Effect of adrenergic blockers, carvedilol, prazosin, metoprolol and combination of prazosin and metoprolol on paracetamol-induced hepatotoxicity in rabbits

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ABSTRACT

Objectives: To evaluate hepatoprotective potential of carvedilol, prazosin, metoprolol and prazosin plus metoprolol in paracetamol-induced hepatotoxicity.

Materials and Methods: Thirty-six male rabbits were divided into six groups, six in each, group 1 received distilled water, group 2 were treated with paracetamol (1 g/kg/day, orally), group 3, 4, 5 and 6 were treated at a dose in (mg/kg/day) of the following: Carvedilol (10 mg), prazosin (0.5 mg), metoprolol (10 mg), and a combination of metoprolol (10 mg) and prazosin (0.5 mg) respectively 1 h before paracetamol treatment. All treatments were given for 9 days; animals were sacrificed at day 10. Liver function tests, malondialdehyde (MDA) and glutathione (GSH) in serum and liver homogenates were estimated. Histopathological examinations of liver were performed.

Results: Histopathological changes of hepatotoxicity were found in all paracetamol-treated rabbits. The histopathological findings of paracetamol toxicity disappeared in five rabbits on prazosin, very mild in one. In carvedilol group paracetamol toxicity completely disappeared in three, while mild in three rabbits. Paracetamol hepatotoxicity was not changed by metoprolol. In metoprolol plus prazosin treated rabbits, moderate histopathological changes were observed. Serum liver function tests and MDA in serum and in liver homogenate were elevated; GSH was depleted after paracetamol treatment and returned back to the control value on prior treatment with prazosin. MDA in serum and liver homogenate, alkaline phosphatase, total bilirubin were significantly decreased after carvedilol and prazosin plus metoprolol treatments.

Conclusion: Carvedilol and prazosin are hepatoprotective in paracetamol hepatotoxicity, combination of prazosin and metoprolol have moderate, and metoprolol has a little hepatoprotection.

KEY WORDS: Antioxidant, carvedilol, liver toxicity, metoprolol, paracetamol, prazosin

Introduction

Paracetamol is a widely used over-the-counter analgesic and antipyretic with a documented potential to damage the liver. In the United States and Great Britain, approximately one-half of all cases of acute liver failure is attributed to paracetamol toxicity.10 It has been found in a model of paracetamol-induced hepatotoxicity in rats that there is an association between paracetamol hepatotoxicity and raised plasma levels of catecholamine,11 and that alpha-adrenergic blockers such as prazosin, doxazosin and terazosin demonstrated a hepatoprotective effect that is attributed to inhibition of elevated catecholamine.12 The present study was designed to investigate the possible protective effects of carvedilol, metoprolol, prazosin in paracetamol induced hepatotoxicity in rabbits.

Materials and Methods

Preparation of Drugs

Paracetamol: 30 tablets of paracetamol (500 mg, GlaxoSmithKline, United Kingdom) were grinded by porcelain mortar, dissolved in 60 ml of distilled water to obtain a suspension with a concentration of (250 mg/ml). An accurate
dose of (1 g/kg) was administered orally for each rabbit by a pediatric nasogastric tube introduced through a hole in wood tongue depressor placed between the teeth to prevent the rabbit chewing the tube.

**Carvedilol**

Five tablets of carvedilol (12.5 mg, Roche, Switzerland) were grinded by porcelain mortar; dissolved in 10 ml distilled water to obtain a suspension concentration of (0.5 mg/ml), and each rabbit received (0.5 mg/kg) orally.

**Prazosin**

Two tablets of prazosin (5 mg, Pfizer, USA) were grinded, dissolved in 20 ml distilled water to obtain a suspension concentration of (0.5 mg/ml), and each rabbit received (0.5 mg/kg) orally.

**Metoprolol**

Two tablets of metoprolol (50 mg, AstraZeneca, Switzerland) were grinded, dissolved in 20 ml distilled water to obtain a suspension concentration of (5 mg/ml) and given to each rabbits in a dose of (10 mg/kg) orally.

**Animal Handling**

The experiments were carried out on 36 locally bred sexually mature domestic male rabbits. Their body weights ranged from 1 to 2 kg. The animals were housed in the main animal house at Basrah College of Medicine. They were kept in a stainless steel cage for acclimatization with a 12:12-h light/dark cycle and free access to food and drinking water. They were not fed for 12 h before the experiment.

**Study Design**

The study protocol was approved by the local institutional Ethical Committee. The study was carried out between November 2012 and April 2013.

The rabbits were randomly divided into six groups, six animals in each group. Group 1 (control group), were treated with distilled water 2 ml daily for 9 days. Group 2 (paracetamol toxicity group) were treated with a single loading dose of paracetamol (1 g/kg/day, orally) for 9 days. Group 3 were pretreated with carvedilol (10 mg/kg/day, orally) once daily 1 h before paracetamol treatment. Carvedilol and paracetamol treatment were continued for 9 days. Groups 4, 5 and 6 followed the same treatment protocol of group 3 but treated with prazosin (0.5 mg/kg/day, orally), metoprolol (10 mg/kg/day, orally), and the combination of prazosin and metoprolol at the same doses for individual drugs.

**Blood Sampling and Tissue Handling**

On the morning of day 10, 5–10 ml of blood was taken directly from the heart under light ether anesthesia, and transferred into non heparinized tube and allowed for few minutes to clot. Serum was separated by centrifugation at 3000 rpm for 20 min. One ml of serum was used freshly to measure serum malondialdehyde (MDA) while the rest of the serum was frozen at −20°C for the analysis of liver function test and glutathione (GSH) measurements. The rabbits were then sacrificed; liver specimens were obtained for the biological measurements and histopathological examination.

**Histopathological Examination**

The specimens were examined by a specialist histopathologist (argininosuccinate synthetase) at the Department of Pathology and Forensic Medicine, Basrah College of Medicine. The examiner was blinded for the treatments.

**Preparation of Liver Homogenate**

Liver tissues were homogenized in cold phosphate buffer saline (potential of hydrogen = 7.4) to obtain 10% of liver homogenate (using Hiedolph electrical homogenizer, Korea) at 6000 rpm for 20 min.

**Laboratory Measurements**

**Estimation of serum malondialdehyde**

Thiobarbituric acid assay of Buege and Aust (1978)[1] was used for measuring serum MDA.

**Estimation of malondialdehyde in liver homogenates**

Malondialdehyde levels in liver homogenates were estimated as described by Ohkawa et al., 1979.[4]

**Estimation of glutathione in serum and liver homogenate**

Enzyme-linked immunosorbent assay method was used for quantitative determination of endogenous GSH concentrations in serum and in liver homogenate using kit specific for rabbits (Cusabio reagents, Cusabio laboratories, Daxueyuan Road, Donghu Hi-Tech development area, Wuhan, China).

**Liver Function Tests**

The activities of serum aspartate aminotransferase (S. AST) and serum alanine aminotransferase (S. ALT) were estimated using commercially available kits (Randox diagnostic reagents, Randox Laboratories, United Kingdom). Serum alkaline phosphatase (S. ALP) and serum total bilirubin were estimated by commercially available kits (Biolabo reagents, Biolabo SA, France).

**Statistical Analysis**

Statistical Package for the Social Sciences computer package version 19 (SPSS Inc. Chicago; USA; available form: http://www.spss.com) was used for statistical analysis. Data were analyzed by one-way analysis of variance. Tukey Honestly Significant Difference test was used to compare between the means. The results were considered significant at $P < 0.05$. The results were expressed as mean ± standard deviation, unless otherwise stated.

**Results**

**Histopathological Examination**

**The control group**

There were no histopathological changes in all liver samples obtained from rabbits in this group ($n = 6$). A representative histopathological slide is presented in Figure 1a.

**Paracetamol group**

The histopathological changes of moderate to severe hepatotoxicity were seen in all rabbits in this group and summarized as follows [Figure 1b]:

- Perivenular necrosis and degeneration of hepatocytes extending to the mid zonal area
- Sinusoidal dilatation with congestion (lobular and portal) profound accumulation of erythrocytes within the sinusoids
- Clear inflammation represented by lymphocytic infiltration (portal and lobular)
- Moderate to severe portal inflammatory cell infiltration.
Carvedilol + paracetamol group

In three rabbits, the liver appears completely normal, in two rabbits the liver showed only mild sinusoidal dilatation, and one rabbit with mild inflammatory cell infiltration of lymphocytes in the portal area [Figure 1c].

Prazosin + paracetamol group

Histopathological examination showed disappearance of paracetamol toxicity in five rabbits pretreated with prazosin. Mild perivenular sinusoidal dilatation was seen in one rabbit.

Metoprolol + paracetamol group

In three rabbits, the liver showed hydropic degeneration ranged from diffuse to mild and perivenular sinusoidal dilatation, two rabbits with mild portal inflammation and sinusoidal dilatation and in one rabbit only sinusoidal dilatation was observed [Figure 2a and b].

Prazosin + metoprolol + paracetamol group

Mild perivenular sinusoidal dilatation and mild inflammatory cell infiltration only in the portal area observed in four rabbits and two rabbits developed mild perivenular sinusoidal dilatation without inflammation [Figure 2c].

Effect of Treatments on Serum Liver Enzymes

**Effect on serum aspartate aminotransferase**

Treatment with paracetamol has resulted in a significant rise in AST level to 31 ± 8.06 U/I compared with 11.5 ± 3.14 U/I in the control group (P < 0.03). The rise by paracetamol in AST was significantly decreased by pretreatment with prazosin to 12.3 ± 2.87 U/I, P < 0.03. In the same direction, pretreatment with carvedilol reduced AST level to 14.6 ± 5.57 U/I which is marginally significant, P = 0.06. While pretreatment with metoprolol or with prazosin plus metoprolol produced less reduction in the mean AST level to 19.3 ± 10.38 and 19.6 ± 9.64 U/I respectively, which is statistically not significant, P > 0.14. The data are presented in Table 1.

**Effect on serum alanine aminotransferase**

Treatment with paracetamol has resulted in a significant elevation in the level of ALT from 6.5 ± 1.87 U/me in the control group to 15.8 ± 8.23 U/me, P < 0.01. Pretreatment with carvedilol reduced ALT to 10.1 ± 7.33 U/I, this reduction did not achieve statistical significance. Pretreatment with prazosin reduced ALT to 10.1 ± 4.3 U/I, which was significantly reduced from the paracetamol value; P<0.05. Pretreatment with metoprolol reduced ALT to 10.1 ± 4.3 U/I, which was significantly reduced from the paracetamol value; P<0.01. The data are presented in Table 1.

**Effect on serum alkaline phosphatase**

Serum ALP level in the control group was 38.2 ± 20.5 IU/L, which was significantly increased by paracetamol treatment to 75.4 ± 27 IU/L and then significantly reduced in the...
groups pretreated with carvedilol, prazosin, metoprolol and the combination metoprolol and prazosin to 41.9 ± 19.7, 45.3 ± 16.1, 40.1 ± 15.3 and 39.2 ± 2.91 μmol/l respectively compared with the value of paracetamol treatment, \( P < 0.01 \).

**Effect on serum total bilirubin**

The serum total bilirubin in the control group was 0.3 ± 0.14 mg/dl which was significantly increased by paracetamol treatment to 0.79 ± 0.56 mg/dl, \( P < 0.02 \). The level of serum total bilirubin then decreased to 0.31 ± 0.22, 0.3 ± 0.15, 0.3 ± 0.07 and 0.34 ± 0.14 mg/dl by carvedilol, prazosin, metoprolol, and the combination prazosin and metoprolol respectively, \( P < 0.03 \) [Table 1].

**Effect of Treatments on Malondialdehyde Level**

**Effect on serum malondialdehyde level**

Serum level of MDA was significantly increased to 0.5 ± 0.29 μmol/l in the group treated with paracetamol compared with 0.2 ± 0.03 μmol/l of the control group, \( P < 0.05 \). Treatment with carvedilol prior to paracetamol reduced MDA level toward the control value from 0.5 ± 0.29 μmol/l in paracetamol-treated group to 0.2 ± 0.1 μmol/l in carvedilol treated group, \( P < 0.05 \). Treatment with prazosin before paracetamol reduced MDA level to 0.21 ± 0.05 μmol/l which was significantly lower than MDA level in rabbits treated with paracetamol, \( P < 0.05 \). Similar to prazosin, the combination of prazosin and metoprolol reduced elevated MDA level by paracetamol to 0.23 ± 0.09 μmol/l, \( P < 0.05 \). While treatment with metoprolol alone slightly and insignificantly reduced elevated serum MDA level by paracetamol to 0.36 ± 0.29 μmol/l. These data are presented in Table 2.

**Effect on malondialdehyde level in liver homogenate**

The mean control value of MDA in liver homogenate was 2206 ± 580 nmol/g, which was significantly increased to 3958 ± 1016 nmol/g in the group of rabbits treated with paracetamol, \( P < 0.01 \). Treatment with carvedilol, prazosin and the combination prazosin and metoprolol before paracetamol has resulted in a significant reduction in the MDA level in liver homogenate to 2113 ± 567, 2247 ± 972, 2356 ± 935 nmol/g respectively. These values were significantly lower than 3958 ± 1016 nmol/g in the paracetamol treated group, \( P < 0.01 \). The level of MDA in liver homogenate in the group treated with metoprolol before paracetamol was to 3242 ± 766 nmol/g which was lower but did not achieve statistical significance from that of paracetamol treated group [Table 2].

**Effect of Treatments on Glutathione Level**

**Effect on serum glutathione level**

Serum GSH was 19.9 ± 10.9 nmol/ml in the control group which was significantly reduced to 10.5 ± 4.03 nmol/ml by paracetamol treatment, \( P < 0.03 \). In the group of rabbits treated with carvedilol before paracetamol, serum GSH increased to 19.1 ± 12.6 nmol/ml. In prazosin and prazosin plus metoprolol treatment before paracetamol, the level of S. GSH was significantly increased to 30.1 ± 8.19, 23.5 ± 10 nmol/ml respectively, \( P < 0.01 \). The level of serum GSH with metoprolol treatment before paracetamol was 12.4 ± 2.38 nmol/ml, which was slightly but insignificantly higher than. GSH level produced by paracetamol. These results are presented in Table 2.

**Effect on glutathione level in liver homogenate**

The mean GSH level in liver homogenate of the control group was 9.77 ± 3.68 nmol/ml, which was insignificantly reduced by paracetamol to 7.84 ± 2.4 nmol/ml. The levels of GSH in liver homogenate in rabbits treated with carvedilol, prazosin, metoprolol and prazosin plus metoprolol when given before paracetamol were 9.51 ± 2.29, 8.94 ± 0.37, 9.27 ± 0.56 and 9.23 ± 0.64 nmol/ml respectively. These treatments did not achieve statistical differences compared with that of paracetamol treatment [Table 2].

**Discussion**

Paracetamol is a widely used nonprescription analgesic and antipyretic drug. Among users, patients suffering from high blood pressure, migraine, myocardial infarction, congestive heart failure and thyrotoxicosis that may frequently and chronically use paracetamol to treat headache or ill-defined pain associated with these diseases. Beta-blockers, from the other hand, may concomitantly be prescribed for these patients as principal therapeutic drugs or as prophylactic, and paracetamol may present its self as a drug, which endanger liver function since it has a liver-damaging potential. Administration of paracetamol in the present study leads to elevation of MDA levels in serum and liver homogenate, suggesting provoked lipid peroxidation leading to tissue damage and failure of endogenous antioxidant defense. The serum level of AST and ALT were significantly elevated in paracetamol treated rabbits compared with the control group. This elevation is attributed to damaged liver cells since these enzymes are located in the cytosol and released into the blood following liver damage. [5] In few studies

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**Table 2:**

**Effect of adrenergic blockers on MDA and GSH in serum and liver homogenate in paracetamol-induced hepatotoxicity in rabbits**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>S. MDA level (μmol/l)</th>
<th>MDA level in 10% liver homogenate (nmol/ml)</th>
<th>S. GSH level (nmol/ml)</th>
<th>GSH level in 10% liver homogenate (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2±0.03</td>
<td>2206±580</td>
<td>19.9±10.9</td>
<td>9.77±3.68</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>0.5±0.29</td>
<td>3958±1016</td>
<td>10.5±4.03</td>
<td>7.84±2.4</td>
</tr>
<tr>
<td>Carvedilol</td>
<td>0.2±0.1*</td>
<td>2113±567</td>
<td>19.1±12.6</td>
<td>9.51±2.29</td>
</tr>
<tr>
<td>Prazosin</td>
<td>0.21±0.05*</td>
<td>2247±972</td>
<td>30.1±8.19</td>
<td>8.94±0.37</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>0.36±0.29</td>
<td>3242±766</td>
<td>12.4±2.38</td>
<td>9.27±0.56</td>
</tr>
<tr>
<td>Prazosin+metoprolol</td>
<td>0.23±0.09*</td>
<td>2356±935*</td>
<td>23.5±10*</td>
<td>9.23±0.64</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD (n=6 in each group). *Significantly different from the paracetamol value; \( P<0.05 \), *Significantly different from the paracetamol value; \( P<0.01 \). MDA=Malondialdehyde, GSH=Glutathione, S. MDA=Serum malondialdehyde, S. GSH=Serum glutathione, SD=Standard deviation.
in the animal model, adrenergic blocker drugs were reported to have some protective effect against paracetamol toxicity,[10-13] and in one study adrenergic blockers were found protective against carbon tetrachloride hepatotoxicity.[14] In the present study carvedilol, a beta blocker; prazosin, an alpha blocker, metoprolol and prazosin combination were used in a rabbit model of hepatotoxicity induced by paracetamol. Adrenergic blockers used at these doses reduced paracetamol hepatotoxicity at varying degrees. On histopathological examination, prazosin treatment has prevented hepatotoxicity in five rabbits and liver sections in these rabbits appeared completely normal. These changes were paralleled with marked reduction in serum AST, ALT, ALP and total bilirubin. These findings are in agreement with the results of Randle et al.[25] The mechanism behind the hepatoprotection of prazosin is not well defined, however, marked reduction in MDA levels both in serum and in liver homogenate, with highly significant increase in serum GSH may suggest antioxidant activity of metoprolol,[10] or a result of drug presence of histopathological signs of paracetamol toxicity degeneration is seen in histopathological examination. The of sinusoidal dilatation with mild inflammation and hydropic changes were paralleled with marked reduction in serum AST, ALT, ALP and total bilirubin. These findings are in agreement with the results of Randle et al.[25] The mechanism behind the hepatoprotection of prazosin is not well defined, however, marked reduction in MDA levels both in serum and in liver homogenate, with highly significant increase in serum GSH may suggest antioxidant activity of metoprolol,[10] or a result of drug presence of histopathological signs of paracetamol toxicity degeneration is seen in histopathological examination. The of sinusoidal dilatation with mild inflammation and hydropic degeneration is seen in histopathological examination. The presence of histopathological sings of paracetamol toxicity in the presence of metoprolol may, in part, reflect lacking of antioxidant activity of metoprolol,[106] or a result of drug interaction between metoprolol with paracetamol on the liver. It is worth mentioning that metoprolol, although rare, occasionally having some toxicity on the liver, characterized by hydropic degeneration.[113] The combination of metoprolol with prazosin produced a better effect on hepatotoxicity of paracetamol, but some features of toxicity still exist. These liver changes were mild, but not completely ameliorated. The combination of metoprolol and prazosin favorably corrected the rise in MDA produced by paracetamol toxicity and significantly corrected depletion of GSH, which is highly in favor of involvement of antioxidant mechanism in hepatoprotection by the addition of prazosin. It can be speculated that paracetamol liver toxicity is more pronounced in diseases accompanied by high catecholamine level such as congestive heart failure[112,113] or thyrotoxicosis.[114] Paracetamol in thess disease is expected to produce liver toxicity at therapeutic doses and that the use of adrenergic blockers may be beneficial in minimizing the toxicity of paracetamol. It can be concluded that adrenergic blockers are not the specific antidotes for paracetamol hepatotoxicity, however, concomitant use with paracetamol may minimize such toxicity. Carvedilol and prazosin have hepatoprotective effects in paracetamol-induced hepatotoxicity, combination of prazosin and metoprol have moderate, and metoprol has little hepatoprotective potential.

References

