EVALUATION OF ANTIFIBROTIC AND ANTIOXIDANT EFFECTS OF OLMESARTAN MEDOXOMIL, A NEW ANGIOTENSIN II BLOCKER, ON EXPERIMENTALLY-INDUCED LIVER FIBROSIS IN RATS

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ABSTRACT

Aim: The aim of the current article was to investigate the possible antifibrotic and antioxidant effects of a new angiotensin II type I receptor blocker, olmesartan medoxomil, using low and high dose on CCl₄-induced liver fibrosis. Methods: Fifty adult male albino rats were randomly divided into five equal groups, including normal control rats (group I), control vehicle rats (group II), model group (group III), and two treated groups with either low (group IV) or high dose (group V) of olmesartan medoxomil. Except for rats in control groups, all rats were injected subcutaneously with 1ml/kg CCl₄ at a ratio of 1:1 with olive oil, twice a week for 12 weeks. Olmesartan groups were given (0.6 and 6 mg/kg/day) via gavage. At the end of the study period, blood samples and liver tissues were collected and subjected to the biochemical and histopathological examination. Liver function, oxidative stress markers in liver tissues and a marker of liver fibrosis (liver hydroxyproline content) were assessed. In addition to histopathological examination of liver tissues. Results: CCl₄-induced liver fibrosis was manifested by a significant elevation in activities of AST, ALT, ALP and serum bilirubin and a significant decrease in serum albumin. In addition to a significant elevation of liver fibrosis marker (hydroxyproline content of the liver). At the same time, there was a significant increase of lipid peroxidation measured as (MDA) and a significant decrease of (GSH) and (SOD) content in the liver tissue homogenate. Our results revealed that the rats treated with low dose of olmesartan concomitant with CCl₄ showed a non-significant decrease in liver enzymes (AST, ALT, ALP) and bilirubin when compared with model group, while the rats treated with high dose of olmesartan concomitant with CCl₄ resulted in a significant decrease in the previous parameters. At the same time, low dose olmesartan treated rats showed a non-significant difference in albumin as compared to model group while high dose olmesartan treated rats showed a significant elevation in serum albumin. Hydroxyproline content of the liver showed improvement evidenced by a significant decrease in its level in both olmesartan treated groups when compared with CCl₄-treated rats. As regarding lipid peroxidation markers, administration of low or high dose of olmesartan concomitant with CCl₄ exhibited a significant decrease in liver MDA and a significant increase in both GSH and SOD. The biochemical observations were supported by histopathological examination of liver sections. Conclusion: olmesartan medoxomil with low or high dose produced a beneficial effects on CCl₄-induced liver fibrosis through its antifibrotic and antioxidant effects.
INTRODUCTION

Liver fibrosis and cirrhosis, represent the final common pathway of virtually all chronic liver diseases (Gines et al., 2004). There are many primary causes of liver fibrosis with the most common being chronic hepatitis B and C, alcohol and the increasingly important problem of non-alcoholic fatty liver disease. The pathways involved in the fibrogenic response to these and other causes of hepatic fibrosis appear to be broadly similar and share many of the features of chronic fibrotic disease in other organs. The modern view of hepatic fibrosis is that of a dynamic and a potentially reversible process and the end result reflects a balance between pathways which lead to matrix accumulation and those which result in matrix degradation and fibrosis resolution (Friedman et al., 2000 and Friedman, 2003).

The disease is characterized by the deposition of excessive amounts of scar tissue in the liver, which disturbs liver structure and function. In an advanced stage, the fibrotic process acquires a self-perpetuating character, and fibrosis will gradually progress into its end-stage called cirrhosis even when the injurious stimulus is removed. Finally, healthy liver cells are largely replaced by connective tissue. This remodeling of the liver parenchyma also result in impaired blood flow through the liver, which subsequently leads to portal hypertension and many secondary problems (Pinzani et al., 2005).

The response of repair by fibrosis is common to most chronic inflammatory diseases of major organs, including the heart, kidneys, lungs, pancreas and liver. It has been argued that the "encapsulation" of a site of injury by fibrosis is designed to restrict further tissue injury (Friedman et al., 2000).

In the liver, the key event in the initiation of fibrosis is activation of hepatic stellate cells (HSCs). This process characterized by de novo expression of α-smooth muscle actin and transformation to a cell of
myofibroblastic phenotype that produce a range of prosclerotic cytokines and matrix proteins (Friedman, 1993). With progressive fibrosis, there are changes in both the quantity and quality of hepatic extracellular matrix proteins such as type I collagen (Paizis et al., 2001).

Apart from the circulating renin-angiotensin system (RAS), the existence of local or intra-organ RASs have been described in a number of organ, including the heart, kidney, lung, pancreas, and liver (Leung, 2004). Researches increasingly show that locally synthesized angiotensin II (Ang II), as a fibrogenetic factor is involved in cardiac fibrosis (Silvestre et al., 1999), renal interstitial fibrosis (Metzger et al., 1999), and pulmonary fibrosis (Wang et al., 1999). Thus treatment with angiotensin-converting enzyme (ACE) inhibitor or angiotensin II type I (AT₁) receptor antagonist may attenuate the cardiac fibrosis that occurs in experimental myocardial infarction (Silvestre et al., 1999), and may also retard the progression of renal glomerulosclerosis and interstitial fibrosis (Hebert et al., 1999).

A growing number of studies have suggested that RAS, an important factor in regulating blood pressure and body fluid homeostasis, is also involved in hepatic fibro-genesis. Patients with chronic liver disease, showed an increased in plasma renin activity (Helmy et al., 2000). There is now considerable evidence that Ang II, the main effector peptide of RAS, is involved in both recruitment of inflammatory cells (Sewnath et al., 2004) and transformation of HSC into an activated phenotype (Bataller et al., 2003). Despite efforts to develop antifibrotic agents, no drugs have been approved as antifibrotic agents in human (Friedman, 2003). Clinical trials and experimental studies have revealed that blockade of RAS with ACE inhibitors or the AT₁ receptor blockers can significantly slow down the progression of fibrotic disease (Yoshiji et al., 2002).

Olmesartan medoxomil - a non-peptide imidazole derivative - is one of the newest addition to the angiotensin II receptor blockers class. It's a
prodrug that is rapidly and completely de-esterified in the intestinal wall into the active metabolite, olmesartan (Grossman et al., 2000).

The aim of the present study was to evaluate the possible antifibrotic and antioxidant effects of olmesartan medoxomil, a new angiotensin II type I receptor blocker, using low or high dose on experimentally-induced liver fibrosis by carbon tetrachloride (CCl₄) in rats.

MATERIAL AND METHODS

Animals:

The study was conducted on fifty adult male albino rats with initial body weight ranging between 180-200 grams. All animals were housed in a well ventilated room with free access to food and water. The rats had been familiarized with the environment for one week before the study.

Drug and Chemical:

- **Olmesartan medoxomil**: It is a product of Sankyo Company Limited, in the form of tablets, which were crushed and suspended uniformly in 1% solution of carboxymethyl-cellulose (CMC).

- **Carbon tetrachloride (CCl₄)**: It was purchased from El-Nasr Chemical Industries Company, in the form of liquid, It was dissolved in olive oil at a ratio of (1:1).

- **Experimental Groups**:

  Fifty rats were divided into 5 groups. Each consisted of 10 rats and distributed as follows:

  **Group (I): Normal control group**, the animals received no treatment.

  **Group (II): Control vehicle group**, each animal received a vehicle for CCl₄ (i.e. olive oil) by subcutaneous route in a dose of 1 ml/kg twice weekly for 12 weeks and simultaneously the animals administered a vehicle for olmesartan medoxomil (i.e. CMC solution) by oral route in a daily dose of 1ml/kg for the same period.
• **Group(III): Model group**, each animal received CCl₄ subcutaneously in a dose of 1 ml/kg twice weekly for 12 weeks *(Zhang et al., 2001).*

**Group(IV): Low dose olmesartan-treated group**, each animal received 1 ml/kg CCl₄ subcutaneously twice weekly concomitant with olmesartan medoxomil in a dose of 0.6 mg/kg/day by gavage for 12 weeks *(Mizuno et al., 2002).*

**Group (V): High dose olmesartan-treated group**, each animal received 1 ml/kg CCl₄ subcutaneously twice weekly concomitant with olmesartan medoxomil in a dose of 6 mg/kg/day by gavage for 12 weeks *(Mizuno et al., 2002).*

**Experimental Parameters:**

Twenty four hours after the end of the experimental period, blood samples were collected from retro-orbital venous plexus and allowed to clot. Serum was separated by centrifugation for spectrophotometric assessment of liver functions. Subsequently, rats were sacrificed and liver tissues were dissected out and washed with ice-cold saline. Parts of the 3 major lobes of each liver were fixed in 10% formalin and embedded in paraffin for histopathological studies, the remainder were homogenized in 0.1 mol/L Tris-HCl buffer (pH 7.4). The homogenate was used for assessment of marker of liver fibrosis and oxidative stress markers.

**Biochemical Analysis:-**

(I) **Serum determinations:**

**Hepatotoxicity indices:**

Serum determination of aminotransferases (AST and ALT) *(Frankel and Gradwohl, 1970)*, alkaline phosphatase (ALP) *(Donald and Ralph, 1990)*, total bilirubin level *(Walter and Gerade, 1970)* and serum albumin level *(Doumas et al., 1971).*
(II)Liver tissue homogenate determinations:

(A)Liver fibrosis marker:
Determination of liver hydroxyproline: As an index of liver fibrosis using the method of Woessner (1961).

(B)Oxidative stress markers:
Determination of hepatic malondialdehyde (MDA) concentration (Ohkawa et al., 1979), antioxidant enzymes: Glutathione (GSH) (Ellman, 1959) and superoxide dismutase (SOD) (Misra and Fridovich, 1972).

Histopathological Examination:
Paraffin sections of 5-7 μm in thickness were prepared and subjected to Hematoxylin-Eosin (Hx&E) according to Drury and Wallington (1980).

Statistical Analysis:
The experimental data are expressed as mean ± S.E. The data were analyzed by the student t-test. The differences were considered to be statistically significant when P < 0.05.

Results
During the experiment; two rats died from CCl₄-treated rats (group III) while one rat died from each low or high dose olmesartan-treated rats (groups IV, V).

Biochemical Analysis:-

(I)Serum determinations results:
Table (1) shows non significant difference in the mean values of AST, ALT, ALP, total bilirubin and serum albumin between normal control group (group I) and control vehicle group (group II).

CCl₄ treatment of the rats (group III) resulted in a significant elevation (P < 0.05) in the mean values of liver function
(AST, ALT and ALP) and total bilirubin compared to control vehicle rats (group II) indicating hepatocellular damage. Concomitant oral administration of low dose of olmesartan with CCl₄ (group IV) showed a non-significant decrease (P > 0.05) in the mean values of liver function as compared with CCl₄-treated rats (group III). While concomitant administration of high dose of olmesartan with CCl₄ (group V) resulted in a significant reduction (P < 0.05) of these parameters as compared to CCl₄-treated rats (group III). While, groups IV and V showed a significant increase (P < 0.05) in liver function parameters when compared with control vehicle rats (group II).

The serum albumin in CCl₄-treated rats (group III) was significantly lower (P < 0.05) than that in control vehicle group (group II). Concomitant administration of low dose olmesartan and CCl₄ (group IV) showed a non-significant difference (P > 0.05) in the mean values of serum albumin while the co-administration of high dose of olmesartan and CCl₄ (group V) resulted in a significant elevation (P < 0.05) of this parameter when compared with CCl₄-treated rats (group III). On the other hand, groups IV and V showed significant decrease (P < 0.05) in plasma albumin when compared with control vehicle rats (group II).
Table (1): Effect of oral administration of low dose of olmesartan medoxomil (0.6 mg/kg/day) and high dose (6 mg/kg/day) on liver functions in rats with liver fibrosis induced by CCl₄ (1ml/kg s.c. twice a week for 12 weeks).

<table>
<thead>
<tr>
<th></th>
<th>Normal control group (Group I) n=10</th>
<th>Control vehicle group (Group II) n=10</th>
<th>Model group (Group III) n=8</th>
<th>Low dose olmesartan-treated group (Group IV) n=9</th>
<th>High dose olmesartan-treated group (Group V) n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>68.94 ± 0.98</td>
<td>69.56 ± 0.99</td>
<td>301.60 ± 4.99*</td>
<td>294.26 ± 1.96*</td>
<td>195.83 ± 3.32*</td>
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<tr>
<td>ALT (U/L)</td>
<td>43.76 ± 1.06</td>
<td>43.87 ± 1.08</td>
<td>149.40 ± 3.18*</td>
<td>145.53 ± 1.53*</td>
<td>98.74 ± 2.94*</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>107.21 ± 2.48</td>
<td>106.89 ± 2.57</td>
<td>219.38 ± 1.94*</td>
<td>215.00 ± 1.39*</td>
<td>169.38 ± 1.88*</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.47 ± 0.02</td>
<td>0.47 ± 0.01</td>
<td>1.71 ± 0.02*</td>
<td>1.62 ± 0.04*</td>
<td>0.98 ± 0.02*</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>3.55 ± 0.06</td>
<td>3.60 ± 0.04</td>
<td>2.97 ± 0.05*</td>
<td>3.02 ± 0.02*</td>
<td>3.49 ± 0.03*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE
* : Significant change (P< 0.05) compared to control vehicle group.
#: Significant change (P< 0.05) compared to model group.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase
**Liver tissue homogenate determinations results:**

**(A) Liver fibrosis marker:**

Table (2) shows non significant difference in the mean values of liver hydroxyproline content between normal control group (group I) and control vehicle group (group II).

CCL₄-induced liver fibrosis in rats (group III) accompanied by a significant elevation (P < 0.05) of liver hydroxyproline concentration when compared with control vehicle group (group II). Co-administration of low or high doses of olmesartan with CCL₄ (group IV, V) exhibited a significant decrease (P < 0.05) in liver hydroxyproline concentration compared with CCL₄-treated rats (group III). On the other hand, there was a significant elevation (P < 0.05) in this parameter in groups IV and V when compared with control vehicle group (group II) (fig 1).

**(B) Oxidative stress markers:**

Table (2) shows non significant difference in the mean values of malondialdehyde (MDA), liver glutathione (GSH) and superoxide dismutase (SOD) between normal control group (group I) and control vehicle group (group II).

CCL₄-treated rats (group III) showed a significant increase (P < 0.05) in MDA as compared to control vehicle group (group II) and also showed a significant decrease (P < 0.05) in liver GSH and SOD levels. Treatment of rats with low or high dose of olmesartan concomitant with CCL₄ (group IV, V) resulted in a significant decrease (P < 0.05) in liver MDA and a significant increase (P < 0.05) in the levels of liver GSH and SOD when compared with CCL₄-treated rats (group III). As regards groups IV and V when compared with control vehicle group, there was a significant increase (P < 0.05) in MDA and a significant decrease (P < 0.05) in liver GSH and SOD levels (figs 2,3,4).
Table (1): Effect of oral administration of low dose of olmesartan medoxomil (0.6 mg/kg/day) and high dose (6 mg/kg/day) on hydroxyproline, MDA, GSH, and SOD in rats with liver fibrosis induced by CCl₄ (1ml/kg s.c. twice a week for 12 weeks).

<table>
<thead>
<tr>
<th></th>
<th>Normal control group (Group I) n=10</th>
<th>Control vehicle group (Group II) n=10</th>
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<th>Low dose olmesartan-treated group (Group IV) n=9</th>
<th>High dose olmesartan-treated group (Group V) n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyproline mg/g</td>
<td>4.73 ± 0.18</td>
<td>4.75 ± 0.19</td>
<td>8.58 ± 0.42*</td>
<td>6.18 ± 0.34*#</td>
<td>5.67 ± 0.33*#</td>
</tr>
<tr>
<td>MDA nmol/g</td>
<td>55.86 ± 1.27</td>
<td>56.11 ± 1.25</td>
<td>124.01 ± 3.86*</td>
<td>82.75 ± 1.77*#</td>
<td>73.08 ± 1.29*#</td>
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<td>GSH mol/mg</td>
<td>10.16 ± 0.5</td>
<td>10.18 ± 0.51</td>
<td>3.15 ± 0.21*</td>
<td>6.90 ± 0.13*#</td>
<td>8.27 ± 0.18*#</td>
</tr>
<tr>
<td>SOD U/mg</td>
<td>134.65 ± 2.43</td>
<td>134.75 ± 2.41</td>
<td>9.71 ± 3.35*</td>
<td>109.50 ± 2.01*#</td>
<td>128.13 ± 1.72*#</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE
* : Significant change (P< 0.05) compared to control vehicle group.
#: Significant change (P< 0.05) compared to model group.
MDA, malondialdehyde, GSH, gluathione, SOD, superoxide dismutase.
Fig 1: Effect of low dose (0.6 mg/kg / day p.o. for 12 weeks) and high dose (6 mg/kg/day p.o. for 12 weeks) of olmesartan medoxomil on liver hydroxyproline levels in rats with liver fibrosis induced by CCl₄ (1 ml/kg s.c. twice a week for 12 weeks).

Data are expressed as mean ±SD.

*: Significant change (P< 0.05) compared to control vehicle group.
#: Significant change (P< 0.05) compared to model group.
Fig 2: Effect of low dose (0.6 mg/kg / day p.o. for 12 weeks) and high dose (6 mg/kg/day p.o. for 12 weeks) of olmesartan medoxomil on liver malondialdehyde level in rats with liver fibrosis induced by CCl₄ (1 ml/kg s.c. twice a week for 12 weeks).

Data are expressed as mean ±SD.

*: Significant change (P< 0.05) compared to control vehicle group.
#: Significant change (P< 0.05) compared to model group.
**Fig3:** Effect of low dose (0.6 mg/kg / day p.o. for 12 weeks) and high dose (6 mg/kg/day p.o. for 12 weeks) of olmesartan medoxomil on liver glutathione levels in rats with liver fibrosis induced by CCl₄ (1 ml/kg s.c. twice a week for 12 weeks).

Data are expressed as mean ±SD.

* : Significant change (P< 0.05) compared to control vehicle group.
#: Significant change (P< 0.05) compared to model group.
**Fig4:** Effect of low dose (0.6 mg/kg / day p.o. for 12 weeks) and high dose (6 mg/kg/day p.o. for 12 weeks) of olmesartan medoxomil on liver superoxide dismutase in rats with liver fibrosis induced by CCl₄ (1 ml/kg s.c. twice a week for 12 weeks).

Data are expressed as mean ±SD.

*: Significant change (P < 0.05) compared to control vehicle group.
#: Significant change (P < 0.05) compared to model group.
**Histopathological Examination:**

Histopathological examination of liver sections of control groups (group I, II) showed normal cellular architecture with radiating hepatic cords, normal sinusoidal spaces and central veins (fig.5, 6). While, the liver specimens obtained from the rats treated with CCl₄ alone (group III) revealed a destruction of normal hepatic architecture and the hepatocytes have become surrounded by fibrous tissue (fig.7). Areas of hepatocellular necrosis and inflammatory cellular infiltration were also observed. Many cells showed degenerative changes in the form of fatty degeneration (fig. 8).

The liver specimens obtained from the rats treated with CCl₄ concomitant with olmesartan medoxomil using low dose (group IV) or high dose (group V) resulted in apparent amelioration of necrosis and inflammatory cellular infiltration in addition to a reduction of fibrous tissue which was more marked in group V (figs 9,10).
Fig 5: A photomicrograph of a liver section of group I (normal control rats) showing radiating liver cords which surround a central vein, normal blood sinusoids and normal hepatocytes (Hx & E X 400).

Fig 6: A photomicrograph of a liver section of group II (control vehicle rats) showing radiating liver cords which surround a central vein, normal blood sinusoids and normal hepatocytes (Hx & E X 400).
Fig 7: A photomicrograph of a liver section of group II (CCl₄-treated rats) showing destruction of hepatic architecture, fatty degeneration and the hepatocytes become surrounded by fibrous tissue (Hx & E X 200).

Fig 8: A photomicrograph of a liver section of group II (CCl₄-treated rats) showing inflammatory cellular infiltration and areas of necrosis (Hx & E X 400).
Fig 9: A photomicrograph of a liver section of group III (CCl₄ + low dose olmesartan-treated rats) showing normal hepatic architecture, decrease in fibrous tissue but the hepatocytes still exhibit fatty degeneration (Hx & E X 200).

Fig 10: A photomicrograph of a liver section of group IV (CCl₄ + high dose olmesartan-treated rats) showing normal hepatic architecture, marked decrease in fibrous tissue and fatty degeneration (Hx & E X 200).
DISCUSSION

In the present study, we investigated the possible antifibrotic and antioxidant effects of low or high doses of olmesartan medoxomil, a new AT₁ receptor antagonist, in a chronic model of liver fibrosis experimentally-induced by CCl₄.

The in-vitro finding that AT₁ receptors are expressed on activated human (Bataller et al., 2000) and rats (Wei et al., 2000) hepatic stellate cells (HSCs), and are thus likely to be increased in number as stellate cells proliferate, provides evidence for a potential pathway through which Ang II might mediate and exacerbate liver injury. Binding of Ang II to AT₁ receptors induces contraction and proliferation of HSCs (Bataller et al., 2000). To date, it is controversial whether blockade of Ang II can attenuate the development of hepatic fibrosis in animal models.

CCl₄-induced fibrosis shares several characteristics with human fibrosis of different etiologies; thus, it is an adequate model of human fibrosis (Liu et al., 2000). The hepatotoxicity of CCl₄ depends on its metabolism in hepatocytes by cytochrome P450 2E1 (CYP 2E1), which generates highly reactive trichloromethyl free radicals, leading to lipid peroxidation and membrane damage (Shi et al., 1998). Kupffer cells (KCs) are activated by free radicals and produce proinflammatory mediators, resulting in the triggering of an inflammatory cascade (Thompson et al., 1998).

In the present study, the hepatotoxicity of CCl₄ in rats was confirmed by a significant elevation of AST, ALT, ALP and total bilirubin. This might be due to the release of these enzymes from the cytoplasm, into the blood rapidly after rupture of the plasma membrane and cellular damage (Sallie et al., 1991). In accord with our findings,
several authors have reported a significant increase in liver enzymes in rats subjected to CCl$_4$ intoxication (Yokohama et al., 1999 and Nkosi et al., 2005).

Data of the present study revealed that CCl$_4$ intoxication produced a significant reduction in albumin serum level compared to control vehicle group, this coincide with that of Vazquez et al. (1990) who observed a reduction in protein synthesis after CCl$_4$ treatment.

To assess liver fibrosis, hydroxyproline was chosen because it is a sensitive marker that increases significantly during liver fibrosis. The increase in this amino acid reflects an increase in the de novo synthesis of liver collagen and an increase in the amount of hydroxyproline (Ala-Kokko et al., 1987).

In the present study, measurement of hydroxyproline confirmed the histological observation of enhanced liver fibrosis by CCl$_4$. The chronic administration of CCl$_4$ resulted in a significant increase in the liver content of hydroxyproline compared with the control vehicle group. Similar observation have been reported previously (Ohishi et al., 2001 and Jeong et al., 2005).

In our study, administration of olmesartan medoxomil in a small dose (0.6 mg/kg) did not reduce hepatocyte injury induced in rats by CCl$_4$ as indicated by the insignificant effect on serum AST, ALT, ALP and total bilirubin levels although it improved liver fibrosis as indicated by significant reduction of liver content of hydroxyproline and histopathological examination of liver tissues. This suggests that olmesartan may have a direct effect on fibrosis. Our results are in accordance with the findings of Kurikawa et al. (2003) who demonstrated that administration of olmesartan in a dose of 1 mg/kg
induced insignificant changes in liver enzymes and significant decrease in liver hydroxyproline content in liver fibrosis induced in rats by common bile duct ligation. They concluded that olmesartan may have direct antifibrotic effect rather than hepatoprotective effect. In contrast, in this study, administration of olmesartan in a high dose (6 mg/kg) was able to improve liver functions and produced hepatoprotection, as indicated by significant reduction of serum AST, ALT, ALP and total bilirubin in CCl₄ intoxicated rats compared with model group, in addition to its antifibrogenic action. Histopathological examination also showed that olmesartan in a high dose evidently alleviated the progression of hepatic fibrosis.

The results are in consistent with the findings of many studies that elucidated attenuation of the progression of liver fibrosis in-vivo by other AT₁ receptor antagonists in different animal models of experimentally-induced liver fibrosis.

In a rat model of pig-serum induced liver fibrosis, administration of candesartan blocked hepatic fibrosis and decreased the expression of α-smooth muscle actin (α-SMA), a marker of activated HSCs (Yoshiji et al., 2001). In another study by Xu et al. (2006), valsartan induced significant improvement in liver functions as indicated by reduction of ALT, AST and increase in serum albumin in addition to significant decrease in serum level of hyaluronic acid on CCl₄-induced liver fibrosis in rats. Fujita et al. (2007) showed that telmisartan markedly attenuated hepatic inflammation and fibrosis in non-alcoholic steatohepatitis in rats.

On the other hand, irbesartan did not result in reduced liver fibrosis or hydroxyproline content, although it suppressed the over-expression of TGF-β₁ and type I collagen gene (Paizis et al., 2001). Valsartan exerted a weak suppressive effect on the development of hepatic fibrosis (Fujita et al., 2007). These conflicting findings could be attributed to
differences in the method of fibrogenesis or in the drugs tested and the doses which was used in each one of the previous studies.

In the present study, we started olmesartan treatment concomitantly with CCl₄ to assess the value of early therapy with AT₁ blocker in chronic liver disease. The reason of choosing this protocol was the finding of Tox et al. (2007) who assessed the hypothesis that the expression of angiotensin II receptor type I in the liver tissue changes with increasing fibrosis, which would influence the antifibrotic efficacy of AT₁ blockers. They demonstrated for the first time that the expression of AT₁ mRNA and protein is negatively correlated with the degree of liver fibrosis. This decrease expression of the receptor may reduce the potential impact of any angiotensin II on the progression of liver fibrosis compared to other profibrotic stimuli like TGF-β₁, endothelin-1 and platelet-derived growth factor. Therefore, blocking AT₁ receptors should become less effective during progressive fibrosis and more effective when initiated early.

Many published papers described an improvement of rat liver fibrosis after therapy with AT₁ receptor blockers without inducing any antihypertensive effects (Paizis et al., 2001 and Yoshiji et al., 2001; 2002). This effect may be of value as we may achieve the beneficial antifibrotic effect without having possible deleterious haemodynamic side effects by using AT₁ receptor blockers in small dose.

Our results revealed that administration of low dose of olmesartan in CCl₄-induced liver fibrosis in rats significantly reduced the fibrogenic marker, hydroxyproline, in the liver tissues. The antifibrotic activity of olmesartan observed in this study could be supported by its antifibrotic effects elucidated in other tissues. Porteri et al. (2005) reported a significant reduction and even normalization of collagen content in the
heart and the kidneys of spontaneously hypertensive rats treated with low and high doses of olmesartan.

In the present study, we explored the hepatoprotective mechanisms of olmesartan by studying markers of oxidative stress. Szymonik-Lesiuk et al. (2003) reported that the exhaustion of intracellular substances, such as glutathione (GSH), which are capable of preferentially conjugated with the toxic metabolites and free radicals, is one of the consequences of CCl₄-induced injury.

In our study, GSH in liver homogenate of CCl₄ model group was remarkably reduced, reflecting that the potency of antioxidation in injured cells was altered. This result agree quite well with that of Ko et al. (1995). Depletion of GSH was found to induce lipid peroxidation, which, in turn, causes increased GSH consumption (Bandyopadhyay et al., 1999). Accordingly, we measured the level of malondialdehyde (MDA) in the liver tissue as an indicator of lipid peroxidation and, hence, the oxidative stress state. We found that chronic administration of CCl₄ induced a significant increase in the liver content of MDA in comparison with the control vehicle group. Similar observations have been reported in previous studies (Kanter et al., 2005 and He et al., 2006).

Superoxide dismutase (SOD) acts as a cellular defense element against potentially harmful effects of superoxide ions by catalyzing the dismutation of these ions (Kamalakkannan et al., 2005). We found that the rats treated with CCl₄ had a significantly lower level of SOD in the liver tissue compared with the control group.

The oxidative stress activates HSCs, induces the secretion of growth factors and profibrogenic cytokines and stimulates collagen synthesis. Angiotensin II is also reported to stimulate the formation of
reactive oxygen species in-vitro and in-vivo. (Rajagopalan et al., 1996 and Torrecillas et al., 2000). These findings indicates that lipid peroxidation induced by Ang II is involved in the progression of hepatic fibrosis.

In the present study, treatment of rats with olmesartan in both low or high dose counteracted GSH depletion and lipid peroxidation induced by CCl₄ in rats. At the same time, administration of low or high dose of olmesartan concomitant with CCl₄ induced a significant increase in SOD level in the liver of rats when compared with the model group. These findings support the premise that olmesartan can guard against the sequences of oxidative stress. So, the antioxidant properties of this drug are involved in the mechanism of action through which is exert its hepatoprotective effect.

To our knowledge, The antioxidant effect of olmesartan has not been examined previously in models of liver injury. In accordance with our results, it was proven experimentally that the antioxidant properties of olmesartan contributed to its beneficial effect in treating many clinical conditions. Takahashi et al. (2007) reported that administration of low or high dose of olmesartan attenuated lipid peroxidation as indicated by significant reduction of MDA in renal tissue of rats exposed to progressive renal injury induced by subtotal nephrectomy.

Other studies was constructed to explore the exact mechanisms underlying the antioxidant effect of olmesartan. Fujimoto et al. (2008) found that angiotensin II induced a dose dependent increase of superoxide production in isolated normal glomeruli. They explored the effect of olmesartan both in-vitro and in-vivo. Olmesartan was able to suppress reactive oxygen species production in glomeruli. It reduced superoxide production both in-vitro and in the glomeruli of rats with
experimentally-induced chronic kidney disease. Moreover, they proved that the effect of olmesartan on superoxide production was independent of its blood pressure lowering effects. In addition, Miyata et al. (2002) demonstrated that olmesartan significantly quenched a number of different types of free radical particularly hydroxyl radicals in-vitro.

In conclusion, we demonstrated that olmesartan medoxomil, a new AT$_1$ antagonist, exerts a protective effects against CCl$_4$-induced liver fibrosis when used in low or high dose through its antifibrotic and antioxidant effects.

References:


تقييم التأثيرات المضادة للتليف و الأكسدة لدواء الأولميسارتان ميدوكسوميل ( دواء جديد مثبت لمستقبلات الأنجيوتنسين 2 ) على الفئران المصابة بتليف الكبد المستحدث

تجربية

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يهدف هذا البحث إلى دراسة التأثيرات المحتملة المضادة للتليف و الأكسدة في الفئران المصابة بتليف التهابي في الكبد المستحدث تجريبيا بدماغ رايع كلوريد الكربون و ذلك باستخدام جرعة منخفضة و عالية من دواء جديد مثبت لمستقبلات الأنجيوتنسين 2 و هو الأولميسارتان ميدوكسوميل.

هذا وقد أجري البحث على 50 فأرا من ذكور الفئران البيضاء تم تقسيمهم إلى خمسة مجموعات متساوية : المجموعة الأولى و الثانية هي المجموعات الضابطة، المجموعة الثالثة: تم إعطاءها رايع كلوريد الكربون بجرعة 1 مل/كجم من وزن الفأر تحت الجلد مرتين أسبوعيا لمدة 12 أسبوعا، المجموعة الرابعة: فئران يتم إعطائها رايع كلوريد الكربون بنفس الجرعة السابقة و في نفس الوقت تم إعطاؤها بجرعة منخفضة من الأولميسارتان (1/2 مجم/كجم من وزن الفأر يوميا عن طريق الفم) و المجموعة الخامسة: فئران تم إعطائها رايع كلوريد الكربون بنفس الجرعة و تم علاجها بجرعة عالية من الأولميسارتان (1 مجم/كجم من وزن الفأر عن طريق الفم يوميا ) و في نهاية المدة البحث أيا هو 12 أسبوعا، تم تجميع عينات الدم و الكبد من المجموعات الأربعة و ذلك لتقييم وظائف الكبد في الدم و أيضا تحتوي الكبد من موثر التليف الكيدي ( هيدروكسي بروتين ) و مستوى الأكسدة القوقية و نشاط الأنزيم المضاد للأكسدة في نسيج الكبد و كذلك الفحص الهستوئلولوجي لعينات الكبد.

و قد أظهرت نتائج هذا البحث أن استخدام دواء الأولميسارتان بجرعة منخفضة لم يحدث تغيير ذو دلالة إحصائية في وظائف الكبد بينما أدى استخدام الدواء بجرعة عالية إلى تحسن ذو دلالة إحصائية في وظائف الكبد أما بالنسبة لمستوى الهيدروكسي بروتين فقد حدث انخفاض ذو دلالة إحصائية في محتوى الكبد من هذه المادة و ذلك في المجموعات المعالجتان بالولميسارتان و قد لوحظ أيضا حدوث انخفاض ذو دلالة إحصائية في مستوى الجلوتاتابون و كذلك في نشاط الأنزيم المضاد للأكسدة و هو السوير أكسيد ديميتوزت في كلا المجموعات المعالجتان بالولميسارتان و ذلك مقابلة بالمجموعة المضادة للتليف الكيدي الغير معالجة بالدواء.

و قد تأكدت هذه النتائج بالفحص الهستوئلولوجي للسج Dodd الجلد حيث حدد تحسن ملحوظ في التليف الكيدي في المجموعات المعالجة بالولميسارتان بالمقارنة بالمجموعة المصابة بالتليف الكيدي الغير معالجة بالدواء.

و من هذه الدراسة نستخلص أن دواء الأولميسارتان له تأثير وافي و هو سميكة الكبد المحتملة تجريبيا بواسطة رايع كلوريد الكربون و أن هذا التأثير يعتمد على تركيز الجرعة و ذلك عن طريق تحسين وظائف الكبد و الخواص المضادة للأكسدة و التليف.