Research Article

Role of Guanosine in Glycerol Induced Acute Renal Failure in Rats

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Abstract
Background: Acute renal failure (ARF) is a serious clinical problem with high rate of mortality and morbidity. Currently used prophylactic and therapeutic strategies to address ARF are limited and warrant further studies. In the present study an attempt was made to investigate the effect of guanosine against glycerol induced AKI in rats. **Methods**: Male Wistar rats were divided in to three groups. After 24 h of water deprivation rats in group 2 received glycerol once whereas rats in group 1 served as control. In group 3 rats received intraperitonial injection of guanosine then animals were sacrificed, blood and kidney were collected for various biochemical and histopathological studies. **Results**: Glycerol treatment produced significant renal structural abnormalities and functional impairment (increased urea and creatinine). Guanosine dose dependently attenuated glycerol induced structural and functional changes in kidney. **Conclusion:** The reversal of glycerol induced AKI by guanosine points towards a role in improve the pathogenesis of renal injury. The result of this study suggests that guanosine may offer an alternative mode of treatment for AKI.
Keywords: renal failure, guanosine, glycerol

Introduction
Acute renal failure (ARF) is characterized by a rapid, potentially reversible, decline in renal function including rapid fall in glomerular filtration rate (GFR) and retention of nitro-genous waste products over a period of hours or days (Case et al., 2013). Glycerol induced ARF is characterized by myoglobinuria, tubular necrosis The pathogenic mechanisms involved in glycerol-induced renal failure include ischemic injury, tubular nephrotoxicity caused by myoglobin, and the renal actions of cytokines released after rhabdomylosis (Willox et al., 1986).

Klotho is a transmembrane protein that acts as a co-receptor for fibroblast growth factor-23 (Kurosu et al., 2006). In the renal tubule, klotho modulates sodium-phosphate co trans-porters, calcium channels and potassium channels (Cha et al., 2009). Several studies confirmed that expression of klotho is decreased in acute renal failure (Seo et al., 2015).

So the aim of this study was to determine the effect of guanosine on altered renal function associated with glycerol induced acute renal failure and to study its effect on expression of kotho gene in renal tissue.

Methods
1. Animals
This study was carried out on 60 male Wistar rats. Rats were kept in animal house under standard conditions of boarding and feeding with free access to water. The rats were divided into 3 groups:

1- Control group (20 rats) received single intramuscular with saline of 0.9% NaCl.
2- 2-Glycerol induced ARF group (20 rats) received single intramuscular inje-ction of glycerol (Sauriyal DS et al., 2011).
3- Guanosine treated group (20 rats) was injected intraperitoneal with guanosine given as daily for 2 weeks before indu-ction of ARF as in group II then completed for one month after glycerol induction (Kim SG et al., 1997). During the injection period, animals were kept in their cages well ventilated, in 12h day/ night cycle. Ethical approval was obtained for the study from Research Ethics Committee, Faculty of Medicine, Minia University.

2. Experimental procedures
Animals were sacrificed at the end of 6^{th} week. The kidney tissue was removed and weighed.

The kidney tissue was immediately frozen in liquid nitrogen, stored at -80°C for real time PCR and other was fixed in formalin for histopathological study (Hematoxylin and Eosin stain). Blood samples were collected to estimate the level of urea and creatinine.

3. Real Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR):
Tissue samples were subjected to RNA extraction by homogenized by ribozol followed by phenol/chloroform extraction and ethanol precipitation. Real Time PCR was performed according to manufacture instructions "Sensi FAST TM SYBR ®Hi – ROX One-Step Kit" (sigma com.) for determination the expression of Klotho genes by real time RT PCR. The forward and reverse primers for B actin were 5'-CCC ATT GAA CAC GGC ATT G -'3 and 5'-GTA CGA CCA GAG GCA TAC A - '3 respectively. Forward and reverse primers for Klotho were 5'- CGT GAA TGA GGC TCT GAA AGC-3'and 5'- GAG CGG TCA CTA AGC GAA TAC G -3' respectively. The PCR conditions were as follows one cycle of initial denaturation at 45°C for 10 min and 95°C for 2 min, 40 cycles of denaturation at 95°C for 5 sec, an annealing step for 60°C for 10sec, and extension at 72°C for 5 sec.

4. Statistical analysis
Data were collected, revised, verified, coded, then entered PC for statistical analysis and graph blotting by using the software Statistical Package for Social Sciences, SPSS version 20 Results are expressed as means ± standard deviation (SD). Differences in groups were compared using analysis of variance (ANOVA) then multiple comparisons were made using post hoc test.

Results

The results showed increase in serum urea and creatinine in glycerol induced ARF group, although showed significantly decrease in expression of Klotho gene in same group. In other hand in guanosine treated group, the results showed decrease in serum urea and creatinine level; also significantly increase in expression of Klotho gene in same group.

Discussion

Glycerol is a chemical compound which used practically for induction acute renal failure through renal cause related to rhabdomyolysis which resulted from skeletal muscle injury and subsequent released of its content (i.e. myo-globin, sarcoplasmic proteins) which may be filtered through the glomeruli, leading to ARF via different mechanisms, such as intratubular obstruction secondary to protein precipitation, renal vasoconstriction, inflammation and tubular damage associated with reactive oxygen species production (Cil O et al., 2012).

Our results revealed that serum level of creatinine and urea significantly increased in response to glycerol administration in rats as compared to control group (P value = 0.001) but their level significantly decrease in guanosine-treated group than glycerol induced ARF group (P value = 0.001). By histo-pathological study of kidney tissue, we found that rats treated by glycerol alone showed that tubular lesion in the form tubular dilatation with hyaline casts, tubular atrophy and clowdy swelling (cells enlarged with abundant eosino-philic cytoplasm) of in the cells lining the tubules in which obliterating the lumen of some tubules, and in guanosine treated group showed that decrease in severity of clowdy swelling with reforming of the lumen and disappearance of the casts. We found that the rats of the glycerol induced ARF group showed less expression of Klotho than guanosine treated group. This indicates that guanosine protects against early ARF.

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