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Novel resveratrol analogues attenuate renal ischemic injury in rats

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ABSTRACT

Background: Renal ischemia–reperfusion (I/R) is a severe clinical complication with no specific treatment. Resveratrol has been shown as a promising experimental agent in renal I/R due to its effect on cellular energy metabolism, oxidative stress, and inflammation. Recently, we identified two biologically active resveratrol analogues (RSVAs), RSVA405 and RSVA314. We hypothesized that both RSAVs would attenuate I/R-induced renal injury.

Methods: Adult male rats were subjected to renal I/R through bilateral renal pedicle clamping for 60 min, followed by reperfusion. RSVA405 (3 mg/kg Body Weight), RSVA314 (3 mg/kg Body Weight), or vehicle (10% dimethyl sulfoxide and 33% Solutol in phosphate buffered saline) were administered by intraperitoneal injection 1 h before ischemia. Blood and renal tissues were collected 24 h after I/R for evaluation.

Results: Administration of RSVA405 and RSVA314 significantly reduced the serum levels of renal dysfunction and injury markers, including creatinine, blood urea nitrogen, aspartate aminotransferase, and lactate dehydrogenase, compared with vehicle. The protective effect of RSVA405 and RSVA314 was also reflected on histologic evaluation. Both RSVAs reduced the number of apoptotic cells by more than 60% as determined by transferase dUTP nick end labeling assay, compared with vehicle. The renal adenosine triphosphate levels of the vehicle group was decreased to 52.4% of control, whereas those of the RSVA405 and RSVA314 groups were restored to 72.3% and 79.6% of control, respectively. Both RSVAs significantly reduced the protein expression of inducible nitric oxide synthase and nitrotyrosine and the messenger RNA levels of tumor necrosis factor-α, interleukin-6, and interleukin-1β.

Conclusions: RSVA405 and RSVA314 attenuate I/R-induced renal injury through the modulation of energy metabolism, oxidative stress, and inflammation.

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1. Introduction

Renal ischemia–reperfusion (I/R) injury is a severe complication commonly occurring during major vascular surgery and is unavoidable in renal transplantation [1–4]. It is the most common cause of acute kidney injury (AKI) in the surgical intensive care unit. Patients with AKI are at an increased risk for chronic kidney disease, end stage renal disease, and mortality [5]. Furthermore, the severity of AKI is predictive of the likelihood for progression to chronic kidney disease [6]. The treatment of AKI in the clinical setting is limited to reversal of the causal insult and managing the complications of decreased renal function [7]. Therefore, the identification of novel therapeutic agents is of priority for this disease.

The pathophysiology of renal I/R can be described as an early energy deficit during ischemia, which is followed by a secondary phase of oxidative injury, inflammation, and metabolic dysfunction during reperfusion [8]. Resveratrol is a polyphenol, which has significant protective effects against I/R injury of various organs [9–14]. Specifically, resveratrol has been shown to be protective in several animal models of renal I/R-induced injury through its antioxidant and anti-inflammatory effects [15–20]. Interestingly, resveratrol is also an activator of the metabolic enzymes sirtuin 1 (Sirt1) and 5′ adenosine monophosphate-activated protein kinase (AMPK) [21]. Pharmacologic activation of these two enzymes has been shown to be protective in renal I/R-induced injury through the enhancement of renal energy metabolism [11,22–24].

Although the beneficial effects of resveratrol have been demonstrated *in vitro* and in animal studies, the poor bioavailability of this molecule has been a major concern in human studies [25]. To identify alternatives, we have screened several molecules with structural similarity to resveratrol as previously described [26]. RSVA405 and RSVA314 were shown to activate AMPK 50 times more potently than resveratrol [26,27]. In addition, RSVA405 and RSVA314 were also shown to have an anti-inflammatory effect through the inhibition of STAT3 function [27,28]. In this study, we hypothesized that administration of these two newly identified resveratrol analogues (RSVAs) would be protective against I/R-induced renal injury. To test this hypothesis, we used a previously established rodent model of bilateral renal I/R injury [29]. Animals subjected to renal I/R were pretreated with RSVA405 or RSVA314. The effects of treatment with these two RSVAs on I/R-induced renal injury were determined by analyzing several markers of renal injury, energy metabolism, oxidative stress, and inflammation.

2. Materials and methods

2.1. Experimental animals

Male Sprague–Dawley rats (300–350 g) were purchased from Charles River Laboratories (Wilmington, MA). Only males were used in this study to eliminate potential gender variability. The rats were housed in a temperature-controlled room, on a 12-h light–dark cycle. Rats were fed a standard Purina rat chow diet and allowed water *ad libitum*.

2.2. Rodent model of renal I/R injury

Rats were randomly assigned into different groups ($n = 5$ per group). Initially, rats were intraperitoneally injected with 0.5 mL of 3 mg/kg Body Weight RSVA405, RSVA314, or 10% dimethyl sulfoxide + 33% Solutol in phosphate buffered saline (PBS) as vehicle. The dose range and route of drug administration were selected based on previous publications using resveratrol in renal I/R [18,19,30]. RSVA405 and RSVA314 were obtained from Chembridge (Hit2Lead compounds # 5113025 and 5194489, respectively; San Diego, CA) and their chemical structures are depicted in Figure 1 [26]. An hour after injection, the rats were anesthetized with isoflurane (Butler Schein, Dublin, OH) inhalation. Using a midline abdominal incision, renal I/R injury was induced by bilateral renal pedicle clamping for 60 min. Then the clamp was removed, and reflow (reperfusion) was visually verified. No anticoagulants were administered at any point during this study. Control animals were euthanized for sample collection and were not subjected to any surgical procedures. At 24 h after reperfusion, animals were anesthetized and plasma and tissue samples were harvested and stored at −80°C until analysis. All experiments were performed in accordance with the guidelines for the use of experimental animals by the National Institutes of Health (Bethesda, MD) and were approved by the Institutional Animal Care and Use Committee of the Feinstein Institute for Medical Research.

2.3. Determination of organ injury variables

Plasma levels of creatinine, blood urea nitrogen (BUN), aspartate aminotransferase (AST), and lactate dehydrogenase

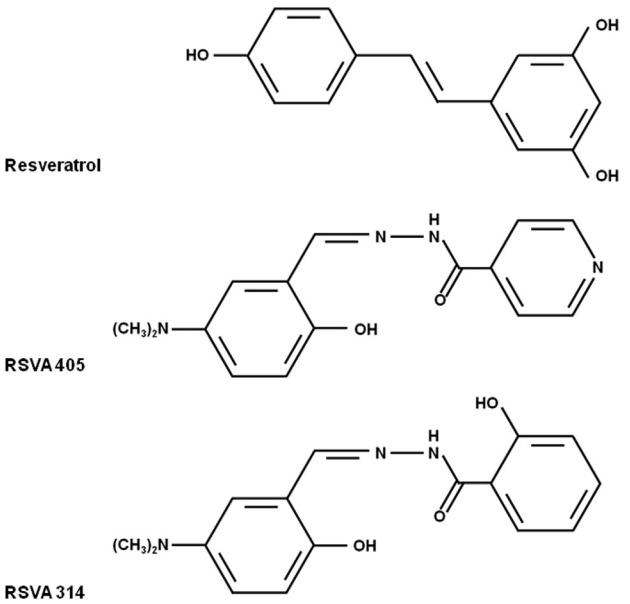

Fig. 1 – The chemical structure of the RSVAs RSVA405 and RSVA314.