Preclinical evaluation of Di-sodium Alpha ketoglutarate against Isoniazid-Rifampicin Induced Hepatotoxicity

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***Abstract*— The combination of Isoniazid (INH) and Rifampicin (RIF) is extensively used for the treatment of tuberculosis, but drug induced hepatotoxicity remains the major clinical challenge during treatment. The hepatoprotective role of AKG (Di-sodium Alpha ketoglutarate) is assessed on experimental animal study model. Male Sprague Dawley rats were divided into three groups of 6 animals each. Group I, (Vehicle control): given distilled water only, Group II (INH-RIF): Isoniazid and Rifampicin (50 mg/kg, orally) for 4 weeks, Group III, Treatment group: was given INH-RIF as in group II followed by AKG treatment at a dose of 2gm/kg, orally for 4 weeks. At the end of the experiment animals were sacrificed, blood samples were collected and biochemical and antioxidant assay were performed. Serum biochemical analysis showed elevated ALT, AST levels following INH and RIF exposure which significantly improved with AKG treatment. Antioxidants parameters were correlative to biochemical and histopathological findings. So from the present study it is concluded that AKG protects against INH and RIF induced hepatic injury.**

***Keywords*— Di-sodium Alpha ketoglutarate, Isoniazid, Rifampicin, Hepatotoxicity.**

I. INTRODUCTION

Tuberculosis (TB) is not only the most prevalent among infectious diseases, but also a leading cause of mortality in the developing world [1]. With the introduction of anti-tubercular drugs; Isoniazid (INH) and Rifampicin (RIF) the incidence of tuberculosis has steadily come down, but the associated side effects notably, drug induced hepatotoxicity remains a major treatment challenge more of so with the emergence of drug resistant tuberculosis [2].

INH and RIF are extensively metabolized in the liver by Phase 1 and 2 enzymes (n-acetyl transferase2 and cytochrome P450 2E1). For INH metabolism, Phase 1 mediated acetylation results in metabolites formation which are largely non-toxic, while Phase 2 mediated hydrolysis leads to formation of reactive electrophilic metabolite such as isoniazid hydrazine, which are highly toxic to hepatocytes. Also RIF by acting on Nuclear pregame X- receptor (PxR) activates cytochrome P 450 (Cyt P450 3A4) resulting in increased hydrolysis of INH into INH-Hydrazine [3]. Thus RIF and its metabolites though directly toxic themselves, does also potentiates the INH induced hepatotoxicity by the formation of excessive electrophilic molecules and reactive oxygen species (ROS).

There is a growing body of literature appreciating the role of exogenous Alpha ketoglutatre (AKG) in detoxification of reactive oxygen species (ROS). This keto-acid neutralizes ROS in non-enzymatic/NADPH independent fashion with concomitant formation of succinate and CO2. The present study evaluates the role of AKG in protecting anti-TB drug induced hepatotoxicity owing to its (ROS) scavenging properties.

II. MATERIAL AND METHOD

A. Experimental Animals

18 male Sprague Dawley rats weighing (200 to 250 g) were obtained from the Experimental animal facility of Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the institute (INM/IAEC/2009/06/009).

Male Albino rats were divided into three groups of 6 animals each. Group I (Vehicle control): Normal Saline, Group II (Drug Group, INH-RIF): Isoniazid and Rifampicin (50 mg/kg, orally) for 4 weeks, INH and RIF were prepared in sterile water and the pH of RIF solution was adjusted to 3.0 with 0.1 mol/L HCl [4]. Group III (Treatment Group, INH-RIF + AKG,), INH-RIF as in group II treated simultaneously with AKG at a dose of 2gm/kg, orally for 4 weeks [5][6]. The animals were sacrificed after 24 h of the exposure and intervention using diethyl ether by cervical dislocation. Blood and liver tissue samples were collected. Serum was separated by centrifugation at 3000 rpm for 10 min for LFT (Liver Profile Test). Liver tissue was placed in formaldehyde solution for routine histopathological examination by light microscopy. The other part of liver was placed in liquid nitrogen and stored in -80º C for evaluating antioxidant status.

B. Measurement of Liver Function test and antioxidant activities

Hepatocellular integrity (ALT, AST) with hepatic functioning (bilirubin) were determined using commercially available kits. The homogenate of frozen liver tissue (stored at -80ºC) was used for evaluating antioxidant activities. Lipid peroxidation, GSH and MPO was determined by the method of Mihara[7], Ellman[8] and Hillegass [9] respectively.

C. Statistical analysis

The data is expressed as mean ±SD. The data was analyzed using SPSS software. Statistical significance was determined

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using one way analysis of variance (ANOVA). $p < 0.05$ is taken as statistical significance value.

III. RESULTS

Effect on Body weight: There was no mortality in any of the groups. The body weight and relative liver weights of the experimental animals calculated at the end of the study had no statistically significant difference when compared to the control animals (Table I).

TABLE I: BODY WEIGHT AND LIVER WEIGHTS OF ANIMALS (N=6)

Treatment	Body weight (g)	Relative liver weight (g)
Control	155 ± 7	4.46 ± 0.53
INH-RIF	153 ± 5*	4.38 ± 0.30
INH-RIF+AKG	154 ± 4*	4.52 ± 0.46

*No significant difference among groups

Effect of AKG on INH + RIF induced hepatotoxicity: As shown in (Table II), the levels of hepatic markers ALT and AST were significantly higher in the INH-RIF group as compared to the control group. AKG treatment significantly reversed the elevations in these parameters. Also the significant increase in serum bilirubin levels in INH-RIF group animals were significantly reversed with AKG.

Effect of AKG on lipid peroxidation: The levels of liver MDA, a parameter for lipid peroxidation showed significant increment in INH-RIF group compared to control group animals. Whereas, AKG treatment following the INH-RIF challenge reversed the alterations (Table III).

Effect of AKG on GSH concentration: In the INH-RIF group there is a significant decrement of hepatic GSH as compared to normal control group. The treatment of AKG achieved a considerably increase in GSH levels as compared to INH-RIF alone group (Table III).

Effect of AKG on MPO levels: Tissue MPO activity was measured as an indirect evident of neutrophil infiltration. INH-RIF caused significant increase in the liver MPO activity, which was significantly higher than the control group, while AKG treatment revered the levels (Table III).

TABLE II: LIVER PROFILE TEST (N=6)

Treatment	Control	INH+RIF	INH-RIF+AKG
ALT (IU/L)	40.57 ± 6.78	128 ± 11.97**	54 ± 7.76#
AST(IU/L)	140 ± 7.53	468 ± 36.9**	191 ± 24.2 #
Bilirubin (mg/dL)	0.502 ±0.13	2.65±0.43*	0.98±0.76#

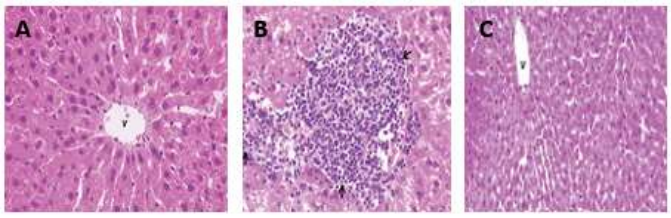
**p<0.01, highly significant, *p<0.05, significant (when compare to control), # values comparable to control

TABLE I: EFFECT OF AKG ON INH+RIF -INDUCED OXIDATIVE STRESS

Parameter	Control	INH-RIF	INH-RIF+AKG
MDA (nmol/g tissue)	44.0 ± 6.4	81.04 ± 6.3**	58.2 ± 0.55#
GSH (nmol/g tissue)	2.51 ± 0.3	0.89 ± 0.1**	1.54 ± 0.2#
MPO (U/g protein)	12.4 ± 2.1	23.2 ±2.5*	15.2 ± 3.4#

**p<0.01, highly significant, *p<0.05, significant (when compare to control), # values comparable to control

FIGURE I: HISTOPATHOLOGY



Photomicrographs of rat liver (H and E, x100, x50) from: (A) control group showing normal hepatic architecture; (B) INH-RIF group showing intense cellular degeneration, cellular inflammation of hepatocytes; (C) AKG treatment group showing normal hepatic architecture; V, represents hepatic portal vein

IV. DISCUSSION

The present study was conducted to evaluate the curative effect of AKG against INH-RIF induced hepatotoxicity in rat. This study gives some scientific evidences on the effect of AKG on enzymatic, antioxidant status and histological observations from oxidative damage induced by INH and RIF treatment in rats.

Oxidative stress mediated liver injury is the main mechanism of antituberculosis drugs induced hepatotoxicity [10]. Unifying the various proposed hypothesis of INH-RIF induced hepatotoxicity reveals that it's not the potential drugs but metabolites generated from phase 1 and 2 enzymes (NAT2, Cytochrome P4502E1), action via nuclear pregame receptor (PxR) causing dysregulation of transporters (ATP binding cassettes Subfamily B, member1) results in the formation of reactive oxygen species (ROS) [11]. Serum hepatobiliary enzymes such as AST, ALT are present in high concentrations in the liver cells under normal conditions and the elevations of these enzymes indicate hepatocytes membrane damage [12]. The present study revealed that serum AST, ALT and Bilirubin were significantly increased in rats intoxicated with INH-RIF in comparison with control group. The treatment of AKG significantly ameliorated the toxic effects. Recent microbial studies on AKG had shed newer light to its antioxidant properties, are in agreement with our result [13]. Oxidative stress is a prominent feature in the pathophysiology of liver disease [14]. Oxidative stress occurs when reactive oxygen species (ROS) are not adequately neutralized by cellular antioxidant defense mechanisms [15]. The present study reveals that treatment with AKG has a critical role in abrogating the oxidative stress-mediated toxicity of INH-RIF.

INH-RIF induced significant lipid peroxidation in rat liver, which was quantified by measuring malondialdehyde (MDA). INH-RIF treated rats showed significantly raised MDA levels, which was in agreement with previous INH-RIF induced hepatotoxic models. AKG significantly suppressed the INH-RIF induced lipid peroxidation as evident from our results. Besides replenishing the intracellular GSH pool, another important function of AKG consists of formation of carnitine, which leads to physiological normalization of fat metabolism offering protection against lipid peroxidation. Our results are supported from a previous investigation on the effects of α-KG on MDA levels, where it was observed AKG prevented lipid peroxidation in rat livers when they were exposed to alcohol [16], ammonium acetate [17] or sodium valproate [18].

The molecular mechanism for AKG to offer protection

against oxidative damages is likely due to its ability to participate in non-enzymatic NADPH independent oxidative decarboxylation of free radicals [19]. Ammonia is known to inhibit antioxidant enzymes, AKG by acting as a scavenger of amino groups transforms the ammonia to nontoxic amino acids like glutamate or glutamine. AKG is also known be involved in GSH synthesis and being a strong ammonia and phosphate binding factor may indirectly stabilize redox state in organism [20]. Thus, the present study shows that AKG offers protection against oxidative damage caused by INH-RIF.

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