ORIGINAL ARTICLE

Ketotifen Effect, A Histamine H_1 Antagonist and Mast Cell Growth Inhibitor, on Glycerol-induced Acute Renal Failure in Rats

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Abstract

Aim: Investigating the healing effect of Ketotifen, a selective stabilizer of mast cells and a histamine H_1 receptor antagonist, on acute renal failure (ARF) model in rat kidney induced by glycerol. **Materials and Methods:** A renal failure was produced by rats deprived of water for 24 h followed by i.m. injection with 50% (vol/vol) glycerol (10 ml/kg of body weight). After 30 min, Ketotifen was used at a dose of 2 mg/kg for 5 consecutive days. On the 6th day, the rats were sacrificed; blood was obtained for blood urea and creatinine assays. The kidney tissue was used for the determination of reduced GSH levels. **Results and Discussion:** The results revealed that simultaneous treatment of glycerol with Ketotifen exhibit a normalizing effect on the main indicators of kidney functions, and reduce the glutathione (GSH) concentration significantly, compared to the glycerol-treated group. **Conclusions:** The anti-inflammatory and antioxidant effects of Ketotifen in treating the ARF are due to its potential to reduce the urea and creatinine, and increase the GSH.

Key words: Acute renal failure, glutathione, glycerol, Ketotifen

INTRODUCTION

The most widely used model of myoglobinuric acute renal failure (ARF) is produced by subcutaneous or intramuscular injection of hypertonic glycerol. In glycerol-induced ARF there is an enhanced generation of hydrogen peroxide, and scavengers of reactive oxygen metabolites and iron chelators provide protection.[1] Rhabdomyolysis may result from substance abuse, toxins, infection, and prescribed and no prescribed medications. It may also occur after traumatic events. Rhabdomyolysis ranges in severe from an asymptomatic elevation of creatinine kinase (CK) levels in the blood to severe, life-threatening cases associated with very high plasma CK levels, myoglobinuria, and ARF.[2] Rhabdomyolysis is based on an increase in free ionized calcium in the cytoplasm. Its main complications include (a) ARF, which is triggered by renal vasoconstriction and ischemia, (b) myoglobin cast formation in the distal convoluted tubules, and (c) direct renal toxic effect of myoglobin on the epithelial cells of proximal convoluted tubules.[3] During rhabdomyolysis, massive amounts of myoglobin are released from muscle cells and filtered by the glomerular filtration barrier. Once filtered, myoglobin is endocytosed by tubular cells through the megalin-cubilin receptors. Inside tubular cells, ferrous (Fe 2+) myoglobin is oxidized to a ferric (Fe 3+) form, leading to the formation of a hydroxyl radical, the most reactive of the reactive oxygen species (ROS). To be stable, ferric myoglobin is transformed to ferryl (Fe 4+) myoglobin by redox cycling, yielding radical species. These radical species promote lipid peroxidation of membrane fatty acids and induce malondialdehyde (MDA) synthesis, which mediates alterations of proteins and DNA.[4]

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Mast cells are distributed in the kidneys under normal circumstances, but the number of mast cells increases dramatically during renal ischemia/reperfusion injury. Under path sparsely illogical conditions mast cells are stimulated and released, numerous mediators, such as histamine, trypsin-like enzyme, chymase, heparin, and a host of cytokines, which they produce a wide array of biological effects.

Altering mast cell activity and function have proven to be a promising technique to reduce intestine, lung, heart, and brain ischemia-reperfusion injury. It has been reported that the histamine antagonist Ketotifen could improve the survival rate of rats subjected to intestinal ischemia-reperfusion injury.[5]

Ketotifen is a selective stabilizer of mast cells and an orally-active prophylactic agent for the management of bronchial asthma and allergic disorders. Ketotifen inhibited lipopolysaccharide-induced edema by reducing the plasma leakage in the skin in rats. Ketotifen has anti-oxidant activity, anticytokine, and anti-chemokine production properties.[6]

To address these issues, the ability of Ketotifen was examined, to promote recovery from glycerol-induced acute kidney injury (AKI), by administering the drug 30 min after the induction of injury and examining the effects of glycerol and Ketotifen on both renal and GSH function.

MATERIALS AND METHODS

Wister rats weighing (250–300 g) were adapted for 1 week before any experimental procedures and were fed with standard commercial rat pellets and allowed water *ad libitum*. They were kept under controlled environmental conditions (temperature 23 ± 2°C, humidity 55 ± 15%, and lighting regimen of 12-h light: 12-h dark). All methods performed in this study were in accordance with regulatory guidance on the care and use of experimental animals.

Rats were dehydrated for 24 h before glycerol injection. Rats were divided randomly into three groups (n = 6 for each group). The first group (untreated, N) was not injected with any treatment; the second (G) and third groups (K) of rats were given intramuscular injections of 50% glycerol (Surechem Products Ltd.) (10 ml/kg) in their hind limbs.[7] The first and second groups received normal saline, and the third group received orally Ketotifen fumarate, obtained from Sigma-Aldrich Co. LLC, (2 mg/kg/day, suspended in normal saline),[6] once daily for 5 consecutive days, applied 30 min after glycerol injection.

An hour after the last dose, the animals were sacrificed under deep ethyl ether anesthesia (Surechem Products Ltd.). Blood samples and kidney tissues were harvested for future biochemical analyses.

Assessment of renal function

Blood samples were collected by heart puncture. Serum was separated for renal function tests (serum urea and creatinine concentrations).

Serum creatinine concentration

According to the manufacturer's instructions, creatinine concentrations in plasma samples were measured with a rate-blanked and compensated picric acid colorimetric assay (CREA, Roche/Hitachi Modular p analyzer). In this enzymatic method, creatinine is converted to creatine under the activity of creatininase. In alkaline solution, creatinine forms a yellow-orange complex with picrate. The absorbance of samples and standard was measured twice (after 30 s and 90 s) spectrophotometrically (Hitachi U-1800) at 505 nm. The concentrations calculated according to the manufacturer's protocol.

Serum urea concentration

The principle of the urea measuring, using the Roche/Hitachi Modular p analyzer kit, is based on the change in the intensity of staining ammonia compounds with sodium salicylate and sodium hypochlorite, which is directly proportional to the concentration of urea in the sample. The colored complex measured by spectrophotometrically at 340 nm, according to the manufacturer's protocol.

Determination of reduced glutathione (GSH)

The nephrectomy was performed rapidly. The kidney was stored at −80°C for biochemical determinations of reduced GSH. GSH Assay Kit (Abnova) for direct assay of reduced GSH in kidney tissue was used. Tissue samples were washed in cold PBS. 150 mg was measured and homogenized in 1–2 ml of cold phosphate buffer (pH = 6–7) containing 1 mM EDTA. The homogenate was centrifuged at 10000 g for 15 min at 4 c. The supernatant was stored at −20°C for later use for GSH assay. The reduced GSH in the kidney tissue was measured spectrophotometrically according to the improved 5,5'-dithiobis (2-nitrobenzoic acid DTNB) method which combined deproteination and detection (reagent A) into one reagent. DTNB reacts with reduced GSH to form a yellow product. The optical density measured at 412 nm is directly proportional to the GSH concentration in the sample.

Statistical analysis

Results were expressed as a mean ± standard deviation. Statistical analysis was performed using the GraphPad Prism (Version 6) statistical package. Comparisons between the groups for parameter were performed using a one-way analysis of variance followed by Dunnett's multiple comparison tests, for serum creatinine, urea concentration, and reduced GSH. Statistical significance was set at $P < 0.0001$.

RESULTS

ARF was induced by injecting 50% of glycerol in water into the muscle of both hind limbs at a total dose of 10 ml/kg of body weight. Within 2 h after this injection, the urine was found to be burgundy red in color due to the presence of heme pigments.

The effects of Ketotifen on kidney function

Serum creatinine and blood urea are two principal clusters of renal function variables.

Serum level of urea

As shown in Figure 1, the serum levels of urea and creatinine in G rats were significantly higher than those in N rats. The level of urea was 33.85 ± 9.237 mmol/L in N rats, whereas the level of urea reached 111.7 ± 21.09 mmol/L in the G rats. Administration of Ketotifen significantly reduced levels of urea (61.67 ± 18.63 mmol/L) compared with that of G rats.

Serum level of creatinine

The level of creatinine was 0.398 ± 0.088 µmol/L in N rats, whereas the level of creatinine reached 1.87 ± 0.265 µmol/L in G rats. In contrast, administration of Ketotifen significantly reduced the levels of creatinine (0.683 ± 0.079 µmol/L) compared with G rats [Figure 2].

Effect of Ketotifen on GSH activities in colon tissues

The endogenous antioxidant, GSH level in the colonic tissue was decreased significantly after glycerol, as compared to the levels measured in the normal group ($P < 0.0001$). However, Ketotifen treatment group significantly reversed the glycerol-induced GSH reduction ($P < 0.0001$), but the renal GSH level was still low compared to the normal group [Figure 3].

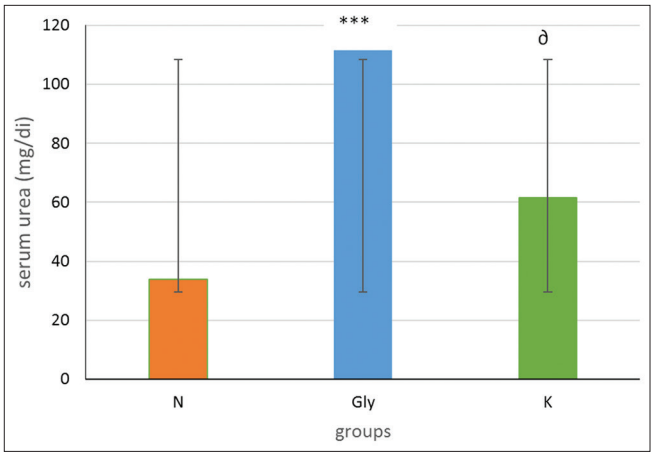

Figure 1: Serum urea level. ***P < 0.0001 versus N, ∂ P < 0.0001 versus G

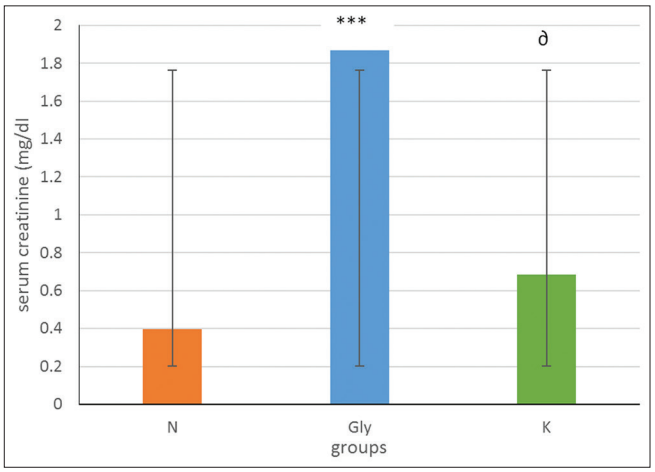

Figure 2: Serum creatinine level ***P < 0.0001 versus N, ∂ P < 0.0001 versus G

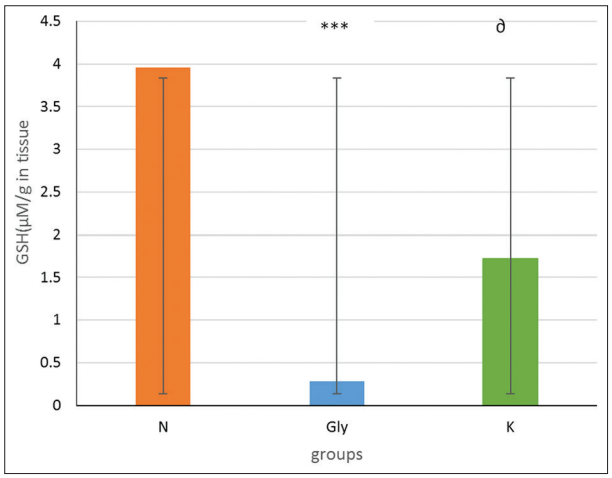

Figure 3: Effects of Ketotifen on renal glutathione content in glycerol-induced acute renal failure. ***P < 0.0001 versus N, ∂ P < 0.0001 versus G

DISCUSSION

There are some experimental data suggesting that nephrotoxic drugs can alter the levels of kidney markers, GSH, and other antioxidant enzymes, which are commonly used to monitor the development and extent of renal tubular damage due to oxidative stress. As well as it was found that ROS may be involved in the impairment of glomerular filtration rate.[8]

Glycerol-induced AKI (Gly-AKI) in rats are characterized by renal ischemia and myoglobin-derived heme-iron-mediated renal oxidative stress, leading to severe tubular injury (necrosis and apoptosis), renal and systemic inflammatory response, preglomerular vasoconstriction, and ARF.[9]

In the present study, the experimental induction of AKI, with the injection of 10 ml of 50% glycerol, was successfully achieved. The deleterious effects of glycerol on the kidney were confirmed by renal functions, the concentration of urea and creatinine, parameters of renal failure were significantly increased in glycerol group, as well as GSH decreased ($P < 0.0001$). Similar results were also observed in earlier studies.[10-12]

In contrast, the results indicate that Ketotifen treatment ameliorated glycerol-induced renal dysfunction by a significant decrease in serum urea and creatinine ($P < 0.0001$). These data correspond with the Tong results that indicate the protective effect of Ketotifen significantly altered renal ischemia-reperfusion injury.[5]

The renal GSH activity following Ketotifen administration was also studied, and its effects on renal tissues were evaluated, which depends largely on its antioxidant effect. Furthermore, Ketotifen reversed the effect of glycerol by significantly increasing the activities of GSH. This shows the curative effect of Ketotifen against the oxidative stress-induced increase in urea and creatinine. At the same time while it facilitated the enhanced activity of antioxidant enzymes, thereby counteracting the effect of glycerol-induced free radicals. That might be due to the ability of Ketotifen to neutralize the increase in free radicals caused by glycerol, as Tong study demonstrated that Ketotifen improved SOD activity and decreased MDA content with a reduced renal oxidative injury.[5]

In addition to histamine-receptor antagonism, some of these effects may be related to the inhibition of the release of mast cell and neutrophil-derived pro-inflammatory mediators. Our results indicate that mast cell stabilizer, capable of increasing tissue levels of GSH, is a promising treatment for diseases characterized by kidney dysfunction AKI. The curative effect of Ketotifen on the kidney markers can be attributed to its antioxidant properties.

CONCLUSION

Treatment with Ketotifen blunted AKI-induced renal dysfunction in rat kidney and reduced oxidative damage. These findings indicate that Ketotifen administration may reduce glycerol-induced renal injury. Therefore, we are proposing for the first time that Ketotifen might be a potential candidate against glycerol-induced nephrotoxicity through its antioxidant and anti-inflammatory properties.

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