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The antifibrotic drug halofuginone reduces ischemia/reperfusion-induced oxidative renal damage in rats

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Abstract *Aim:* The objective of the present study was to evaluate the protective effects of halofuginone against renal ischemia/reperfusion (I/R) injury.
Materials and methods: Male Wistar albino rats were unilaterally nephrectomized and the left renal pedicles were occluded for 45 min to induce ischemia and then reperfused for 6 h (early) or for 72 h (late). The rats were treated intraperitoneally with either halofuginone (100 μg/kg/day) or saline 30 min prior to ischemia and the dose was repeated in the late reperfusion groups. In the sham groups, rats underwent unilateral nephrectomy and were treated at similar time points. The animals were decapitated at either 6 h or 72 h of reperfusion and trunk blood and kidney samples were obtained.
Results: I/R injury increased renal malondialdehyde levels, myeloperoxidase activity and reactive oxygen radical levels, and decreased the renal glutathione content. Halofuginone treatment was found to reduce oxidative I/R injury and improve renal function in the rat kidney, as evidenced by reduced generation of reactive oxygen species, depressed lipid peroxidation and myeloperoxidase activity, and increased glutathione levels.
Conclusions: The present findings demonstrate the anti-inflammatory and antioxidant effects of halofuginone in renal I/R injury, supporting its potential use where renal I/R injury is inevitable.

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Introduction

Ischemia/reperfusion (I/R) injury, which occurs in shock, vascular surgery, renal transplantation and during early allograft rejection subsequent to renal transplantation, is a major cause of early kidney damage associated with significant morbidity and mortality. Based on models of renal I/R injury in small animals, it is well known that tubular epithelial injury, inflammation, and oxidative stress play major roles in the pathophysiology of acute kidney injury, but it remains to be established whether this paradigm holds true for humans [1], while a better understanding of the cellular and molecular mechanisms is required for the improvement of current therapeutic approaches to renal I/R injury.

Renal I/R injury is initiated by the cellular depletion of energy substrates during a discrete ischemic interval. The re-establishment of blood flow during reperfusion leads to a complex series of events, including cytoskeletal disruption, increased microvascular permeability, interstitial edema, impaired vasoregulation, inflammatory cell infiltration, alteration of cellular ionic homeostasis with subsequent oncosis and induction of proteolytic and phospholipolytic pathways, production of reactive oxygen and nitrogen species, leukocyte infiltration, and generation of inflammatory mediators [2—4]. The interactions between leukocytes and resident tissue cells in inflammation highlight many of the complexities of cell—cell and cell—matrix interactions. Leukocyte adhesion is an important event in leukocyte recruitment as it causes dynamic interactions among cells that promote antigen presentation, leukocyte activation, transcellular generation of inflammatory mediators and maintenance phases of inflammation [5,6]. The activated neutrophils release reactive oxygen species (ROS) and cytotoxic proteins such as myeloperoxidase (MPO) or lactoferrin into the extracellular fluid [7]. Therefore, antioxidants and anti-inflammatory agents that limit leukocyte recruitment and ROS generation have gained attention as attractive targets for specific pharmacologic interventions in renal inflammatory diseases including ischemic acute renal failure.

Halofuginone is a nontoxic plant alkoloid [7-bromo-6-chloro-3-(3-hydroxy-2-piperidine)-2-oxopropyl-4(3H)-quinazoline] isolated from the roots of *Dichroa febrigua*, and is used as an antiparasitic drug [8]. Independent of this effect, halofuginone was found to be a potent inhibitor of collagen type α 1 (I) gene expression [9], which was demonstrated in various cell types, both *in vitro* and *in vivo* [8—12]. The discovery of the inhibitory effect of halofuginone on collagen synthesis and extracellular matrix (ECM) deposition led to intensive studies that were aimed at controlling many conditions associated with excessive deposition of collagen, such as pulmonary, pancreatic and renal fibrosis [13—15], liver cirrhosis [16,17], scleroderma and chronic graft-versus-host disease [18], postoperative peritendinous and abdominal adhesions [19,20], urethral and esophageal strictures [21,22], wound repair [23], colitis [24], and injury-induced arterial intimal hyperplasia [25]. Although the exact antifibrotic mechanism of halofuginone is not well understood, it was found to be associated with inhibition of transforming growth factor-β [26], which is known to promote mesengial cell proliferation and ECM deposition [27,28]. Halofuginone also regulates cell growth and differentiation, apoptosis, cell migration, and immune cell function [29]. Treatment with halofuginone effectively inhibited the delayed-type hypersensitivity response, indicating suppression of T-cell-mediated inflammation *in vivo*. Moreover, it was shown that halofuginone is a potent inhibitor of nuclear factor (NF)-κB, proinflammatory cytokines and p38 mitogen-activated protein kinase phosphorylation in activated T-cells *in vitro* [30]. Recently, we have demonstrated that halofuginone exerts beneficial effects in rats with colonic inflammation via the suppression of neutrophil accumulation, preservation of endogenous glutathione (GSH) and inhibition of ROS generation, accompanied by an antifibrotic action through the inhibition of tissue collagen production [24]. Similarly, it was shown in rats with gentamicin-induced renal injury that halofuginone treatment alleviates acute toxicity [31]. However, there have been no studies published to date on the effects of halofuginone on renal I/R injury. The present study was designed to investigate the putative short-term protective effects of halofuginone against I/R injury in the kidney, using functional, biochemical and histological parameters for evaluation of the extent of oxidative damage.

Materials and methods

Animals

Male Wistar albino rats (200—250 g) supplied by the Marmara University (MU) Animal Center (DEHAMER) were housed in an air-conditioned room with 12-h light and dark cycles, where the temperature (22 $\pm$ 2 °C) and relative humidity (65—70%) were kept constant. Rats were fed with standard laboratory chow with free access to water. All experimental protocols were approved by the MU School of Medicine Animal Care and Use Committee.

Surgery and experimental design

Briefly, an upper abdominal midline incision was made and right nephrectomy was performed under anesthesia (100 mg/kg ketamine and 0.75 mg/kg chlorpromazine; intraperitoneally, i.p.). The left renal pedicle was occluded for 45 min to induce ischemia and then subjected to reperfusion for 6 h (early reperfusion; $n = 16$) or 72 h (late reperfusion; $n = 16$). The I/R rats were treated i.p. with either saline or halofuginone (100 μg/kg) at 30 min prior to ischemia and the doses were repeated daily in the late reperfusion group. In the sham groups ($n = 24$), rats underwent right nephrectomy and were treated with either saline or halofuginone at similar time points.

The animals were decapitated at either 6 h or 72 h of the reperfusion period and trunk blood samples were collected for the analysis of blood urea nitrogen (BUN), creatinine and lactate dehydrogenase (LDH) levels. Renal tissue samples obtained from each animal were stored at −80 °C for the subsequent measurement of malondialdehyde (MDA), GSH and MPO activity. Formation of ROS in the tissue samples was monitored using a chemiluminescence (CL)