DOI: 10.21276/sjmps.2017.3.5

Saudi Journal of Medical and Pharmaceutical Sciences **ISSN 2413-4929 (Print)**
Scholars Middle East Publishers **ISSN 2413-4910 (Online)**
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

Original Research Article

In vivo effect of antituberculous drugs on methimazole induced hepatotoxicity in BALB/c mice

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Abstract: The effect of isoniazid and rifampicin on metabolism dependent hepatotoxicity of methimazole (MMI) was studied in BALB/c mice. Hepatic damage was induced by single intraperitoneal dose of MMI (1000mg/kg). Pretreatment with isoniazid and rifampicin was carried out in separate groups for ten days and six days respectively prior to administration of methimazole. The extent of liver injury was determined by measuring alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) along with histopathological analysis of liver samples. The elevation of liver enzyme levels caused by MMI was augmented by rifampicin however; isoniazid exhibited a protective role by preventing MMI induced escalation in serum ALT, AST and ALP. The biochemical findings were also reflected in histological examination. These findings suggest that activity of microsomal enzymes can influence the hepatotoxic potential of MMI.

Keywords: Methimazole, isoniazid, rifampicin, hepatotoxicity

INTRODUCTION

Methimazole (MMI) is the most widely used antithyroid drug for management of hyperthyroid states. It exerts its effect via inhibiting thyroid peroxidase; the key enzyme involved in the synthesis of thyroid hormone. Its greater efficacy and better safety profile has attributed to its preferred use in clinics. However, its administration is associated with lung, liver and renal toxicity in 5% of patient [1]. Hepatotoxicity is important because of liver's pivotal role in biotransformation and detoxification of drugs. The incidence of liver dysfunction induced by methimazole in adults is 6.6% presenting as hepatitis, cholestasis and acute liver failure [2]. Although the precise mechanism of MMI induced hepatotoxicity requires further investigation, formation of reactive metabolites and induction of oxidative and carbonyl stress seems to play a role in this harmful complication. Studies suggest that metabolic activation of MMI by cytochrome P450 and Flavin mono-oxygenase to N-methylthiourea and glyoxal is a necessary step for generation of liver damage. These reactive metabolites disrupt cellular function resulting in glutathione depletion and adduct formation [3].

Metabolism dependent hepatotoxicity of compounds like MMI can be influenced by co-administration of enzyme inducers and inhibitors by altering reactive metabolite formation. This is exemplified by augmented toxicity of paracetamol by phenobarbitone induced hepatic enzymes while simultaneous administration of cimetidine with paracetamol proved to be hepatoprotective[4].Similarly, MMI hepatotoxic potential is largely dependent upon the amount and activity of metabolizing enzymes and can be modified depending upon the nature of concomitantly used drug.

Isoniazid (INH) and rifampicin (RIF) are first line antituberculous drugs with the ability to induce entirely different isoforms of CYP 450. INH is an established inducer of CYP 2E1 working by increasing de-novo synthesis or decreasing enzyme degradation [5]. RIF is prototypical inducer acting through pregnane X receptor (PXR) and constitutive androstane receptor (CAR) to induce various cytochrome and non-cytochrome enzymes [6]. This enzyme inducing property of INH and RIF give rises to drug interactions

ranging from loss of therapeutic effect 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 INH and RIF in mice.

MATERIALS AND METHODS

ANIMALS

Male BALB/c mice, 8-10 weeks of age weighing between 30-40 grams obtained from National Institute of Health (NIH) were caged under standard laboratory conditions with diet and drinking water *ad libitum.* The study protocols for research was approved by ethical committee of "Centre of Research in Experimental and applied Medicine (CREAM)".

CHEMICALS

Methimazole of analytical grade was purchased from Sigma-Aldrich (USA). Isoniazid and rifampicin in pure powder form was provided through courtesy of Novartis Pharmaceuticals (Karachi, Pakistan). Dimethyl-sulfoxide (DMSO) was obtained from scientific center (Rawalpindi, Pakistan).

INDUCTION OF HEPATOTOXICITY

MMI induced hepatotoxic model was standardized in our laboratory using different doses and time interval before animal sacrifice [7, 8]. Elevated serum ALT and ALP levels and significant histopathological changes five hours after single intraperitoneal dose of 1000mg/kg confirmed liver injury. So, to induce hepatotoxicity, the toxic dose of MMI was taken as 1000mg/kg.

EXPERIMENATL DESIGN

Forty eight male mice were divided into eight groups, each comprising six animals and given following treatment:

Group I: This group served as control for MMI treated mice and received normal saline i.p

Group II: received MMI 1000 mg/kg i.p dissolved in normal saline

Group III: This group received plain drinking water for ten days and served as control for Group IV

Group IV: This group was given INH at the dose of 0.1%w/v in drinking water for ten consecutive days [9]

Group V: Mice were pretreated with INH for ten days. At eleventh day, MMI (1000mg/kg) was injected intraperitoneally to these animals.

Group VI: served as control for RIF treated mice and received DMSO by oral gavage for 6 days

Group VII: received RIF10 mg/kg in DMSO by oral gavage for 6 days [10]

Group VIII: Mice were treated with RIF for 6 consecutive days. At seventh day, mice were injected MMI (1000 mg/kg i.p)

Sample collection

Sampling of Group I and II was carried out five hours after administration of normal saline and MMI. Blood was collected from Group III and IV, 24 hours after completion of ten day course of INH. Sampling was done in Group VI and VII was on seventh day 24 hours after last dose of RIF. Cardiac puncture was done in Group V and VIII on eleventh and seventh day five hours after MMI.

BIOCHEMICAL INVESTIGATION

After collection of blood samples, serum was separated from clotted blood by centrifugation at 4000rmp for 10 minutes. Separated serum was used for estimation of ALT, AST and ALP by commercially available kits.

HISTOPATHOLOGICAL ANALYSIS

After cardiac puncture, liver was removed after dissection and fixed in 10% formalin. Hematoxylin and eosin (H & E) stained slides were studied microscopically for histopathological changes at the Department of Pathology, Army Medical College.

STATISTICAL ANALYSIS

Data was analyzed using SPSS 21. All data was expressed as Mean ± S.E.M. Significance of difference between two groups is calculated by students "t" test. Results were considered significant when $p< 0.05$.

RESULTS

Biochemical analysis

All control groups (G-I, G-III and G-VI) displayed normal range of liver enzymes levels during experimental period. Single toxic dose of MMI significantly raised levels of ALT, AST and ALP as compared to saline treated mice of G-I. Ten days of INH alone treatment in G-IV produced an unsubstantial p value ($p > 0.05$) after comparison with G-III. INH pretreatment significantly inhibited MMI induced elevations of hepatic biomarker unveiling INH protective effect. Values of ALT, AST and ALP remain unaffected in RIF treated G-VI as revealed by inconsequential results of biochemical comparison with DMSO injected animals (G-V). The hepatotoxic effects of MMI were drastically potentiated with RIF pretreatment as compared to MMI alone group (G-II) suggested by $p < 0.05$ (Table 1).

Table-1: Effect of Pretreatment of Isoniazid and Rifampicin on Methimazole induced Hepatotoxicity

GROUPS	ALT (U/L)	AST (U/L)	ALP (U/L)
G-I	45.17±10.03	127.66±7.66	184±15.69
G-II	261.33±20.30 [a*]	223±31.29 [a]	251.17±17.61[a]
G-III	48.33±7.32	128.33±7.29	188.67±8.42
G-IV	48.5±8.06 [b]	97±20.58 [b]	214.17±32.90 [b]
G-V	60.67±8.03 [c**]	97.5±31.15 [c*]	213.83±18.44
G-VI	54.83±7.79	127.83±8.19	184.5±8.92
G-VII	80.17±11.93 [b]	170.33±19.03 [b]	169.83±13.45 [b]
G-VIII	360.83±32.33 [c]	325.66±20.25 [c]	300.83±24.32 [c]

Values expressed as Mean ± S.E.M.
[a] $p < 0.05$, [a *] $p < 0.001$ when compared to G-I,
[b] $p > 0.05$ when G-III compared to G-IV and G-VI compared to G-VII
[c] $p < 0.05$,[c] * $p < 0.01$,[c] ** $p < 0.001$ when compared to G-II

HISTOPATHOLOGICAL EXAMINATION

Microscopic examination of H & E stained slides of G-I, G-III and G-VI showed normal hepatic organization consisting of portal triad and cords of hepatocytes radiating from central vein (Figure 1).

MMI caused destruction of liver architecture with loss of radial distribution of hepatocytes. The central veins were congested with blood cells and surrounded by necrotic hepatocytes. Moderate portal inflammation was also observed (Figure 2).

Histopathological study of INH (G-IV) and RIF (VII) alone treated groups showed maintenance of characteristic lobular appearance with inflammatory cell infiltration (Figure 3 and Figure 5).

Hepatoprotective effect of INH pretreatment was revealed by preservation of cellular continuity with minimal inflammatory changes (Figure 4).

RIF administration prior to MMI resulted in extensive inflammation and necrosis of parenchymal cells in G-VIII (Figure 6).

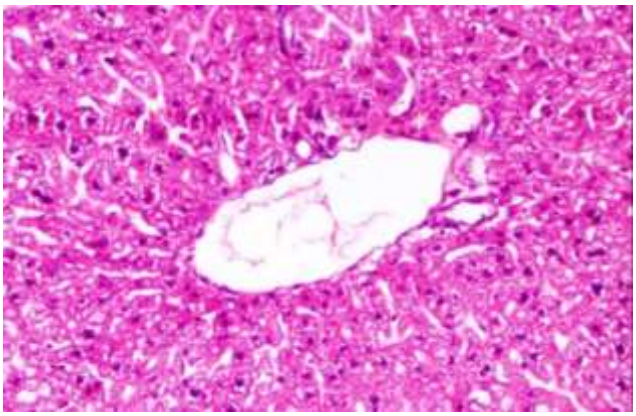

Fig-1: Control groups (G-I, G-III & G-IV) showing normal liver morphology

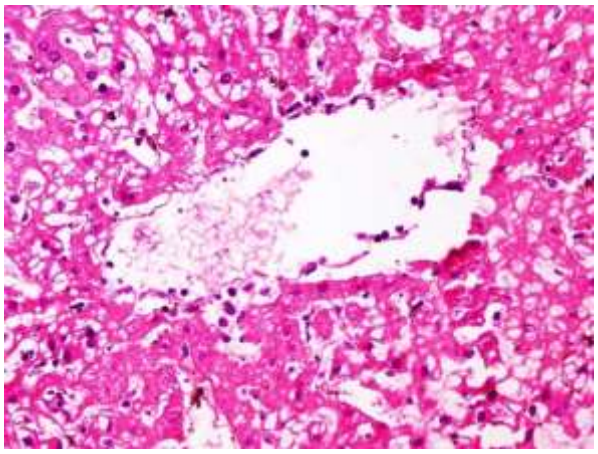

Fig-2: G-II MMI treated group revealing cellular discontinuity, necrosis and loss of hepatocytes

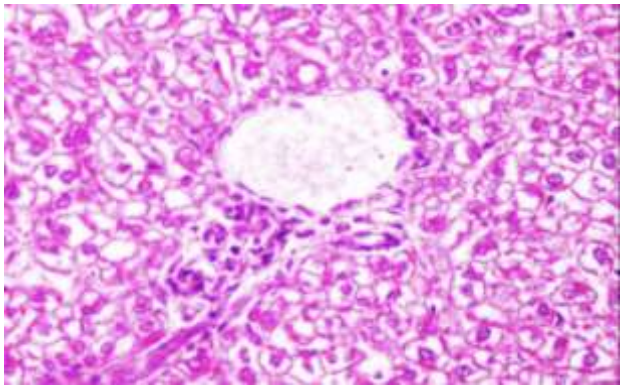

Fig-3: INH treated G- IV with minimal inflammatory changes

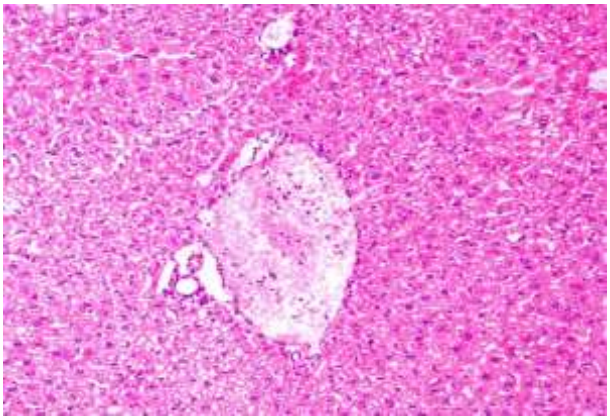

Fig-4: Group V (INH +MMI) showing preservation of hepatic architecture with few inflammatory cells

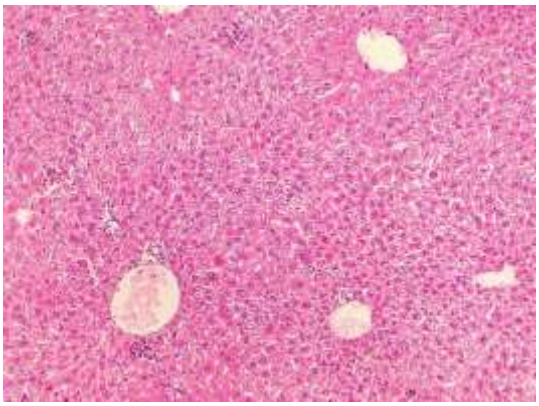

Fig-5: RIF alone in G- VI displayed near normal liver organization with minimal inflammatory cell infiltration

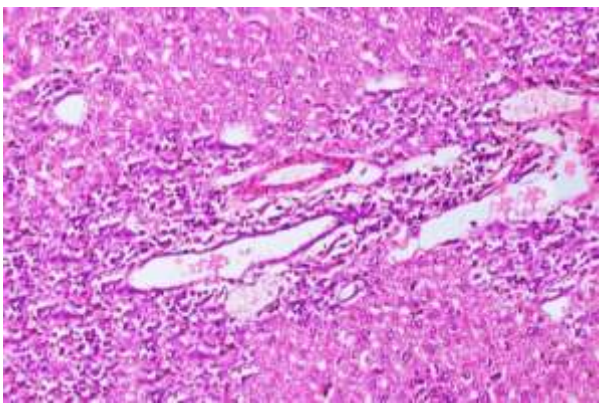

Fig-6: G- VIII (RIF +MMI) showing marked portal inflammation

DISCUSSION

Development of an understanding of the mechanism of drug-induced hepatotoxicity has always been hampered by the lack of an appropriate animal model. In this study, a successful hepatotoxic model of MMI-induced liver injury was developed after conduction of a pilot project with previously mentioned experimental doses and duration. Single intraperitoneal injection of MMI (1000mg/kg) resulted in significant elevation of ALT, AST and ALP with loss of cellular architecture and portal inflammation. These findings were authenticated by studies of Heidari [7, 8] and Koyabashi [11]. The magnitude of increase in serum ALT and AST was more than that of ALP. Similar increment was witnessed in previous study where MMI produced a 202% increase in ALT as compared to a percentage increase of 191 in ALP [12].

MMI induced liver injury and cell is due to CYP 450 and FMO dependent formation of reactive metabolites. Although the exact isoforms of MMI metabolizing enzymes remains blur till date, role of CYP2A5 [13] and FMO 1, 2, 3 [14] and FMO 4[15] have been implicated. Thus, the portion of MMI to be converted into its hepatotoxic metabolites can be modified by the amount and activity of metabolizing enzymes.

INH and RIF were employed for their enzyme inducing properties. INH alone in Group IV at dose of 0.1% w/v in drinking water for ten days was unable to significantly change the morphology and cellular function of liver. Inconsequential results of biochemical and histological comparison with Group III affirmed the non-hepatotoxic potential of INH at the selected dose and duration. Yue and his mates also validated our results by demonstrating lack of of liver dysfunction with almost 3 fold increase in hepatic CYP 2E1 levels after 10 days treatment of INH [16]. RIF followed a similar trend to that of INH with absence of substantial derangement of serum biomarkers along with minimal histopathological changes. Although the antituberculous dose of 10mg/kg/day for six days is capable of enzyme induction [17], increased dose [18] and prolong duration [19] is required for RIF's hepatotoxic manifestations.

Literature reveals that induction of particular enzymes by one enzyme inducing drug may facilitate increased generation of toxic intermediates of a second drug thus, enhancing hepatotoxic potential of second drug. This effect was evaluated by administration of MMI in INH and RIF pretreated mice. The extent of MMI induced liver injury was much greater in RIF pretreated animals as evident by drastic rise of liver transaminases. The biochemical severity of liver damage corroborated with marked necroinflammatory changes on histology. Since RIF alone didn't profoundly alter liver enzymes, intrinsic hepatotoxic ability of RIF can be excluded as a potentiating factor of MMI induced hepatotoxicity. This exaggerated hepatotoxic response can be attributed to formation of excessive metabolites owing to augmentation of MMI metabolizing enzymes i-e CYP2A5 [20] and FMO-4 [21] in RIF pretreated livers of Group VIII. Coherent with these findings, Heidari and his mates observed the deteriorating effects of phenobarbitone on different parameters of MMI induced liver injury. Their research demonstrated striking elevation of ALT and thiobarbituric acid (TBA) levels with reduction of glutathione levels marking potentiation of hepatotoxicity [7].

Surprising results were obtained when INH pretreated group V received MMI. In contrast to G-VIII (RIF+MMI), INH prevented MMI induced escalation of ALT, AST and significantly preserved hepatic organization. Thus, the protective effect of INH was disclosed which was thought to reflect inhibition of P450 1A2, 2A6, 2C19 and 3A4 [22] by INH, preventing generation of hepatotoxic species. This defensive role of INH was comparable to its preventive role in N-nitrosodimethylamine [23] and halothane [24] induced liver damage where INH acts as enzyme inhibitor. Studies have also documented ameliorative capacity of enzyme inhibitors against MMI induced hepatic effects through suppression of enzyme mediated bio-activation of MMI [25]. However, these protective findings of INH contradict its amplifying effect on acetaminophen [26] and thioacetamide induced hepatotoxicity [27] owing to CYP 2E1 induction.

CONCLUSION

RIF potentiated MMI induced biochemical and histological changes while INH proved protective against it by altering the level of exposure to reactive metabolites. However, the beneficial and toxicological effect will be most apparent for isozyme selective inducers and inhibitors. Further investigations on the specific enzyme responsible for converting MMI to reactive metabolites may provide new strategies to protect and treat MMI induced hepatotoxicity.

ACKNOWLEDGMENTS

We would like to thank Department of Pharmacology and Therapeutics and Department of Pathology, Army Medical College for providing technical support for carrying out this research project.

CONFLICT OF INTEREST

There is no conflict of interest

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