Potentiating effect of rifampicin on methimazole induced hepatotoxicity in mice

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Abstract: Methimazole (MMI) is a widely used drug for hyperthyroidism. However, its clinical use is associated with hepatotoxicity. Though the precise mechanism of hepatic damage is still far from clear, role of metabolic activation and reactive metabolites have been implicated. The present study was designed to investigate the role of enzyme induction in bioactivation based hepatotoxicity of methimazole in mice. Thirty male mice were randomly divided into five groups. Hepatotoxicity was induced by single intraperitoneal injection of methimazole (1000mg/kg). Pretreatment with rifampicin which is a potent enzyme inducer was carried out for 6 days prior to administration of methimazole. The extent of hepatic damage was determined by measuring serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) along with histopathological grading of liver samples. The elevated levels of biochemical markers by methimazole were potentiated by pretreatment with rifampicin. This potentiation of hepatic injury was also observed in liver histopathological examination. These findings suggest induction of microsomal enzymes as a potentiating factor of methimazole induced hepatotoxicity.

Keywords: Methimazole, rifampicin, hepatotoxicity, reactive metabolites.

INTRODUCTION

Thionamide antithyroids which include propylthiouracil, methimazole and carbimazole have continued to be the mainstay treatment of hyperthyroidism for more than five decades. Methimazole (MMI) has become the most frequently prescribed antithyroid due to its greater efficacy, better compliance and superior side effect profile (Emiliano *et al*., 2010).

However, epidemiological studies have shown that 5% of hyperthyroid patients taking MMI can present with renal, splenic, pulmonary and hepatic injury (Sefi *et al*., 2014).In animal studies, MMI exposure of rodents confirms these organ damage by modifying their histology (Cano-Europa *et al*., 2010).

Liver injury is important from toxicological viewpoint due to its role in metabolism and detoxification of xenobiotics. Although, the exact mechanism of MMI induced hepatotoxicity remains blurred till date, formation of reactive metabolites and induction of oxidative stress seem to play role in this complication (Mizutani *et al*., 2000). MMI is metabolized by cytochrome P450 and Flavin mono-oxygenase to glyoxal, N-methylthiourea and its oxidation products sulfinic and sulfenic acid (Zuniga *et al*., 2012).

Data studies have suggested CYP2A5 (Xie *et al*., 2011) and FMO 1, 2, 3 (Siddens *et al*., 2008) and FMO 4 (Itagaki *et al*., 1996) as specific MMI metabolizing isoforms. These hepatotoxic products of metabolism (N-methylthiourea & glyoxal) have the ability to disrupt cellular function resulting in glutathione depletion and irreversible adduct formation. These intermediates-protein complexes can also act as haptens resulting in activation of inflammatory system (Heidari *et al*., 2015).

Enhanced metabolism dependent hepatotoxicity in animals pretreated with enzyme inducers have been observed in many previous studies where induction of particular enzymes by one drug may facilitate increased formation of toxic metabolites by a second drug. This is exemplified by augmented toxicity of isoniazid from activated metabolites owing to enzyme induction by rifampicin (Kalra *et al*., 2007). Similarly portion of MMI to be converted into reactive metabolites is largely dependent upon the amount and activity of metabolizing enzymes (Mohutsky *et al*., 2010).Thus, concomitant use of enzyme inducing drugs with MMI in multimorbidity can modify it hepatotoxic potential.

Among the list of enzyme inducers, rifampicin (RIF) is a prototypical inducer of many cytochrome and non-cytochrome enzymes (Chen and Raymond, 2006).

It acts through pregnane X receptor (PXR) and constitutive androstane receptor (CAR) to induce various phase I and phase II enzymes. Binding of rifampicin to these nuclear receptors result in dimerization with retinoic acid receptor (RXR) followed by attachment of heterodimer to respective response elements in target

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promoter regions (Ma *et al*., 2008). This potent enzyme inducing property of RIF give rises to many drug-drug interactions ranging from loss of therapeutic efficacy to increased drug toxicity.

In this study, we aimed to assess the effects of MMI on hepatic biochemical and histopathological parameters alone and in combination with RIF in mice.

MATERIALS AND METHODS

Chemicals

Methimazole was purchased from Sigma-Aldrich (USA). Rifampicin was provided through courtesy of Novartis Pharmaceuticals (Karachi, Pakistan). Dimethylsulfoxide (DMSO) was obtained from Scientific Center (Rawalpindi, Pakistan). Commercially available kits of Cormay, (Poland) and Linear (Spain) were used for determination of ALT and ALP.

Animals

Thirty male BALB/c mice of 8-10 weeks and weighing 30-40grams were obtained from National Institute of Health, Islamabad. Animal cages were maintained under standard husbandry conditions i-e (temperature 20^0-25^oC, humidity 40-60% and 12 hour light/dark cycle) with diet and water *ad libitum* at the animal house of Army Medical College. Animal care and research was carried out in accordance with protocols of ethical committee of "Centre of Research in Experimental and applied Medicine (CREAM)" at Army Medical College, Rawalpindi.

Experimental design

Animals were randomly divided into five groups with at least six mice in each group. The treatments were as follows: Group I: This group served as control for MMI treated mice and received normal saline i.p .Group II: served as control for RIF treated mice and received DMSO by oral gavage for 6 days. Group III: Received MMI 1000mg/kg i.p dissolved in normal saline. Group IV: RIF10mg/kg in DMSO by oral gavage for 6 days (Cheng *et al*., 2009).

Biochemical and histological parameters were assessed 24 hours after last administered dose. Group V: Mice were treated with RIF for 6 consecutive days. At seventh day, mice were injected MMI (1000mg/kg i.p).The experimental duration of Group I and G-III was of 5 hours while that of G-II, IV and V lasted seven days.

Serum biochemical parameters

Serum ALT and ALP were used as biomarkers of hepatic injury. At the end of experiment, five hours after administration of MMI, blood samples were collected by cardiac puncture. Serum was separated by centrifugation and ALT and ALP was determined using commercially available kits.

Histopathology

Liver specimens were fixed in 10% buffered formalin solution. Paraffin embed sections were stained with hematoxylin and eosin for evaluation by histopathologist.

STATISTICAL ANALYSIS

Data was expressed as Mean ± SEM. Statistical analysis was carried out using SPSS 21. One way analysis followed by Post hoc Tukey was used to analyze significance of data. Results were considered significant when $p<0.05$.

RESULTS

Biochemical analysis

Mice were treated with 1000mg/kg i.p of MMI which was taken as the appropriate toxic dose after conduction of pilot study with various previously mentioned experimental doses (Kobayashi *et al*., 2012, Heidari *et al*., 2014). Prior studies indicated that maximum rise in transaminase levels occurred five hours after MMI administration. So evaluation of serum markers and histology was carried out five hours post methimazole injection.

Serum ALT and ALP of the control groups G-I and G-II remained within normal range during the experimental period. These biochemical markers were also unaffected in RIF alone treated group (G-IV). An insignificant *p* value was obtained after comparison between G-II and G-IV.

Single intraperitoneal injection of MMI produced marked increase in ALT and ALP in comparison with G-I. The increment seen was more in levels of ALT than ALP. This elevation of liver enzymes was amplified in RIF pretreated group suggested by *p* value <0.01 (fig. 1 and 2).

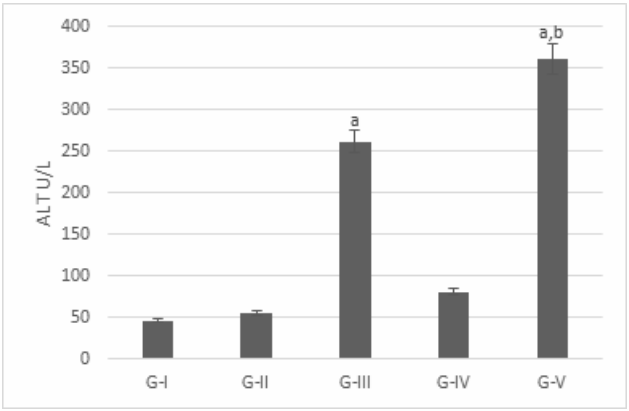

Fig. 1: Effect of Methimazole on serum ALT
[a] Significantly higher than control animals (G-I and G-II)
[b] Significantly higher than methimazole treated group

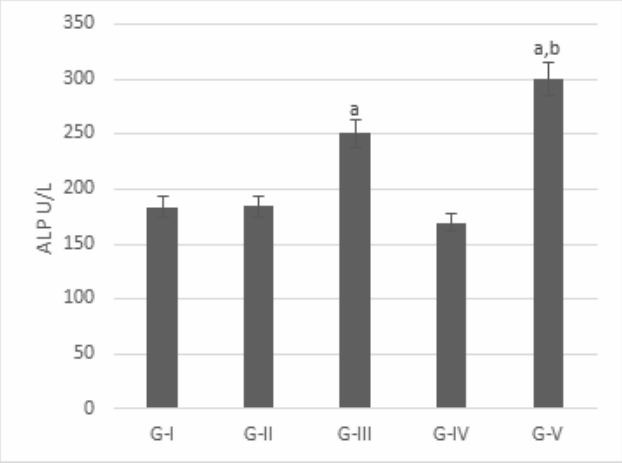

Fig. 2: Effect of Methimazole on serum ALP
[a]Significantly higher than control animals (p<0.05)
[b]Significantly higher than methimazole treated group (p<0.05)

Histopathology analysis

Histopathological evaluation of liver samples revealed that MMI caused cellular discontinuity, congestion of blood vessels, loss of radial distribution of hepatocytes and mild to moderate inflammatory cell infiltration (fig. 3, Part C). RIF treated group resulted in preservation of hepatic organization with minimal inflammation (fig. 3, Part D).When RIF pretreated group was injected MMI, extensive inflammation and necrosis of parenchymal cells was seen (fig. 3, Part E).

DISCUSSION

Antithyroid drugs (ATD) have been extensively used for the treatment of pediatric and adult hyperthyroidism with preferential usage of MMI over PTU. Both the drugs are very effective and well tolerated but hepatotoxicity concerns associated with them limit their usefulness. Thus, the choice of ATD is dependent on consideration of side effects and risk of profound adverse clinical consequences.

All antithyroid medicines cause distinct pattern of hepatic dysfunction. MMI induced liver injury is mainly cholestatic (Akmal and Kung, 2014) but hepatocellular and mixed picture has also been reported (Yang *et al*., 2015). Toxicological studies have established that MMI causes time and dose dependent liver injury (Mizutani *et al*., 1999). Single intraperitoneal injection of MMI resulted in significantly raised ALT and ALP levels with loss of normal cellular architecture and moderate portal inflammation on microscopy. Marked elevation of biochemical markers with histopathological distortion affirm the hepatotoxic potential of MMI investigated in researches of Kobayashi (Kobayashi *et al*., 2012) and Heidari (Heidari *et al*., 2013). Almost five fold rise in ALT was more than the witnessed increment in ALP.

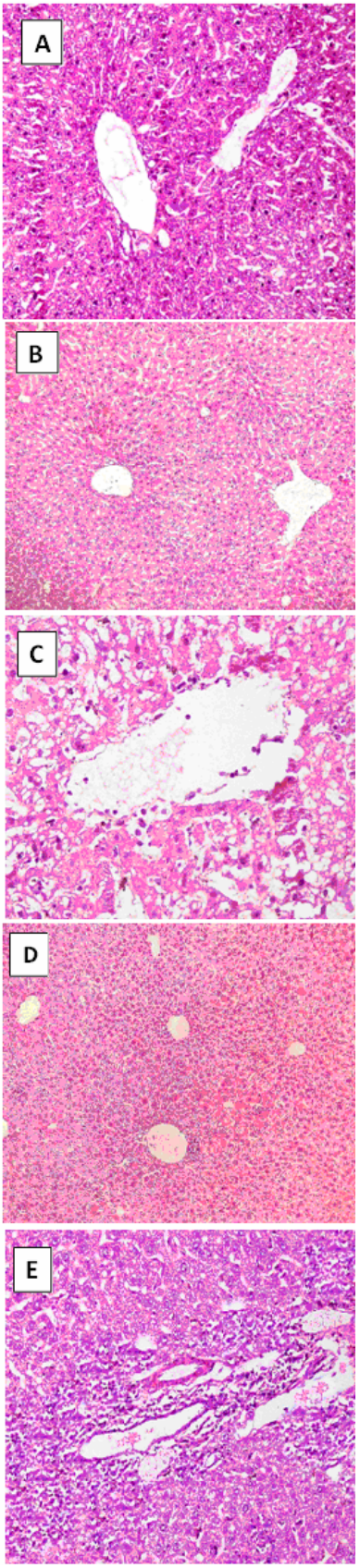

Fig. 3: Histological evaluation of liver samples stained with Hematoxylin and Eosin (H & E) A: Group I, B:

Group II, C: Group III (MMI group), D: Group IV (RIF group), E: Group V (MMI +RIF group)

These observations were authenticated in a study by Tashkandi where administration of MMI produced 202% increase in ALT as compared to a percentage increase of 191% in ALP (Tashkandi *et al*., 2014). Absence of cholestasis on histology with concomitant increase in ALP can be the consequence of limited time of conducted study. It is supported by the fact that the shortest reported duration after intake of MMI for increase in biochemical parameters and appearance of cholestasis in liver biopsy is 24 hours (Kim *et al.*, 1999).

RIF was employed at therapeutic antituberculous dose of 10mg/kg for its enzyme inducing property. This dose and duration of six days is capable for induction of microsomal enzymes in humans (Miguet *et al*., 1977). Results of RIF alone group didn't differ significantly from its control group as revealed by lack of derangement of liver enzymes with minimal inflammatory changes on microscopy. Literature reveals that RIF causes considerable increment of serum transaminases at increased dose (Chen *et al*., 2009) or long duration (Upadhyay *et al*., 2007).

Huq claimed evidence that bio-activation of MMI by CYP 450 and FMO to N-methylthiourea and glyoxal is a prerequisite for hepatotoxicity (Huq, 2008). So role of metabolic pathways and reactive metabolites was evaluated by administration of MMI in RIF pretreated mice.

MMI showed enhanced toxic profile by drastic increase in ALT and ALP. The biochemical severity of hepatic damage paralleled marked necroinflammatory changes on histology. Since RIF alone didn't profoundly raise liver markers, intrinsic hepatotoxic ability of RIF can be excluded as a potentiating factor of MMI induced liver injury. Thus, augmentation of MMI induced hepatotoxicity can be attributed to generation of excessive metabolites owing to RIF induced MMI metabolizing enzymes i-e CYP2A5 (Donato *et al*., 2000) and FMO-4 (Rae *et al*., 2001). RIF is a very broad spectrum inducer, thus biotransformation of MMI by one or more induced CYP activities not demonstrated here cannot be excluded.

Phenobarbital and β naphthoflavone have been demonstrated to increase MMI metabolism by inducing CYP-450 responsible for it (Kedderis and Ricket, 1985). Heidari and mates observed deteriorating effects of same enzyme inducers on different parameters of MMI induced liver damage in mice. Potentiation of hepatotoxicity was evident by striking escalation of ALT and malionaldehyde (MDA) levels with significant reduction of glutathione reservoirs. Histomorphological appearance also correlated with chemical findings (Heidari *et al*., 2014). These findings reinforce the amplifying effect of enzyme induction on metabolism dependent hepatotoxicity of MMI.

CONCLUSION

Concomitant administration of enzyme inducing drugs with MMI in case of comorbidity can modify its hepatotoxic potential and render patients more susceptible to this injurious adverse effect. However, the toxicological effect will be most apparent for isozyme selective inducers of drug metabolism. Further investigations on the specific enzyme responsible for converting MMI to reactive metabolites may provide new strategies to protect and treat MMI induced hepatotoxicity.

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