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Hepatorenal protection during renal ischemia by quercetin and remote ischemic perconditioning

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ARTICLE INFO

Article history:
Received 17 February 2018
Received in revised form
15 April 2018
Accepted 23 May 2018
Available online xxx

Keywords:
Oxidative stress
Quercetin
Reperfusion injury
Remote ischemic perconditioning

ABSTRACT

Background: Pathogenesis of renal ischemia/reperfusion injury (IRI) involves oxidative stress response in the kidney and remote organs. Both quercetin and remote ischemic perconditioning (RIPerC) can protect partially against IRI. This study determined whether combined quercetin and RIPerC could provide an augmented hepatorenal protection against renal IRI.

Materials and methods: I/R was induced by clamping renal arteries for 45 min followed by 24-h reperfusion. RIPerC consisted of four cycles of 2 min of left femoral artery ischemia followed by 3 min of reperfusion administered at the beginning of renal ischemia. Rats were divided into five groups: sham, I/R, RIPerC, quercetin (Q + I/R), and combined quercetin and RIPerC (Q + RIPerC). At the end of reperfusion period, blood, urine, and tissue samples were collected.

Results: I/R caused kidney dysfunction, as proved by significant decrease in creatinine clearance, and a significant increase in liver functional indicators as evidenced by increased plasma alanine aminotransferase and aspartate aminotransferase activity. This was accompanied by a decrease of glutathione peroxidase and catalase activities with an increase of malondialdehyde levels and histological damages in renal and hepatic tissues. Treatment with RIPerC and quercetin reduced all these changes. However, the measure of improvements was enhanced by combined quercetin and RIPerC treatment.

Conclusions: This study demonstrated protective effects of quercetin and RIPerC strategy on the both kidney and liver after renal I/R. The results suggest that combined quercetin and RIPerC provides an enhanced protection against renal IRI by reduction of lipid peroxidation and augmentation of antioxidant systems.

Introduction

It is well known that renal ischemia results in tissue injury and kidney dysfunction. Ischemic kidney injury is generally attributed to tissue hypoxia and consequently to the depletion of cellular ATP.[1] Reperfusion, although serves to minimize the magnitude of the hypoxic insult, results in a unique type of injury response that is commonly called “reperfusion injury”. Reperfusion injury reduces the gain of renal reperfusion and paradoxically leads to renal dysfunction.[2] Besides, liver injury has been seen after reperfusion of ischemic kidney, which proposes that different interorgan pathways from ischemic kidney mediate remote organ injuries. Thus many basic and clinical researches have been focused to limit the ischemia/reperfusion injury (IRI). It has been shown that ischemic preconditioning can protect robustly against renal IRI,[3] but it has

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0022-4804/$ – see front matter
https://doi.org/10.1016/j.jss.2018.05.036

time limitations in clinical settings because renal IRI is unpredictable in most of the patients. Furthermore, local ischemic conditioning, which disconnects blood flow to the kidney, may create renal dysfunction.[4]

Limb remote ischemic perconditioning (RIPerC), triggered by brief I/R of a distant organ and applied during the main organ ischemia, can generate protection for organs engaged in lethal ischemia.[5,6] This method eliminates additional manipulation of the main visceral organ.[4]

Increased reactive oxygen species (ROS) is one of the processes implicated in the pathogenesis of IRI.[2] ROS could react with cellular membrane components such as phospholipids, which leads to lipid peroxidation.[7] Natural cellular defense system against ROS injury involves the enzymes glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutases and the antioxidant molecules glutathione, α-tocopherol, vitamin C, and urate.[8] ROS play a major role in IRI.[9]

Some of plant constituents such as curcumin,[10] crocin,[11] and quercetin[12] can obviously reduce the renal IRI when administered at the time of early reperfusion. The strategy of pharmacologic conditioning can be used for alleviating renal IRI and is particularly promising.[13] Quercetin is a plant polyphenolic flavonoid, found in many fruits and vegetables such as apples, onions, berries, broccoli, and black and green tea.[14] Quercetin has been proven to possess anti-inflammatory,[15] antioxidant,[16] and oxygen radical–scavenging[17] activities. Quercetin is an antioxidant, which had been reported to have protective effect on several organs.[18,19] Quercetin can protect cells against apoptosis and necrosis by inhibiting oxidative stress.[20] Recent studies showed that quercetin had a potent therapeutic effect on liver in rats.[21] Although the sole use of quercetin conditioning cannot provide perfect protection against renal IRI,[22] some investigations have demonstrated that quercetin can augment the renoprotection by other interventions such as curcumin[23,24] and mycophenolate mofetil.[23]

The available evidence suggests that quercetin increases the resistance of the kidney against IRI.[22] Furthermore, the reduction of oxidative stress has a fundamental role in both ischemia- and quercetin-induced renoprotection.[24,25] That is, ischemic and quercetin conditioning partake similar signaling pathways to induce renoprotection, and combining these strategies can generate an additive renoprotective effect.

To the best of our knowledge, however, there is no published report regarding the hepatorenal protective effect of combined quercetin and RIPerC. Therefore, this experiment was performed to assess the hypothesis that combined quercetin and RIPerC can produce an enhanced hepatorenal protection against renal IRI by antioxidant in an *in vivo* rat model.

Materials and methods

Experimental animals

All procedures (i.e., anesthesia and surgery) were in accordance with the Animal Ethics Committee at Shiraz University. Adult male Sprague–Dawley rats (250-300 g body weight) were maintained at room temperature (22°C), 12 h light–12 h dark cycle with free access to water and standard laboratory food.

Surgical procedures

Rats were anaesthetized using sodium pentobarbital (60 mg/kg, i.p.). Each rat was placed on a heating surgical table to maintain the animal body temperature at 37 ± 1°C. After a midline incision, renal artery and vein of both kidneys were carefully cleared and separated from each other under the surgical microscope. The rats were randomly divided into five groups ($n = 7$): (1) in sham group, renal arteries were not occluded; (2) in I/R group, the renal arteries were clamped for 45 min; (3) in RIPerC group, rats were subjected to 45 min ischemia by bilateral clamping the renal artery and four cycles of 2 min of left femoral artery ischemia followed by 3 min of reperfusion administered at the beginning of renal ischemia.; (4) in Q + I/R group, 10 mg/kg body weight quercetin was administered (i.p.) at the beginning of early reperfusion; (5) in Q + RIPerC group, 10 mg/kg body weight quercetin was administered (i.p.) at the onset of early reperfusion.

At the end of the surgery, rats were placed in metabolic cages, and their urine was collected over a period of 24 h. After 24 h of reperfusion, rats were anaesthetized, and blood samples were taken from the inferior vena cava. Rats were killed (under anesthesia) and their kidneys and livers were quickly dissected on dry ice. For oxidative stress assays, parts of the kidney and liver tissues were frozen in liquid nitrogen quickly. Other parts of both organs were fixed in 10% formalin for histological assessments.

Renal functional assessments

The collected urine from the 24 h reperfusion period was measured gravimetrically and total volume was recorded. Plasma and urinary creatinine were measured by colorimetric methods (autoanalyser; Prestige, Biolis 24i, Japan) and were used in conjunction with urine flow to calculate creatinine clearance (C_{Cr}) as an indicator of renal glomerular function. Plasma and urinary Na^+ was measured by a flame photometer (JENWAY, U.K.) and used to evaluate the fractional excretion of Na^+ (FE_{Na}) as an indicator of tubular dysfunction.[26]

Liver functional assessments

The activities of plasma liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were assessed spectrophotometrically (autoanalyser; Prestige, Biolis 24I, Japan).

Renal and hepatic redox markers assessments

Malondialdehyde levels

Tissue malondialdehyde (MDA) levels were determined in the kidney and liver homogenates by spectrophotometry.[27] MDA reacts with thiobarbituric acid to produce a pink color with a maximum absorption at 532 nm.

GPX activity

GPX activity was measured spectrophotometrically. Cumene hydroperoxide was used as the substrate. The assay mixture consisted of tris buffer (pH 7.6), 3 mM NADPH, 20 mM cumene hydroperoxide, 40 mM reduced glutathione, and 10 U/mL