Pharmacological studies on the potential hepatoprotective effects of lisinopril, fosinopril & losartan in the rats.

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Heba Mohammed
Abstract

The present study was designed to investigate the antioxidant and antifibrotic mechanisms of two angiotensin converting enzyme (ACE) inhibitors, lisinopril and fosinopril and the angiotensin receptor blocker, losartan in experimental rat model of liver injury using carbon tetrachloride (CCl₄).

To fulfill this aim, the study was divided into two parts. First, screening the potential hepatoprotective dose of each drug against CCl₄-induced acute hepatotoxicity. Second, studying the hepatoprotective mechanisms of these drugs in chronic model of hepatotoxicity induced by CCl₄.

In the first part, CCl₄ was given in a single oral dose (2.8ml/kg). Then after 6 hours, drug treatment was started. Lisinopril (0.9, 1.8, 9mg/kg), fosinopril (0.9, 1.8, 9mg/kg) or losartan (4.5, 9, 18mg/kg) were given orally, daily for three consecutive days. Then liver functions (serum AST, ALT, ALP, total bilirubin, and total protein) were assessed as well as histopathological examination was done. According to the results of the first part, we chose the minimum hepatoprotective dose of each drug in the second part to study the mechanisms involved in such hepatoprotection.

In the second part, CCl₄ was given in a single initial dose (1ml/kg, orally). Then after one week, CCl₄ (0.5 ml/kg, orally) was given twice weekly for 8 weeks. After one week of starting CCl₄ intoxication, animals were treated orally with lisinopril (1.8mg/kg), fosinopril (1.8mg/kg) or losartan (9mg/kg) daily for 8 weeks.

Liver functions were assessed as well as histopathological examination was done. In addition, the oxidative stress markers (reduced glutathione and lipid peroxides level) and fibrosis markers (hydroxyproline content and liver fibrosis area) were also assessed.

It was found that treatment of animals with lisinopril, fosinopril or losartan concurrently with CCl₄ showed a hepatoprotective effect on liver functions. Also, they counteracted the histopathological changes induced by CCl₄. In addition, all drugs significantly counteracted lipid peroxidation and reduced
glutathione depletion (except fosinopril) as compared to CCl\textsubscript{4} intoxicated group. Moreover, the studied drugs significantly reduced liver hydroxyproline level and area of fibrosis as compared to CCl\textsubscript{4} intoxicated group.

The results of the present study demonstrate the hepatoprotective effect of lisinopril, fosinopril and losartan. This hepatoprotection was performed via antioxidant and antifibrotic mechanisms.
AIM OF THE WORK

In the last two decades, a great concern has been targeted to the role of free radical-mediated oxidative stress in the pathogenesis of a variety of human diseases (Stohs, 1995; Young and Woodside, 2001). Strong evidence exists for the relation between the free radical damaging reactions and liver diseases (Britton and Bacon, 1994, Kaplowitz, 2000). Moreover, it was found that the oxidative stress is the key factor in progression of hepatic fibrosis (Poli, 2000).

Several mechanisms have been proposed to mediate the oxidative stress-induced hepatic injury and consequently liver fibrosis. It was found that free radical-initiated lipid peroxidation plays a role in hepatic fibrogenesis. This finding was supported by the observation that dietary supplementation with vitamin E has a protective effect on carbon tetrachloride (CCl4)-induced hepatic fibrosis (Britton and Bacon, 1994). Furthermore, free radicals could effectively activate hepatic stellate cells and fibroblast proliferation (Murrell et al., 1990), hence play a significant role in reparation toward synthesis of extracellular matrix components (Friedman, 2000).

In the light of these circumstances, a great importance has been attributed to the potential role of antioxidants in the prevention and treatment of liver diseases (Britton and Bacon, 1994; Hagymasi and Blazovics, 2004). However, antioxidants can not be used as monotherapy for liver diseases especially if it is accompanied with fibrosis. Combined therapy with antifibrotic drugs must be considered (Albanis et al., 2003; Hagymasi and Blazovics, 2004).
Until now there is no standard treatment for liver fibrosis. The efficacy of most treatments has not been proven in humans (Bataller and Brenner, 2005). Furthermore, current therapies are often ineffective in treating the underlying fibrosis and are associated with many side effects. Hence, there is an increased demand for potent and safe antifibrotic therapies to prevent the progression to end-stage liver disease (Albanis et al., 2003).

Recently it was found that inhibition of renin-angiotensin system (RAS) either by angiotensin converting enzyme (ACE) inhibitors, or angiotensin receptor blockers (ARBs) is probably a promising strategy in treating liver fibrosis (Bataller and Brenner, 2005). These drugs are clinically used in the treatment of essential hypertension, congestive heart failure, myocardial infarction, renal insufficiency and diabetes mellitus (Bicket, 2002; Burnier, 2001).

In fact, the success of these drugs in treating the above conditions is not only due to their ability to inhibit angiotensin II (Ang II) vasoconstrictor effect, but it is related also to their antioxidant properties (Khaper and Signal, 2001; Jahovic et al., 2005). It was found that these drugs act either by increasing the activity of antioxidant enzymes (Mira et al., 1992), or as direct free radical scavengers (Ravati et al., 1999). In addition, they can ameliorate the deleterious effects following oxidative stress, such as lipid peroxidation (Kedziora-Kornatowska, 1999), and glutathione depletion (Massoudy et al., 1995).

Moreover, number of studies has demonstrated that RAS inhibitors could effectively limit the progression of pulmonary (Uhal et al., 1998), renal (Hebert et al., 1999),
and cardiac fibrosis (Taniyama et al., 2000) and appear to be safe when administered for prolonged periods of time. These effects result from the fact that they suppress the elevated level of the Ang II, which is the powerful promoter of oxidative stress, hence fibrosis (Bechara et al., 2005).

Furthermore, strong accumulating evidence indicates that Ang II is an important mediator in liver fibrosis. It was observed that Ang II and other key components of the RAS are locally expressed in chronically injured livers (Paizis et al., 2002). In last five years some studies tried to explore the potential hepatoprotective effect of certain ACE inhibitors (e.g. captopril, perindopril) and ARBs (e.g. losartan) in different experimental models of liver fibrogenesis (Ramalho et al., 2002; Yoshiji et al., 2002; Tuncer et al., 2003).

Accordingly, the present study was designed to investigate the potential antioxidant and antifibrotic mechanisms of two ACE inhibitors, lisinopril and fosinopril and the angiotensin receptor blocker, losartan in experimental rat model of liver injury using CCl₄.
List of Abbreviations

ACE: Angiotensin II converting enzyme
ALT: Alanine aminotransferase
ALP: Alkaline phosphatase
Ang I: Angiotensin I
Ang II: Angiotensin II
ANOVA: Analysis of variance
ARBs: Angiotensin II receptor blockers
AST: Aspartate aminotransferase
AT₁: Angiotensin II receptor 1
CCl₃⁻: Trichloromethyl radical
CCl₃OO⁻: Trichloromethyl peroxyl radical
CCl₄: Carbon tetrachloride
CYP₄₅₀: Cytochrome P₄₅₀
CYP₂E₁: Cytochrome P₄₅₀-2E₁
DNA: Deoxyribonucleic acid
ECM: Extracellular matrix
Fig.: Figure
g/dl: Gram per deciliter
GSH: Reduced glutathione
g tissue: Gram tissue
H₂O₂: Hydrogen peroxide
LDL: Low density lipoprotein
LOOH: Lipid hydroperoxide
MDA: Malondialdehyde
mg: Milligram
mg/dl: Milligram per deciliter
min: Minute
ml: Milliliter
NADPH: Nicotinamide adenine dinucleotide phosphate
nmol: Nanomole
•O$_2^-$: Superoxide anion
•OH: Hydroxyl radical
PUFA: Polyunsaturated fatty acid
RAS: Renin-angiotensin system
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
TBA: Thiobarbituric acid
TBARs: Thiobarbituric acid reactive substances
TCA: Trichloroacetic acid
U/L: Units per liter
VLDL: Very low density lipoprotein
μg: Microgram
μmol: Micromole
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Table (1): Effect of lisinopril, fosinopril, and losartan on liver functions in rats subjected to acute intoxication with CCl₄.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>Total bilirubin (mg/dl)</th>
<th>ALP (U/L)</th>
<th>Total protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85ᵇ ± 5.4</td>
<td>39ᵇ ± 3.5</td>
<td>0.26ᵇ ± 0.01</td>
<td>88ᵇ ± 2.4</td>
<td>9.5 ± 0.6</td>
</tr>
<tr>
<td>CCl₄**</td>
<td>155ᵃ ± 7.8</td>
<td>93ᵃ ± 2.9</td>
<td>0.88ᵃ ± 0.02</td>
<td>105ᵃ ± 7.8</td>
<td>9.1 ± 0.5</td>
</tr>
<tr>
<td>CCl₄ + Lisinopril (0.9mg/kg)</td>
<td>140ᵃ ± 8.4</td>
<td>85ᵃ ± 2.5</td>
<td>0.81ᵃ ± 0.05</td>
<td>105ᵃ ± 3.0</td>
<td>9.4 ± 0.2</td>
</tr>
<tr>
<td>CCl₄ + Lisinopril (1.8mg/kg)</td>
<td>121ᵃᵇ ± 8.3</td>
<td>70ᵃᵇ ± 1.6</td>
<td>0.5³ᵃᵇ ± 0.04</td>
<td>97 ± 2.7</td>
<td>9.6 ± 0.6</td>
</tr>
<tr>
<td>CCl₄ + Lisinopril (3.6mg/kg)</td>
<td>114ᵇ ± 8.1</td>
<td>69ᵃᵇ ± 3.5</td>
<td>0.39ᵇ ± 0.02</td>
<td>96 ± 4.4</td>
<td>9.9 ± 0.5</td>
</tr>
<tr>
<td>CCl₄ + Fosinopril (0.9mg/kg)</td>
<td>142ᵃ ± 2.9</td>
<td>84ᵃ ± 4.2</td>
<td>0.85ᵃ ± 0.04</td>
<td>102 ± 1.4</td>
<td>10.2 ± 0.7</td>
</tr>
<tr>
<td>CCl₄ + Fosinopril (1.8mg/kg)</td>
<td>121ᵃᵇ ± 4.2</td>
<td>69ᵃᵇ ± 1.7</td>
<td>0.6ᵃᵇ ± 0.05</td>
<td>99 ± 0.6</td>
<td>10.3 ± 0.4</td>
</tr>
<tr>
<td>CCl₄ + Fosinopril (3.6mg/kg)</td>
<td>119ᵃᵇ ± 2.9</td>
<td>67ᵃᵇ ± 5.4</td>
<td>0.5ᵃᵇ ± 0.03</td>
<td>98 ± 1.4</td>
<td>10.0 ± 0.2</td>
</tr>
<tr>
<td>CCl₄ + Losartan (4.5mg/kg)</td>
<td>146ᵃ ± 5.2</td>
<td>87ᵃ ± 4.4</td>
<td>0.92ᵃ ± 0.05</td>
<td>107ᵃ ± 2.1</td>
<td>9.1 ± 0.4</td>
</tr>
<tr>
<td>CCl₄ + Losartan (9mg/kg)</td>
<td>123ᵃᵇ ± 7.1</td>
<td>71ᵃᵇ ± 5.0</td>
<td>0.6ᵃᵇ ± 0.04</td>
<td>99 ± 1.9</td>
<td>8.9 ± 0.3</td>
</tr>
<tr>
<td>CCl₄ + Losartan (18mg/kg)</td>
<td>122ᵃᵇ ± 4.4</td>
<td>70ᵃᵇ ± 4.3</td>
<td>0.6ᵃᵇ ± 0.05</td>
<td>97 ± 3.7</td>
<td>10.1 ± 0.3</td>
</tr>
</tbody>
</table>

*Six rats from each group were sacrificed 3 days after CCl₄ injection.
** CCl₄ was given in a single oral dose (2.8 ml/kg), then treatment schedule of drugs was started 6 hours after CCl₄ injection as the following:-
- Lisinopril was given in dose of 0.9, 1.8, and 3.6mg/kg, orally, daily for 3 days.
- Fosinopril was given in dose of 0.9, 1.8, and 3.6mg/kg, orally, daily for 3 days.
- Losartan was given in dose of 4.5, 9, and 18mg/kg, orally, daily for 3 days.
• Data are presented as means ± SE.
• a or b: Significantly different from control or CCl₄ group respectively, at P ≤ 0.05 using ANOVA followed by Tukey test as a post ANOVA test.
Fig. (10): Effect of lisinopril, fosinopril, and losartan on serum AST level in rats subjected to acute intoxication with CCl₄.

- Data are presented as means ± SE.
- CCl₄ was given in a single oral dose (2.8 ml/kg), then treatment schedule of drugs was started 6 hours after CCl₄ injection as the following:
  - Lisinopril was given in dose of 0.9, 1.8, and 3.6 mg/kg, orally, daily for 3 days.
  - Fosinopril was given in dose of 0.9, 1.8, and 3.6 mg/kg, orally, daily for 3 days.
  - Losartan was given in dose of 4.5, 9, and 18 mg/kg, orally, daily for 3 days.
- a or b: Significantly different from control or CCl₄ group respectively, at P ≤ 0.05 using ANOVA followed by Tukey test as a post ANOVA test.