely.

5. Conclusions

PD may be a potential therapeutic for MODS, because it has diverse pharmacologic effects that counter each event in the development of MODS. The mechanisms of action of PD may be linked to the suppression of oxidative stress, reduction of inflammatory response, attenuation of apoptosis, and protection against mitochondrial injury.

6. Author Contribution

Zhenhua Zeng and Zhongqing Chen carried out the molecular genetic studies, drafted the manuscript and participated in the design of the study. Tao Li carried out the immunoassays. Junli Zhang and Youguang Gao participated in the animal model study. Siqi Xu and Shumin Cai and performed the Histopathological test and statistical analysis. Ke-seng Zhao conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

7. Acknowledgements

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414

Figure Legends

Schema 1. Molecular structure of resveratrol (left) and polydatin (right).

Fig. 1. Cumulative survival of MODS rats. After the induction of MODS, 48 rats were returned to individual cages, and their survival was monitored once an hour. All animals had access to food and water *ad libitum*. Apnea for >1 min was considered to indicate the death of the animal, and the surviving animals over 48 h were euthanized by cervical dislocation. The median survival time was found to be longer in the PD groups than the MODS+NS group, and 45 mg/kg body weight PD showed the best results. MODS+NS = MODS + normal saline; MODS+PD15 = MODS + 15 mg/kg body weight of PD; MODS+PD30 = MODS + 30 mg/kg body weight of PD; MODS+PD45 = MODS + 45 mg/kg body weight of PD; MODS+PD65 = MODS + 60 mg/kg body weight of PD. N = 8 per group.

Fig. 2. Pathological changes in the kidney, liver, and lung at 24 h following MODS induction. The kidney, liver and lung tissues were stained with hematoxylin and eosin. No pathological alterations were observed in the control group (I, IV, and VII), whereas significant changes were observed in the kidneys (II), livers (V), and lungs (VIII) of the MODS+NS group; these changes were apparently attenuated in the MODS+PD group (III, VI, IX. $\times 200$).

Fig. 3. Pathological scores of the kidney (A), liver (B), and lung (C) at 24 h following the induction of MODS. The degree of multiple organ damage was scored blindly using ten randomly selected fields (original magnification $\times 200$). N = 8 per group, *P < 0.05 vs. control group; #P < 0.05 vs. MODS+NS group.

Fig. 4. Serum levels of AOPPs 24 h following MODS induction. The AOPP levels

significantly increased in the MODS+NS group, and they reduced in the MODS+PD group.
N = 8 per group, *P < 0.05 vs. control group; #P < 0.05 vs. MODS+NS group.

Fig. 5. Bax and Bcl-2 expression levels in the kidney and liver tissue at 24 h following the induction of MODS. (A) Immunohistochemical demonstration of the pro-apoptotic factor Bax in kidney, liver, and lung tissues 24 h following MODS induction ($\times 200$); (B) Western blotting measurement of Bax in kidney and liver tissues 24 h following MODS induction. Bax levels in the MODS+NS group were higher than those in the control group, and they reduced significantly in the MODS+PD group; (C) Immunohistochemical demonstration of the anti-apoptotic factor, Bcl-2, in the kidney, liver, and lung tissues 24 h after the induction of MODS ($\times 200$); (D) Western blot assays of Bcl-2 expression in renal and liver tissue specimens 24 h following the induction of MODS. Bcl-2 levels in the MODS+NS group were lower than those in the control group, and increased significantly in the MODS+PD group; (E) Caspase-3 activity in the kidney and liver 24 h after the induction of MODS. Caspase-3 activity was higher in the MODS+NS group and significantly reduced in the MODS+PD group ($P < 0.01$ vs. MODS+NS group). GAPDH was used as the reference for western blotting. N = 8 per group. *P < 0.05, *P < 0.01 vs. control group; #P < 0.05, ##P < 0.01 vs. MODS+NS group.

Fig. 6. Kidney and liver tissue caspase-3 activity following the induction of MODS. Caspase-3 activity was higher in the MODS+NS group and was significantly reduced in the MODS+PD group ($P < 0.01$ vs. MODS+NS group). N = 8 per group. **P < 0.01 vs. control group; #P < 0.05 vs. MODS+NS group; ##P < 0.01 vs. MODS+NS group.

Fig. 7. Serum levels of inflammatory cytokines (IL-1 β , IL-6, and TNF- α) 24 h following

465 MODS induction. Compared to those in control group, the levels of all three cytokines were
466 higher in the MODS+NS group and were significantly restored in the MODS+PD group. N =
467 8 per group. **P < 0.01 vs. control group; #P < 0.05 vs. MODS+NS group; ##P < 0.01 vs.
468 MODS+NS group.

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Table 1. Effects of PD on blood biochemical parameters in MODS rats

Organ	N	Index	Control	MODS+NS	MODS+PD (mg/kg body weight)			
					15	30	45	60
Kidney	6	BUN (μmol/L)	8.2 ± 0.8	16.2 ± 3.1**	15.2 ± 3.9**	14.2 ± 3.9**	12.2 ± 3.9*#	13.1 ± 4**
	6	Cr (U/L)	22.3 ± 6.4	37.1 ± 2.9**	35.7 ± 3**	31.9 ± 2.8**#	25.6 ± 2.9###	30.6 ± 2.7***
Liver	6	AST (U/L)	48.5 ± 19.4	200.4 ± 44.1**	189.3 ± 42.3**	175.5 ± 36.0**	148.4 ± 38.1***#	158 ± 41.3**
	6	ALT (U/L)	75.9 ± 8.8	545.5 ± 33.7**	538.5 ± 27.3**	522.5 ± 32.5**	486.9 ± 61.8***#	498.4 ± 34.2**
	6	TBIL (μmol/L)	0.4 ± 0.1	2.7 ± 0.5**	2.5 ± 0.4**	2.1 ± 0.2***##	1.5 ± 0.2***##	1.6 ± 0.4***##
	6	LDH (U/L)	204.9 ± 60.2	417.6 ± 66.9**	405.3 ± 42**	368.6 ± 46.7**	355.7 ± 14.4***#	364.5 ± 33.8**
Lung	8	pH	7.4 ± 0.1	7.2 ± 0.2**	7.2 ± 0.2**	7.3 ± 0.2	7.3 ± 0.1	7.3 ± 0.2
	8	pO ₂ (mmHg)	98.1 ± 1.4	78.5 ± 4.6**	79.8 ± 2.1**	82.4 ± 4***##	83.50 ± 2.7***##	81.4 ± 1.8***#
	8	pCO ₂ (mmHg)	37.5 ± 4.1	43.9 ± 3.4**	45.8 ± 1.8**	42.4 ± 1.5**	42.8 ± 2.1**	43.6 ± 1.9**

470 Data are mean ± SD. Blood urea nitrogen: BUN; Creatinine: Cr; Total bilirubin: TBIL; Lactate dehydrogenase: LDH; Alanine aminotransferase:

471 ALT; Aspartate aminotransferase: AST. P < 0.05 for all. *P < 0.05, **P < 0.01 vs. control group; # P < 0.05, ##P < 0.01 vs. MODS+NS group.

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Table 2. Effects of PD on survival time and MODS incidence following MODS induction

Group	Incidence			Survival		
	Liver dysfunction	Renal dysfunction	MODS (two-organ dysfunction)	Median survival time (h)	Survival rate 24 h	Survival rate 48 h
Control	0/8	0/8	0/8	48	8/8	8/8
MODS+NS	8/8	6/8	6/8**	29**	8/8	0/8**
MODS+PD15	7/8	5/8	5/8**	31**	8/8	1/8**
MODS+PD30	5/8	4/8	3/8**	37#	8/8	2/8**
MODS+PD45	3/8	1/8	1/8#	48##	8/8	7/8##
MODS+PD60	4/8	3/8	3/8**	36#	8/8	2/8**

**P < 0.01 vs. control group; # P < 0.05, ## P < 0.01 vs. MODS+NS group. N = 8 in each group. MODS+PD15, MODS+PD30, MODS+PD45, MODS+PD60 = 15, 30, 45, and 60 mg/kg body weight PD.

Schema 1

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

A

B

Figure 7