Ameliorative Effect of Mepacure Against Rimactazid-Induced Hepatotoxicity in Rats

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Abstract: Tuberculosis is a dangerous disease and its death toll is increasing year by year. Rimactazid (rifampicin + isoniazid) is one of the effective drugs in the treatment of tuberculosis. However, the use of this drug is associated with toxic reactions in tissues, particularly in the liver. Mepacure (DDB+silymarin) is known to be an effective agent for liver protection and liver regeneration. The aim of this study was to investigate the protective action of mepacure against hepatotoxicity induced by rimactazid drug with respect to the changes in the levels of serum total protein, albumin, total globulins, bilirubin, glucose, triglycerides, total cholesterol, as well as the activities of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Treatment of rats with rimactazid alone, daily for six weeks, induced hepatotoxicity as evidenced by serum biochemical measurements: total protein, albumin, bilirubin, glucose contents were significantly elevated, and the levels of triglycerides and AST activity were significantly decreased. Co-administration of mepacure was found to significantly ameliorate the rimactazid drug-induced alterations in the levels of total protein, albumin, bilirubin, glucose and triglycerides. However, the concurrent administration of mepacure and rimactazid enhanced the toxic effect induced by rimactazid alone on the level of serum total cholesterol and the activities of AST and ALP. From the results obtained, it can be concluded that mepacure is beneficial against hepatotoxic actions of drug used in chemotherapy of tuberculosis in animal models, at least from the view point of parameters examined in this study.

Key words: Tuberculosis, Rimactazid, Mepacure, total protein, albumin, total globulins, AST

INTRODUCTION

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals, or microbiologic agents. However, inflammation is sometimes inappropriately triggered by an innocuous agent, such as pollen, or by an autoimmune response, as in asthma or rheumatoid arthritis. In such cases, the defense reactions themselves may cause progressive tissue injury and anti-inflammatory or immunosuppressive drugs may be required to modulate the inflammatory process (Mycek et al., 2000). Tuberculosis continues to be a serious disease worldwide and is believed to be present in about one third of the world's population (Sahbazian and Weis, 2005). Hepatotoxicity is one of the most serious adverse effects of anti-tuberculosis drugs. (Rana et al., 2006).

Rimactazid is a drug for all forms of pulmonary and extra-pulmonary tuberculosis, one tablet of rimactazid containing 300 mg rifampicin (RIF) and 150 mg of isoniazid (INH). According to Pal et al. (2008), rifampicin and isoniazid are the most effective drugs available for the treatment of tuberculosis. The use of these drugs is associated with hepatotoxicity in some individual. Changes in serum biochemical measurements may take as one evidence of hepatotoxicity (Eminzade et al., 2008).

Mepacure is a drug which protects the liver cells during administration of otherwise hepatotoxic drugs by acting as a cell membrane stabilizer by blocking the entrance of harmful toxins and helping to remove these toxins from the liver cells. In addition, mepacure is used for the treatment of chronic hepatitis, cirrhosis and fatty degeneration (Huber et al., 2004). Each Mepacure capsule contains 30 mg dimethyl 4,4’- dimethoxy-5,6,5’,6’-dimethylene dioxybiphenyl-2,2’- dicarboxylate (DDB) and 50 mg Silymarin.

Therefore, the aim of the present work was to investigate, biochemically, the efficacy of the hepatoprotective drug Mepacure on liver injury which may be induced by the anti-tuberculosis drug Rimactazid. Some serum biochemical parameters concentrations (total protein, albumin, total globulins, glucose, triglycerides, total cholesterol, and bilirubin) and serum enzymes activity (ALT, AST and ALP) were measured to assess the possible effects of these drugs on liver function.
MATERIALS AND METHODS

A-Experimental Animals:
24 adult male albino Sprague- Dawely rats weighing 100-120 g were used. Animals were obtained from the animal house of National Organization of Drug Control and Research, (NODCAR), Cairo, Egypt. Animals were maintained on a standard diet containing: Crushed wheat (46%) shredded barley (40%), fishmeal powder (9%), dried milk (3%), yeast (1%), minerals and vitamins (1%). Animals were housed 6 per cage and were kept in air-conditioned room (temperature 25±1°C). Food and drinking water were available *ad libitum* during the experimental periods.

B-Drugs:
Two Drugs Were Used During this Study. These Drugs Are:
1- Rimactazid® 300 coated tablets: each tablet contains 300mg rifampicin and 150 mg isoniazid. Drug was purchased from the local market as a tablet form manufactured by Novartis Pharma S.A.E. Co, Cairo; under license from Biochemie, Kundl-Austria.
2- Mepacure® capsules: each capsule contains 30 mg Dimethyl Dicarboxylate Biphenyl (DDB) and 50 mg silymarin. Drug was purchased from the local market as a capsule form manufactured by Arab Company for Pharmaceutical and Medicinal plants (MEPACO), Egypt.

C-Dosage and Experimental Design:
Experimental animals were classified into four groups consisting of six rats each:

Group 1: (as control, C): received 1ml/100 g b.wt of vehicle (water and 2% tween 80) daily.

Group 2: received a daily dose of rimactazid (rifampicin 162 mg/kg.bwt and isoniazid 81 mg/kg b.wt). This dose is three times as much as the human therapeutic dose.

Group 3: received a daily dose of mepacure (13.5 mg/kg b.wt silymarin and 8.1 mg/kg b.wt DDB). This dose is equivalent to the human therapeutic dose as extrapolated relative to the body surface area tables according to the surface area ratio between man and rat (Paget and Barnes, 1964).

Group 4: received a daily dose of rimactazid (as group 2) and mepacure (as group 3).

In all treatments described in this study, drugs were prepared in a water solution containing 2% tween 80 and the appropriate dose of each drug was administered orally by gastric intubations to each rat daily for six weeks.

Blood samples were collected from the retro-orbital plexus and put into nonheparinized tubes, which were centrifuged at 3000 rpm for 10 min at 4 °C. The sera were frozen at – 20 °C for the following measurements. The appropriate kit (Stanbio laboratory reagent kit, USA) was used for the determination of serum total protein(Gornall et al.,1949), albumin(Duma,1971), bilirubin(Michaelsson,1961), glucose(Trinder, 1969), triglycerides(Wahlefeld,1974), total cholesterol(Flegg,1973) as well as the activities of aspartate transaminase (AST),alanine transaminase(ALT) (Reitman and Frankel,1957)and alkaline phosphatase(ALP) (Klein et al., 1960). Total globulins was obtained by subtracting the albumin value from the total protein content of the same sample.

The data obtained were analyzed by one-way analysis of variance (ANOVA) and Student's *t*-test for the possible significant interrelation between the various groups. Probability levels of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The administration of rimactazid alone for six weeks caused significant increase in total protein, albumin, bilirubin and glucose contents(the percentage of elevation was 32.15,24.21, 200.0 and 239.84 ,respectively) paralleled with significant decrease of triglycerides content and AST activity in the serum(the percentage of decrease was 32.84 and 31.28 ,respectively), when compared with the control group. The serum content of total globulin and total cholesterol as well as ALT and ALP activities were not significantly changed after rimactazid treatment (Table 1). However, the percentage of change was found 29.28(for total globulins), -4.81(for total cholesterol), 10.05 (for ALT) and -5.86 (for ALP) (Table 1).
Treatment of rats with mepacure drug alone for six weeks resulted in a significant decrease in total globulin content and AST activity (percentage of decrease was 25.31 and 31.28, respectively), as well as significant elevations in serum glucose and total cholesterol contents (percentage of increase was 193.8 and 46.94, respectively) when compared to the control group. Serum total protein, triglycerides, albumin, bilirubin contents and ALT and ALP activities were not significantly changed after mepacure administration alone for six weeks as compared to the control group (Table 1).

The administration of mepacure drug concurrently with rimactazid drug ameliorated the rimactazid-induced elevations in the contents of total protein (32.15 to 5.11), albumin (24.21 to -5.56), total globulins (29.28 to 21.09), bilirubin (200.0 to 71.53), glucose (239.84 to 71.53) as well as the activity of ALT (10.05 to 7.36) (Table 1). Serum triglycerides content of rats administered mepacure with rimactazid showed insignificant change compared to rimactazid group. However, the percentage of decrease was 21.64 instead of 32.84 in case of rimactazid group, both as compared to the control group. On the other hand, administration of both drugs was found aggravate the toxic effects of rimactazid in cholesterol content and the activities of AST and ALP (Table 1). The percentage of changes were -4.81 to -19.40 (cholesterol), -31.28 to 39.21 (AST) and -5.86 to -19.40 (ALP).

Table 1: Serum levels of total protein, albumin, total globulins, bilirubin, glucose, triglycerides, total cholesterol as well as activities of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in various animal groups.

<table>
<thead>
<tr>
<th>Control (C)</th>
<th>Rimactazid (R)</th>
<th>Mepacure (M)</th>
<th>(R + M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>8.21 ± 0.27a</td>
<td>10.85 ± 1.04a (32.15)</td>
<td>7.07 ± 1.03a (13.88)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.13 ± 0.17a</td>
<td>5.13 ± 0.25a (24.21)</td>
<td>4.17 ± 0.17a (9.96)</td>
</tr>
<tr>
<td>Total globulins (g/dl)</td>
<td>4.03 ± 0.19a</td>
<td>5.21 ± 0.72a (32.84)</td>
<td>3.01 ± 0.36 (25.31)</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.20 ± 0.02a</td>
<td>0.60 ± 0.05a (200.00)</td>
<td>0.14 ± 0.02a (30.00)</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>98.0 ± 7.5a</td>
<td>333.05 ± 9.53a (239.84)</td>
<td>287.93 ± 5.0 (193.80)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>65.56 ± 0.93a</td>
<td>44.03 ± 3.40a (32.84)</td>
<td>69.09 ± 2.11b (5.38)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>67.76 ± 8.37a</td>
<td>64.50 ± 4.87a (4.81)</td>
<td>99.57 ± 6.05a (19.40)</td>
</tr>
<tr>
<td>ALT (U/ml)</td>
<td>19.00 ± 3.79a</td>
<td>20.91 ± 2.21a (10.05)</td>
<td>20.13 ± 2.22 (5.94)</td>
</tr>
<tr>
<td>AST (U/ml)</td>
<td>28.00 ± 1.95a</td>
<td>19.24 ± 3.11a (31.28)</td>
<td>15.06 ± 3.13a (21.09)</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>112.00 ± 3.83a</td>
<td>105.43 ± 8.11a (5.86)</td>
<td>108.78 ± 5.29a (2.87)</td>
</tr>
</tbody>
</table>

Note: All values are expressed as mean ± SEM of 6 animals with % change from control indicated in brackets. Means in the same row sharing the same letter are not significantly different (P > 0.05).

The importance of RIF dose on the frequency and severity of hepatic side effects is not clear (Prince et al., 2002). The dose of rimactazid used (RIF: 162mg/Kg b.wt, INH: 81mg/Kg b.wt) is very high compared to those used in the treatment of tuberculosis in human subjects. Higher doses of drugs are required in animal models to produce hepatotoxicity, because rats metabolize the drugs at a faster rate and the duration of treatment is much shorter compared to the treatment of tuberculosis in humans (Santhosh et al., 2007).

Some authors have studied INH toxicity in other animals such as dog (Frankel et al., 2002). Attri et al. (2000) studied INH-RIF induced liver injury in Wister rats, but their route for administration of drug was intraperitoneal. The intragastric route for administration of INH-RIF in the present study was chosen because this is the most frequent route of drug administration in human beings (Rifandin, 2000).

INH is rapidly and almost completely (90-95%) absorbed from gastrointestinal tract and the peak plasma concentration reached within 1 to 2 hours after ingestion (Ellenhorn and BarCeloux, 1988). RIF is also rapidly absorbed from the gastrointestinal tract (90%) and the peak plasma concentration occurs at 1.5 to 4 hours after an oral dose (Ellenhorn and BarCeloux, 1988). INH is metabolized in the liver primarily by acetylation and hydrolysis, and it is these acetylated metabolites that are thought to be hepatotoxins (Peretti et al., 1987). Findings of Yue et al., 2004 in rats suggested that the hydrazine metabolite of INH and its subsequent effect on CYP2E1 induction was involved in the development of INH-induced hepatotoxicity.

Santhosh et al. (2007) suggested that INH+RIF lead to fatal hepatotoxic condition as indicated in the changes in the levels of serum protein, albumin and bilirubin. The present finding that the administration of rimactazid alone caused significant elevation in the serum total protein and albumin levels may be related to dehydration resulting from reducing water intake or possibly nutritional factor, as a side effects of the drug.
Meanwhile, these findings may be an indication that rimactazid caused membrane damage in rat hepatocyte resulting in leakage of such components (Tasduq et al., 2007). The present finding that treatment with rimactazid alone caused a significant increase in serum bilirubin content is consistent with earlier observation. Rana et al. (2006) reported that treatment of Wister rats with INH+RIF induced hepatotoxicity as judged by elevated serum bilirubin as compared with their base line. In contrast, Eminzade et al. (2008) mentioned that treatment of male Wister albino rats with INH+RIF induced hepatotoxicity as evidenced by the decrease in the levels of total protein and albumin.

William and Petri (2001) recorded hyperglycemia and metabolic acidosis in case of overdosage of INH in man. This observation was found partially in accordance with the present study, since rimactazid (INH+RIF) caused a significant elevation in serum glucose level of rats.

The present study showed that administration of mepacure alone caused non significant changes in most of the studied biochemical parameters, except for the levels of serum total globulins, glucose, total cholesterol and the activity of AST. Consistent with these results Mereish et al. (1991) noticed that silymarin did not cause any modification in serum enzyme activities and had no effect on other parameters. Again, El-Sawy et al. (2002) stated that DDB (the other component of mepacure) when given to rats had a non significant effect on liver enzymes.

Simultaneous administration of mepacure with rimactazid was found to attenuate the biochemical evidence of hepatic damage induced by INH+RIF (rimactazid) with respect of serum total protein, albumin, total globulins, bilirubin, glucose contents as well as the activity of ALT. Pari and Kumar (2002) and Tasduq et al. (2005) reported that silymarin (one of the component of mepacure) appeared to enhance the recovery from hepatic damage induced by anti-tuberculosis drugs. Recently, Eminzade et al. (2008) concluded that the active components of silymarin had protective effects against hepatotoxic actions of drugs used in the chemotherapy of tuberculosis in animal models, since it significantly decreased the biochemical changes induced by the drugs.

The present finding that the co-administration of mepacure and rimactazid aggravate the toxic effects of rimactazid in serum total cholesterol content and activities of AST and ALP was found in partial agreement with Tasduq et al. (2007) who reported that INH alone or in combination with other drugs produced a progressive enhancement of toxicity over 15-90 days. Gao et al. (2005) mentioned that DDB (one of the component of mepacure) markedly reduced the elevation in serum ALT and caused an elevation in serum AST of rats after 3 weeks of treatment.

An interesting question arises from this study: why are the hepatotoxicity rates and the hepatoprotective effects reported in this study different to those from previous studies?. Perhaps the different animal’s species and the different experimental conditions (dose level, treatment period) as well as the impaired host immunity (Jasmer and Daley, 2003) plays a role in such conflicting data. Therefore, further studies are needed to improve our understanding of this observation, especially those that examine the molecular basis for hepatotoxicity and hepatoprotective.

REFERENCES


