

Aminophylline, Adenosine Antagonist Attenuates Glycerol-Induced Acute Renal Failure in Rats

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Abstract
we investigate the effect of Aminophylline against acute renal failure (ARF) in female Albino Wister rats.
ARF was produced by glycerol as a simple method for induction ARF so this achieved by injection of glycerol as single dose (10ml/kg, 50%V/V with tap water, IM) in rats which were restricted to drinking water for 24 hours. Aminophylline (5mg/rat I.P) was administered as a single dose at (60min.'s) before ARF. The blood urea nitrogen (*BUN*) and serum creatinine (*Scr*) concentrations were markedly elevated in glycerol-ARF group on the 1st day and 3rdday, but the elevation was significantly suppressed by Aminophylline. Also Aminophylline markedly attenuated the severe impairment of creatinine clearance (*Crcl*) caused by glycerol.
Conclusion: These findings suggest that Aminophylline can protect the renal damage caused by glycerol.
Key words: ARF, glycerol, Aminophylline.

1. Introduction

Acute renal failure (ARF) has become one of the important issues in clinical cases of operation, such as organ transplantation and drug administration e.g. cisplatin, cyclosporin and radiocontrast media(Joseph *et.al*, 2003). The most common cause of ARF is renal ischemia, which result in rapid decrease in tissue ATP(Hems *et.al*,1970; Yuan-Ji Day *et.al*, 2005) and a rise in the ATP degradation products: adenosine, inosine and hypoxanthine(Osswald H *et.al*,1977; Miller WL *et.al*,1978; Bauerle JD *et.al*,2011). The decreased filtration fraction and glomerular filtration rate (GFR) characteristic of renal failure could be produced, at least in part, by increased concentration of an endogenous substance (adenosine) which constrict afferent and dilates efferent arterioles(Churchill PC *et.al*,1982). Hypoxia increases adenosine produced and released by many organs, including the kidney(Miller WL *et.al*,1978;Almut Grenz *et.al*,2007). Released adenosine causes vasodilatation of many vascular beds (coronary, cerebral, skeletal muscle) and extracellular adenosine is thought to play physiologically important roles in controlling blood flow through some organs(Miller WL *et.al*,1978; Merrill GF *et.al*,1981). It was reported that methylxanthines dilate pre-glomerular and constrict postglomerular resistance vessels(Chen *et.al*,1984), and not too long after the development of the clearance concept, it was discovered that methylxanthine increase filtration fraction and GFR(Hartmut Osswald *et.al*, 2011; Timo Rieg *et.al*,2005). In humans, therapeutic plasma levels of Aminophylline more than double GF. Timo Rieg *et.al*, 2005 suggests that the methylxanthines produce afferent arteriolar dilation and efferent arteriolar constriction by antagonizing the effect of an endogenous adenosine, (Which cause reverse effect), Although methylxanthines inhibit various phosphodiasterases including C-AMP Phosphodiasterases(Gaal K *et.al*,1978 ; Amanda J Baker *et.al*,1992).

Since Aminophylline dissociated into theophylline in biological fluids and since theophylline is an adenosine receptor antagonist, it was attributed to the ameliorating effect to antagonism of the hemodynamic effect of endogenous adenosine. Bidani AK *et.al*, 1986 were reported that Aminophylline has ameliorating effect on the course and severity of glycerol-induced myoglobinuric ARF in rats.

The presented study is aimed to shed the light on the mechanism involved in the prevention of glycerol induced-ARF and to explain these mechanisms, so for this reason *Scr*, *BUN*, *Crcl* and mortality rate are used as parameters.

2.Materials and methods

Experimental animals.

Female Albino Wister rats (weighing100-200g, brought from animal house in college of medicine) were used for these experiments and they were kept at 25±2C° and in a 12hr light-dark cycle. They had free access to tap water and commercial chow.

Experimental protocols:

All rats were divided into 4 groups;

Control group: rats in this group were received distilled water (10ml/kg.IM)(Ali A. Razzak *et.al*,2004).
Aminophylline group: rats were received Aminophylline (5mg/rat I.P) as a single dose at (60min.'s) before ARF(Bidani AK *et.al*, 1986).
Glycerol-induced ARF group: ARF was produced by injection of glycerol as a single dose (10ml/kg, 50%V/V with tap water, IM) under light ether anesthesia(Min Wang *et.al*,2011)
Aminophylline+Glycerol-induced ARF group: Aminophylline (5mg/rat I.P) was administered as a single dose at (60min.'s) before ARF [which was produced by injection of glycerol as a single dose (10ml/kg, 50%V/V with tap water, IM) under light ether anesthesia] and 3 days after ARF(Bidani AK *et.al*, 1986; Min Wang *et.al*,2011)
Urine output for 24hr was collected on the second day after injection in any above group to determine the urinary creatinine (U*cr*) and urine volume (UV). To determine the serum parameters, blood samples were obtained from the tail vein at the end of each urine collection period on the 1st, 3rd and 8thday after injection. Urinary and serum creatinine (U*cr*&*Scr)* were measured by *Jaffe* method(Chrom V *et.al*,2008), while *BUN* was measured by *monoxime* method(Robert JX *et.al*,2009). Creatinine clearance was estimated as follows; *Crcl* (ml/min/100g) =

$$\frac{\text{UV X U}cr}{Scr \text{ X day time in minutes (1440)}}$$

Statistical analysis
All data represented as a mean S.E.M. The means of the groups were compared by one-way analysis of variance (ANOVA). The level of statistical significance was accepted as ($P < 0.05$). Calculations were performed using the SPSS statistical package (version 17).

3. Results
Table (1) shows the changes of *Scr*, *BUN*, and *Crcl* concentrations on the 1st, 3rd and 8th days after ARF. Sham operation with or without Aminophylline treatment had no detrimental effects during all days of experiment, but those in the glycerol group increased as the 1st, 3rd day and decreased at 8th day after induction.
In the Aminophylline treated glycerol group the elevation of those parameters was smaller than those in the glycerol group.
On the 1st day, the concentration of *Scr* and *BUN* decreased markedly to 1.47±0.046 mg/dl ($P<0.001$) and 65.039±0.972 mg/dl ($P<0.01$) respectively as compared with 3.025±0.036 mg/dl and 73.825±1.293 mg/dl in glycerol group respectively (Table 1). *Crcl* was increased markedly from 0.601±0.007 (in glycerol group) to 3.447±0.117 ml/min./100gm ($P<0.001$)(Table 1).
On 3rd day after induction of ARF by glycerol also the concentration of *Scr* and *BUN* were lowered to 1.237±0.047 mg/dl ($P<0.01$) and 47.398±1.422 mg/dl ($P<0.001$) respectively as compared with 3.22±0.062 mg/dl and 05.89±0.926 mg/dl in glycerol group (Table 1). *Crcl* was reduced than its value on 1st day, but it was remained more than in the glycerol group as 0.524±0.021 ($P<0.051$) vs. 0.257±0.11 ml/min./100gm in glycerol group (Table 1).
On the 8th day of this experiment the *Scr* and *BUN* were decreased markedly to subnormal values as 0.709±0.038 mg/dl ($P<0.01$) and 23.966±0.726 mg/dl ($P<0.001$) respectively, compared with 1.309±0.007 mg/dl and 55.84±1.53 mg/dl in glycerol group respectively (Table 1). *Crcl* was reached to 1.635±0.049 ml/min./100gm ($P<0.001$) as compared with 0.169±0.009 ml/min./100gm in glycerol group (Table 1).
As time passed and the experiment had finished there was no death in rats of Aminophylline treated glycerol group and no oliguria or hemoglobinuria were recorded as compared with glycerol alone.

4.Discussion
In the present study Aminophylline was effective in ameliorating some of biochemical and functional correlate of glycerol-induced ARF in rats. This renal protective effect of Aminophylline is consistent with the report by Kellett *et.al* (1989), who was shown that adenosine-receptor blockade by DPCPX ameliorates the severity of glycerol induced ARF. The result of this study demonstrated that after injection of glycerol, *Crcl* decreased markedly and *Scr* and *BUN* increased markedly, but Aminophylline was showing a marked and significant amelioration of the severity of glycerol induced ARF on 1st, 3rd and the 8th day after induction by glycerol.
Also, there was no death in rats of Aminophylline treated glycerol group and the mortality rate was at zero level i:e there was a significant difference with glycerol treated rat group. Our results are according to results by Mizumoto *et.al*,(1993).
It was postulated that adenosine has a deleterious effect by increasing afferent arteriolar resistance, probably via an action at the adenosine receptors(Rossi N F *et.al*,1988 ; Rossi NF *et.al*,1987). Thus, it seems that Aminophylline exerted renal protective effects by inhibiting the decrease in renal blood flow caused by adenosine receptor activation.
These finding suggest that the induction of glycerol-induced ARF is largely due to the effect of adenosine released from the glycerol-injected muscle via hemolysis or rhabdomyolysis in addition to myoglobin and

hemoglobin combined with dehydration(Isao Ishikawa *et.al*,1993).

In conclusion, the results of this study indicate a major role for adenosine in glycerol-induced ARF. Adenosine antagonism can prevent renal function decline in patients with and without renal failure. The use of adenosine antagonists may be beneficial in patients with pre-existing renal function impairment. Further studies have to clarify the clinical significance of this observation in a larger number of patients, and in patients with diabetic nephropathy as an example, since these patients run the highest risk of nephrotoxic renal failure.

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Table 1:The effect of Aminophylline on creatinine clearance, blood urea nitrogen and serum creatinine on 1st, 3rd and the 8th day in glycerol induced–ARF

Group		n	Creatinine clearance ml/min/100g	Blood urea nitrogen mg/dl	Serum creatinine mg/dl
1st day	**Control**	10	3.537±0.209	16.208±0.843	0.318±0.02
	Aminophylline	10	1.969±0.114	14.69±0.742	0.054±0.031
	Glycerol	11	0.601±0.007	73.825±1.293	3.025±0.036
	Aminophylline+Glycerol	14	3.447±0.117***	65.039±0.972	1.47±0.046**
3rd day	**Control**	10	3.575±0.245	17.950±0.576	0.389±0.028
	Aminophylline	10	1.635±0.104	26.704±1.004	0.539±0.033
	Glycerol	9	0.089±0.025	105.89±0.926	3.22±0.062
	Aminophylline+Glycerol	14	0.524±0.021***	47.398±1.422***	1.237±0.047***
8th day	**Control**	10	3.726±0.279	17.34±0.709	0.441±0.033
	Aminophylline	10	2.123±0.142	16.435±0.845	0.549±0.036
	Glycerol	7	1.169±0.009	55.840±1.53	1.309±0.067
	Aminophylline+Glycerol	14	1.635±0.094***	23.699±0.726***	0.709±0.038**

The values represent mean ± S.E.M.
**: P < 0.01, ***P<0.001 as compared with glycerol group.

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