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Polydatin ameliorates renal ischemia/reperfusion injury by decreasing apoptosis and oxidative stress through activating sonic hedgehog signaling pathway

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ABSTRACT

Polydatin, a glucoside of resveratrol, recently has been demonstrated possibly to exert its biological effects by targeting sonic hedgehog (Shh). However, whether Shh signaling pathway is involved in the therapeutic effects of polydatin for renal ischemia/reperfusion (I/R) injury has not been evaluated. Our results showed that I/R induced the secretion of Shh, upregulated Patched and Smoothened, and enhanced the nuclear translocation and target gene transcription of Glioblastoma 1 in renal I/R injury models, which were further upregulated after the administration of polydatin significantly and in turn exerted prominent nephroprotective effects against cell apoptosis and oxidative stress. The treatment with cyclopamine (a specific inhibitor of Smoothened) or 5E1 (an anti-Shh antibody) not only markedly inhibited the activation of the Shh pathway, but also dramatically suppressed the nephroprotective effects of polydatin above-mentioned. These results advance our knowledge that polydatin can provide protection for kidneys against I/R injury by enhancing antioxidant capacity and decreasing cell apoptosis through activating Shh signaling pathway.

1. Introduction

Renal ischemia/reperfusion (I/R), which occurs under various clinical circumstances such as kidney transplantation, trauma, sepsis and hypovolemic shock, not only contributes to the life-threatening acute renal failure but also dampens the long-term prognosis of patients (Liu et al., 2012; Yoshida and Honma, 2014). Oxidative stress induced by excessive production of reactive oxygen species plays critical role in renal I/R injury by initiating subsequent lipid peroxidation, deoxyribonucleic acid, and protein damage, which resulting in cell apoptosis in ischemic tissues (Munshi et al., 2011; Xia et al., 2014). Therefore, developing adjunctive therapeutic modalities to reduce oxidative stress may be an effective treatment in attenuating renal I/R injury.

Polydatin ($C_{20}H_{22}O_8$, 3,4′,5-trihydroxystibene-3-β-mono-D-glucoside, Fig. 1a), a glucoside of resveratrol, is a polyphenolic phytoalexin extracted from the dried roots of the perennial herb *Polygonum Cuspidatum Sieb.* Et Zucc (Du et al., 2013; Kitada and Koya, 2013). It has been validated in many studies that polydatin exhibited beneficial effects in ischemic organs such as heart, brain, lungs after I/R injury (Deng et al., 2012; Ji et al., 2012b; Li et al., 2014). Recently, our study have found that polydatin exerts nephroprotective effects by anti-oxidative stress and anti-inflammation mechanisms in renal I/R injury models (Liu et al., 2015). However, the mechanism behind polydatin-induced nephroprotection is not completely understood.

The ischemic tissue produces numerous chemokines, cytokines, growth factors and secreted proteins that may influence organ-specific repair (El Sabbahy and Vaidya, 2011). Sonic hedgehog (Shh), the most studied member of the hedgehog (Hh) signaling pathway ligands, is a secreted extracellular signal protein that participates in a series of cellular processes such as differentiation,

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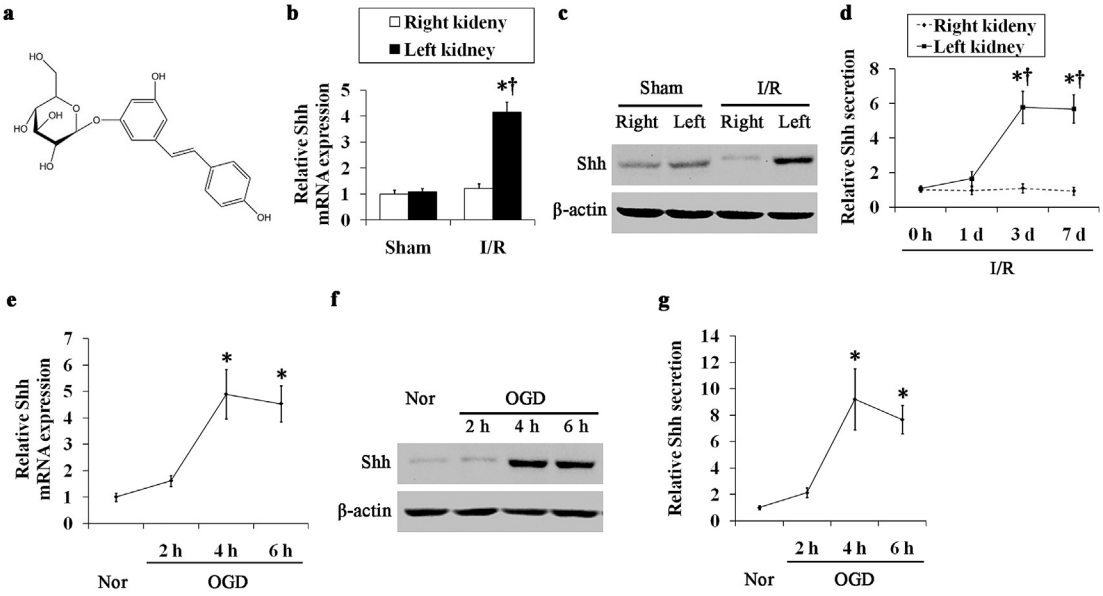

Fig. 1. Hypoxia increased the Shh expression in vitro and in vivo. (a) Chemical structure of polydatin. (b) Shh mRNA level in the ischemic (left) and contralateral (right) kidneys of Sham- and I/R-mice (n = 10 per group). $^{*}P < 0.05$ vs sham; $^{\dagger}P < 0.05$ vs right kidney. (c) Western blot analysis for Shh protein isolated from the left and right kidneys of Sham- and I/R-mice (n = 10 per group). (d) ELISA analysis for Shh protein at the indicated periods of post-surgery time (n = 10 per group). $^{*}P < 0.05$ vs I/R 0 h; $^{\dagger}P < 0.05$ vs right kidney. (e) Shh mRNA level in primary RTECs treated by OGD for 2, 4, and 6 h $^{*}P < 0.05$ vs normoxia. (f and g) Western blot analysis (f) and ELISA analysis (g) for Shh protein in primary RTECs treated by OGD for 2, 4, and 6 h $^{*}P < 0.05$ vs normoxia. Shh, Sonic hedgehog; I/R, ischemia/reperfusion; RTECs, renal tubular epithelial cells; Nor, normoxia; OGD, oxygen-glucose deprivation.

proliferation, and apoptosis in variety tissues and plays fundamental role in regulating proper embryonic development in mammals (Bambakidis and Onwuzulike, 2012; Choudhry et al., 2014). Apart from, recently, it has been demonstrated that Shh cascade was involved in the regeneration of injured tissues, including kidney (Ding et al., 2013; He et al., 2013; Ji et al., 2012a; Ozturk et al., 2007). In normal adult kidneys, the Shh gene is relatively quiescent, and its expression is extremely low and barely detectable (Zhou et al., 2014). However, Shh is clearly induced in some ischemic tissues, regulating a diverse array of biologic processes, such as anti-apoptosis, anti-oxidative stress, and pro-vascularization, to promote the injury repair after tissue damage (Dai et al., 2012; Ghanizadeh, 2012; Huang et al., 2013; Ji et al., 2012a; Li et al., 2013a; Zhou et al., 2014). Most recently, it has been demonstrated by several studies that polydatin and its aglycone, resveratrol, regulated Shh signaling pathway to exert various biological activities in different models (Cheng et al., 2015; Ji et al., 2012b; Meng et al., 2015; Zhou et al., 2015), which suggested that Shh signaling pathway is promising to be a new therapeutic target of polydatin in treating various diseases. However, the effects of Shh signal pathway in acute renal I/R injury still remains unknown; especially, the role of the Shh signal pathway in the therapeutic effects of polydatin for renal I/R injury has not been evaluated in vivo. On the basis of these previous findings, we hypothesized that polydatin exerting its beneficial effects on treating I/R injury might be associated with the activation of Shh signaling pathway.

In this study, we demonstrate, for the first time, that polydatin contributes to protection against renal I/R injury by anti-apoptosis and anti-oxidative stress through regulating Shh signal pathway.

2. Materials and methods

2.1. Ethics statement

Male BALB/c mice (7–9 weeks of age, weight 20–25 g) were provided by the Experimental Animal Center of the Fourth Military Medical University (Xi'an, China), and bred in an experimental animal room of specific-pathogen-free grade. All animal experiments were carried out in strict accordance with the Guidelines of National Institute of Health for the Care and Use of Laboratory Animals, and approved by the Scientific Investigation Board of the Forth Military Medical University. All efforts were made by us to reduce the number of animals and to minimize animals' suffering in this study.

2.2. Cell culture and treatment

Primary renal tubular epithelial cells (RTECs) were isolated as previously described (Yuan et al., 2011). Briefly, kidneys were flushed in situ with 0.9% NaCl to eliminate blood, then removed from adult BALB/c mice, and cortex tissue was excised, minced into pieces of approximately 1 mm^3, and digested for 30 min at 37 °C in 0.5 mg/ml collagenase (Sigma) and washed through a 40 μM cell strainer (BD, San Jose, USA) in renal epithelial growth medium (Lonza, Walkersville, MD). After centrifugation at 300 *g* for 5 min, the pellet was resuspended in renal epithelial growth medium supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (FBS, Invitrogen), and incubated at 37 °C for 24 h. Then, cell flasks were washed several times using phosphate buffer saline (PBS) to remove unattached cells, and added fresh culture medium into them. RTECs were removed from the flasks at day 5 by brief incubation in trypsin/Ethylene Diamine Tetraacetic Acid (EDTA). In accord with previous study reported, RTECs prepared as above were identified by morphology and specific expression of an epithelial marker, cytokeratin 18 (CK18) by using an anti-CK18 antibody (Santa Cruz).

In accordance with our previous report (Liu et al., 2014), we use oxygen-glucose deprivation (OGD) followed by reoxygenation (OGD/R) to simulate an in vitro model of I/R injury. Briefly, cells were first exposed to OGD for 2, 4, 6 h and then resumed the supply of oxygen, glucose and FBS for 24 h. Cells for OGD treatment were cultured in glucose-free medium without FBS in a hypoxic chamber