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Polydatin: a new therapeutic agent against multiorgan dysfunction

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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–induced sepsis. The rats were divided into three groups as follows: MODS + PD, MODS + normal saline, and a control group (no treatment). Survival time, blood biochemical indexes, and histopathologic changes in various organs were evaluated; serum oxidative stress (advanced oxidative protein products [AOPPs]) and proinflammatory cytokines (tumor necrosis factor-α, interleukin 1β, and interleukin 6) were assayed using enzyme-linked immunosorbent assay. Apoptosis-related protein expression (B-cell lymphoma-2 [Bcl-2] and Bax) was assayed by immunohistochemical and Western blotting methods, whereas 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 proinflammatory 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.

1. Background

Polydatin (PD; 3,4′,5-trihydroxystibene-3-monoglucoside) is a monocrystalline drug (Schema 1, left) 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 after severe shock [1–6]. PD is now approved

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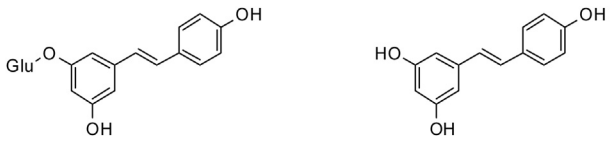

Schema 1 – Molecular structure of PD (left) and resveratrol (right).

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 pharmacologic investigations of PD have mainly focused on antioxidation [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 after severe shock. These are closely related to the pathogenesis of multiple-organ dysfunction syndrome (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" model (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 B cell lymphoma 2 [Bcl-2] and Bax were obtained from Epitomics (Burlingame, CA), whereas the Caspase 3 Activity Assay Kit and horseradish peroxidase–conjugated secondary antibodies were obtained from Beyotime Biotech (Beijing, China). Enzyme-linked immunosorbent assay (ELISA) kits for inflammatory cytokines (tumor necrosis factor-α [TNF-α], interleukin [IL]-1β, and 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).

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 the 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 U/mL, 0.1 mL/1 mL blood volume) within 10 min until the MAP stabilized to 45–50 mm Hg 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 + normal saline (NS) group were subjected to hemorrhage and CLP as described previously, followed by administration of 0.3-mL 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 previously 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 intraperitoneal 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