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Protective Effect of Phosphodiestrase Inhibitor Against Acute Renal Failure in Laboratory Rats (*Rattus norvegicus*)

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Abstract

Glycerol injection in rats can lead to rhabdomyolysis, with the release of the intracellular muscle content to the extracellular compartment and acute kidney injury (AKI). Oxidative stress and the inflammatory processes contribute to the disturbances in renal function and structure observed in this model. This study evaluated the effect of calcitriol administration in AKI induced by rhabdomyolysis and its relationship with oxidative damage and inflammatory process. Male rats were treated with calcitriol (6 ng/day) or vehicle (0.9% NaCl) for 7 days and were injected with 50% glycerol or saline 3 days after the beginning of calcitriol or saline administration. Four days after glycerol or saline injection, urine, plasma and renal tissue samples were collected for renal function and structural analysis. The oxidative stress and the inflammatory processes were also evaluated. Glycerol-injected rats presented increased sodium fractional excretion and decreased glomerular filtration rates. These alterations were associated with tubular injury in the renal cortex. These animals also presented increased oxidative damage, apoptosis, inflammation, higher urinary excretion of vitamin D-binding protein and decreased cubilin expression in renal tissue. All these alterations were less intense in calcitriol-treated animals. This effect was associated with decreases in oxidative damage and inflammation.

Keywords

Rhabdomyolysis/ Glycerol/ Acute renal failure/ Phosphodiesterases

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1-Introduction

Acute renal failure (ARF) is a syndrome distinguished by an acute loss of renal function, it is characterized by a rapid decline in renal function including rapid fall in glomerular filtration rate (GFR), and retention of nitrogenous waste products over a period of hours or days.[1]. Rhabdomyolysis is a syndrome characterized by breakdown of striated muscle with a massive release of intracellular muscle components including myoglobin, creatine kinase, aldolase, as well as electrolytes into the bloodstream and extracellular fluid leading to filtration of myoglobulin to renal tubules.[2], which forms obstructing tubular casts, thus Rhabdomyolysis provokes acute tubular necrosis (ATN).[3]. Myoglobin is an oxygen and iron binding protein found in the muscle tissue of vertebrates, has a higher affinity for oxygen than hemoglobin and assists myocytes to acquire energy, the diagnostic criteria for myoglobinuria at concentrations >20 μg/l.[4]. The three different mechanisms of renal toxicity by myoglobin are usually reported as renal vasoconstriction, formation of intratubular casts and the direct toxicity of myoglobin to kidney tubular cells [5]. Phosphodiesterases are enzymes that catalyze a phosphodiester bond, although the term is usually reserved for cyclic nucleotide phosphodiesterases, PDE4, 7, and 8 are selective for cyclic 3-5-adenosine monophosphate (cAMP); PDE5, 6, and 9 are selective for cyclic 3-5-guanosine monophosphate (cGMP), while PDE1, 2, 3, 10, and 11 can hydrolyze both cAMP and cGMP[6]. PDE5 is expressed by vessel walls, glomeruli, mesangial cells, cortical tubules, and inner medullary collecting duct cells of rat kidney [7]. Phosphodiesterase inhibitors (PDE5) inhibitors block a particular enzyme (phosphodiesterase type 5), found in blood vessel walls. [8]. PDE5 helps control blood flow to the arteries, PDE 5 inhibitors cause the blood vessels to relax and increases blood flow [9]. Several selective PDE5 inhibitors (tadalafil) increase cGMP availability, by preventing cGMP degradation. cGMP is the second messenger for both the natriuretic peptide system and nitric oxide (NO) intracellular signaling. Increased cGMP results in prolongation of NO signaling in vascular smooth muscle cells, resulting in relaxation and vasodilation [10]. Dipyridamole is a phosphodiesterase inhibitor primarily used as a vasodilator for coronary artery disease and peripheral arterial disease by inhibition of phosphodiesterase 3 and 5, resulting in increased cyclic adenosine monophosphate (cAMP) and vasodilation.[11]. Glycerol-induced AKI is an animal model used to understand the clinical syndrome and the general mechanisms of renal lesions which leading to tubule necrosis and obstruction with loss of renal function and renal structural damage.[12]. Mannitol is a nonreabsorbable sugar alcohol that acts as an osmotic diuretic, inhibiting sodium and water reabsorption in the proximal tubule (increasing urinary loss of both sodium and electrolyte-free water) and more importantly in the loop of Henle. [13]. Immunohistochemistry (IHC) is the most common application of immunostaining. It involves the process of selectively identifying antigens (proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological

tissues.[14].IHC detects specific proteins by mono or polyclonal antibodies raised against that protein in biopsy [15], Aims of the study: To Evaluate protective activity of phosphodiesterase inhibitor against acute renal failure and To establish the increase renal blood flow provide protectively against protein leakage by glomeruli.

2-Methods

Animals and experimental protocols. Rats (weighing 190–200 g) were provided by the Animal House of the University of Basra, Iraq and were maintained in groups of five per cage in a 12-h-12-h dark-light cycle under standard environmental conditions (22 °C), with water and chow provided ad libitum. Acute Kidney Damage was induced by intramuscular (IM) injection of 50% glycerol (8 ml/kg; Sigma Chemical Company, St. Louis, USA) diluted in saline (0.9% NaCl) into the hind limbs. Control animals received vehicle (saline) injection via the same route. Some animals received calcitriol (6 ng/day; Abbott, USA); the others received vehicle via mini-osmotic pumps (Model 2004, Alzet, Cupertino, CA) implanted subcutaneously (SC) into the back of the animals under anaesthesia with isoflurane. This calcitriol dose was selected according to Kuhlmann et al. studies [16] that showed the beneficial effect of calcitriol in a subtotal nephrectomy model and in a previous study from our laboratory[17]. Treatment with calcitriol started three days before the administration of glycerol or vehicle and continued until the fourth day after the injections. Rats had water restriction 24 h before the injection of glycerol or saline and 1 h after administration. The animals were divided into four groups: (a) control (n = 7), receiving vehicle SC and injection of saline IM; (b) control +calcitriol (n = 7), receiving calcitriol SC and injection of saline IM; (c) glycerol (n = 13), receiving vehicle SC and IM glycerol injection; and (d) glycerol + calcitriol (n = 10), receiving calcitriol SC and injection of glycerol IM.

3-Results

1-Renal function and plasma creatinine kinase and calcium levels. The animals injected with glycerol showed higher sodium fractional excretion (FENa+), increased plasma creatinine (Cr), plasma creatine kinase (CK) and lower glomerular filtration rate (GFR) than did the control groups, indicating decreased renal function and muscle injury. In addition, increased urinary flow and decreased urinary osmolality (Uosm) were observed in these animals. All these changes, except the urinary osmolality and plasma creatinine, were less intense in animals treated with glycerol + calcitriol. There was no difference in plasma calcium between groups, 96 hours of glycerol injection (Table 1).

Table 1- Creatine kinase and calcium plasma levels and renal function parameters of control and experimental Groups, 4 days after saline or glycerol injections. CK, plasma creatine kinase (U/L); Ca2+, plasma calcium (mg/dL); Cr, plasma creatinine (mg%); GFR, glomerular filtration rate (ml min−1100 g−1); FENa+, fractional sodium excretion (%); Uosm, urinary osmolality (mOsm kg H2O−1); V, urinary flow (mL/min). The data are expressed as the mean ± SEM (GFR, FENa+ and Uosm) or median and interquartile range (25–75%). n = 7–12 per group. *P < 0.05 compared to Control; +P < 0.05 compared to Control +Calcitriol; #P < 0.05 compared to Glycerol.

	Control	Control + Calcitriol	Glycerol	Glycerol + Calcitriol
CK	26.79 ± 1.59	24.57 ± 4.89	44.23 ± 7.51*,+	45.42±3.13*,+
Ca2+	10.04 ± 0.50	9.507 ± 0.41	8.144 ± 0.50	8.373 ± 0.19
Cr	0.610 ± 0.03	0.620 ± 0.05	2.73 ± 0.39*,+	2.07±0.43*,+
GFR	0.421 ± 0.06	0.490 ± 0.03	0.113 ± 0.02*,+	0.240 ± 0.04*,+,#
FENa+ (%)	0.290 ± 0.06	0.317 ± 0.05	3.713±0.51*,+	1.96±0.64+,#
Uosm	2080±241.6	1809±117.9	541.5±25.49*,+	
V	0.004 (0.002;0.01)	0.005 (0.003;0.01)	0.020 (0.01;0.02)*,+	

2- **microscopic examination .** In animals of both groups treated with glycerol, the histological studies revealed the presence of acute tubular necrosis (ATN), characterized by necrotic cells in the tubular lumen, loss of the brush border of tubule cells and increases in the tubular lumen 4 days after glycerol injection (Fig. 1 a–d).

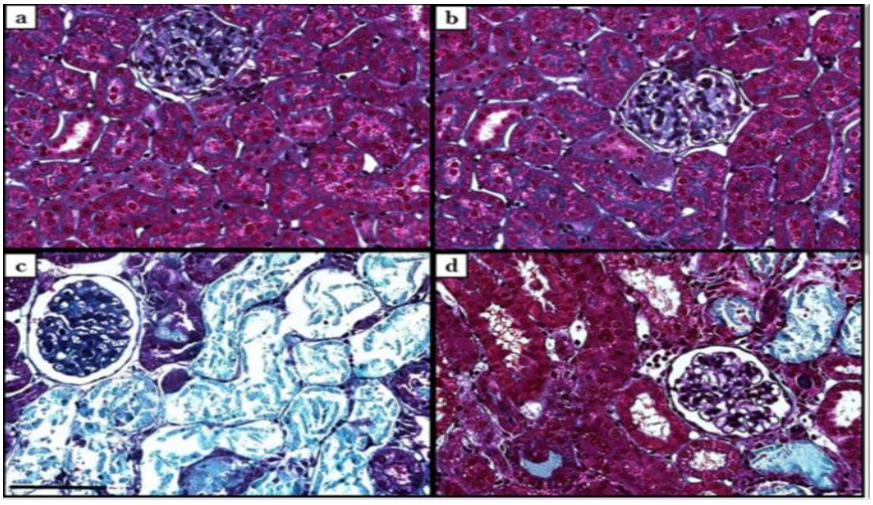

Fig. 1-Histological sections stained with Masson's Trichrome representative of control (a), control + calcitriol (b), glycerol (c) and glycerol + calcitriol (d) animals. Note that tubulointerstitial lesions are more intense in (c) than in (d). Magnification, ×400.

4-Discussion

Glycerol-induced rhabdomyolysis was evidenced by increased plasma creatine kinase concentrations compared to control animals. These animals had alterations in renal function characterized by increased fractional excretion of sodium and urinary volume, as well as decreased GFR and urinary osmolality. Treatment with calcitriol attenuated the increases in urinary volume and fraction excretion of sodium and the decrease in GFR caused by glycerol injection. There was no difference in the calcium plasma levels at day four after the injury induced by glycerol. However, it has been already observed muscle regeneration at the third day after the injury in this model and the lesion is less intense at this time[18]. Morphological data showed that structural damage such as injury tubular in the renal cortex in the animals injected with glycerol was also less intense in animals treated with calcitriol, showing recent lesions of these cells [19].

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