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Mechanisms of nephroprotective effect of mitochondria-targeted antioxidants under rhabdomyolysis and ischemia/reperfusion

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ABSTRACT

Oxidative stress-related renal pathologies apparently include rhabdomyolysis and ischemia/reperfusion phenomenon. These two pathologies were chosen for study in order to develop a proper strategy for protection of the kidney. Mitochondria were found to be a key player in these pathologies, being both the source and the target for excessive production of reactive oxygen species (ROS). A mitochondria-targeted compound which is a conjugate of a positively charged rhodamine molecule with plastoquinone (SkQR1) was found to rescue the kidney from the deleterious effect of both pathologies. Intraperitoneal injection of SkQR1 before the onset of pathology not only normalized the level of ROS and lipid peroxidized products in kidney mitochondria but also decreased the level of cytochrome c in the blood, restored normal renal excretory function and significantly lowered mortality among animals having a single kidney exposed to ischemia/reperfusion. The SkQR1-derivative missing plastoquinone (C12R1) possessed some, although limited nephroprotective properties and enhanced animal survival after ischemia/reperfusion. SkQR1 was found to induce some elements of nephroprotective pathways providing ischemic tolerance such as an increase in erythropoietin levels and phosphorylation of glycogen synthase kinase 3β in the kidney. SkQR1 also normalized renal erythropoietin level lowered after kidney ischemia/reperfusion and injection of a well-known nephrotoxic agent gentamicin.

1. Introduction

Acute renal injury (ARI) in critically ill patients is highly associated with poor prognosis, and despite the increasing efforts to alleviate fatal consequences of ARI, the mortality rate among these patients remains a severe problem [1–3]. Moreover, not only ARI itself but also extrarenal complications accompanying or predisposing to ARI often result in multiorgan failure and greatly contribute to the fatality of a kidney malfunctioning [4,5]. In general, current strategies to treat ARI or its consequences include four principal methods. The most common and widespread method is hemodialysis, which is based on the artificial, external removal of harmful wastes and excess salt and fluids from the blood. Another approach includes the induction of natural mechanisms of cell protection such as modulation of the immune system or ischemic preconditioning. The third approach uses pharmacologic intervention to prevent or alleviate the deadly effects of ARI. And finally, when all three listed approaches fail, the only option remains a kidney transplant. Without going into the details of these four lines of treatment for ARI, we conclude that all four are still very costly, complicated, and inconvenient while often resulting in an imperfect outcome. However, pharmacologic treatment of ARI and associated pathologies has demonstrated appreciable progress and has potential that has yet to be exhausted. For example, where certain kidney pathologies can be attributed to the consequences of oxidative stress, antioxidant treatment is an attractive approach. Thus, in a great number of cases, patients with kidney failure due to these pathologies may potentially benefit from exposure to antioxidants when carefully and wisely used [6–11], reviewed in Koyner et al. [12].

Mitochondria-targeted antioxidants have been developed to provide specific delivery of antioxidant molecules to the interior of the mitochondrion, which potentially suffers from oxidative stress more than other cellular compartments. The chimeric molecule of such an antioxidant in principle contains a cation, bearing delocalized charge to allow movement into the mitochondrial matrix conjugated with an antioxidant moiety (e.g., coenzyme Q10 [13] or plastoquinone [14,15]). The proton motive force existing in the inner mitochondrial membrane becomes the driving machinery for a transport of these cationic antioxidants into mitochondria, thus achieving a drug concentration 10,000 times higher in the mitochondrial matrix than in the cytosol [13–16]. The beneficial effect of these compounds has been demonstrated in a number of cell pathologies, although the mechanism of cell protection remains poorly understood [13,16–21].

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In this study, we evaluated a mitochondria-targeted chimeric compounds either carrying an antioxidant moiety or without it as a potential agent to efficiently alleviate the deleterious consequences of ARI arising from two distinct pathologies, both of which apparently involve oxidative stress: kidney ischemia/reperfusion (I/R) and rhabdomyolysis (also called myoglobinuria or crush syndrome as a specific case of rhabdomyolysis). Spontaneous myoglobinuria is caused by necrotic degradation of striated muscles, resulting in the appearance of the muscle protein myoglobin in the bloodstream with subsequent kidney dysfunction. In earlier studies we [22,23] and others [24] demonstrated the key role of mitochondria as a source and a target of oxidative stress and apparent involvement of the mitochondrial permeability transition in both pathologies. Particularly, we demonstrated that myoglobin supplementation to kidney tubules caused apparent oxidative stress evidenced by a rise in ROS level in the tubules and significant loss of the mitochondrial transmembrane potential [23]. For kidney I/R, we have also identified some features of endogenous protective pathways against tissue damage caused by this intervention involving the beneficial role of the inhibition of glycogen synthase kinase-3 (GSK-3). To facilitate the future design of directed pharmacologic interventions to normalize renal function subsequent to ARI, we explored the underlying mechanisms of nephroprotection and the role of mitochondria in these two pathologies using a positively charged membrane-permeable, mitochondrial-targeted compounds carrying an antioxidative moiety [15] or without it [25]. These compounds, named SkQR1 and C12R1, have structures presented in Fig. 1. In an earlier study [26], we have shown that injection of SkQR1 results in a drop of blood creatinine level elevated under I/R and experimental rhabdomyolysis. These findings demanded to run a comprehensive study to explore in detail the nephroprotective potential of mitochondria-targeted drugs under ARI.

2. Materials and methods

2.1. Modeling of glycerol-induced rhabdomyolysis in rats

Experiments were performed on outbred white male rats (180–200 g) fed ad libitum. Animal protocols were approved by the institutional review boards. Rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal). Rhabdomyolysis was induced by a standard method by injection of 10 ml/kg of 50% water solution of glycerol (ICN, USA) into the leg muscles of rats as described earlier [27]. Control animals were untreated. The therapeutic protocol of SkQR1 used to treat this pathology was next: i/p injection of 100 nmol/kg SkQR1 1 hr after induction of rhabdomyolysis with subsequent injections at 13, 25, and 37 hrs; in total, each rhabdomyolytic animal received 400 nmol/kg SkQR1. On the second day after the injection, blood samples were taken and kidneys were excised for the mitochondria isolation with further determination of malondialdehyde in the tissue and mitochondria. Blood creatinine and urea concentrations were determined using the CellTac blood analyzer. The rhabdomyolysis model experiments were performed with at least eight animals in each group.

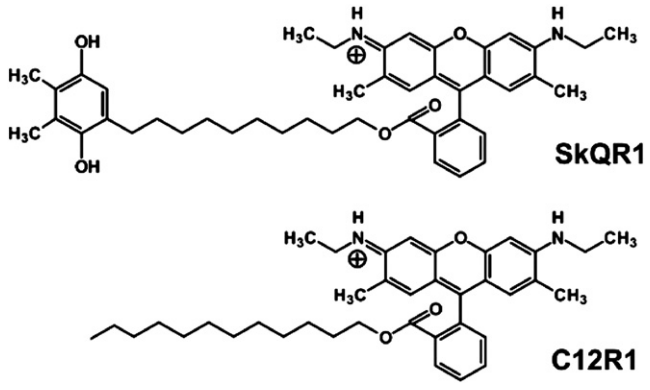

Fig. 1. The chemical structure of SkQR1 (a chimeric molecule combining a mitochondria-targeting rhodamine derivative with plastoquinone, a plant-derived antioxidant) and C12R1 representing SkQR1 without quinone moiety.

2.2. Ischemia/reperfusion protocol

The animals were subjected to 40-min warm ischemia of the left kidney as described in Plotnikov et al. [26]. Briefly, unilateral renal arteries were clamped by a microvascular clip for 40 min, and then circulation was restored by removing the clip. Nephrectomy of the right side was executed together with ischemia. During operation, the body temperature of the rat was maintained at 37 ± 0.5 °C using a thermoregulatory heating unit connected to a rectal probe. On the second day after ischemia blood samples were taken to determine creatinine and urea concentrations using a CellTac blood analyzer (Nihon Kohden Corp., Japan). The therapeutic protocol of SkQR1 treatment: i/p injection of 100 nmol/kg SkQR1 3 hrs before I/R, 1 hr after I/R, and subsequent injections at 13, 25, and 37 hrs; in total, each animal received 500 nmol/kg SkQR1. Sham-operated rats were used as controls. Rats were allocated into the following groups: (1) sham ($n = 12$), (2) I/R + saline ($n = 12$), (3) I/R + SkQR1 ($n = 12$).

2.3. Renal histology

The kidney was isolated immediately after sacrificing the animal and washed with ice-cold phosphate-buffered saline. It was then fixed in a 10% neutral buffered formalin solution, embedded in paraffin and used for histopathological examination. Five micrometer thick sections were cut, deparaffinized, hydrated, and stained with hematoxylin and eosin. The renal sections were examined in blinded fashion for tubular necrosis, hemorrhagic and hyaline casts in the kidneys of all treated animals. A minimum of 10 fields for each kidney slide were examined and scored for pathologic severity. A score from 0 to 4 was given for each pathological sign (necrosis, casts and dilatation): 0, normal histology; 1, from 5% to 25% of tubules have pathology; 2, moderate damage, from 25% to 50% of tubules have pathology; 3, severe, from 50% to 75% have pathology; and 4, almost all tubules in field of view are damaged. The average histological score for each sample was calculated.

2.4. Gentamicin nephrotoxicity protocol

Animals were randomly divided into two groups, each containing 6 animals. The first group (GM-group) of rats received gentamicin intraperitoneally in a single daily dose of 150 mg/kg. The second (GMS-group) of rats received SkQR1 intraperitoneally in a daily dose of 100 nmol/kg 3 h before gentamicin in the same dose as in G-group. Animals in the third (C-group), serving as a negative control, received saline 1 ml/d intraperitoneally. All groups were treated over a period of 6 consecutive days. Following the last application, all animals were sacrificed, and the kidneys were subsequently removed for Western blotting analysis.

2.5. Experiments with renal tubular epithelium cell cultures and kidney slices

Kidneys were excised aseptically from 3- to 7-day-old rats, then homogenized and placed in balanced Hank's solution at pH 7.4. After several washes, the dispensed tissue was placed in 0.1% collagenase and incubated for 20–30 min at 37 °C. Large pieces were removed, and