

# Perinodopril, an angiotensin converting enzyme inhibitor, attenuates experimental hepatic fibrosis

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**Abstract:** Liver fibrosis is considered as a progressive pathological process involving multiple cellular and molecular events that lead to deposition of excess matrix proteins in the extracellular space. When that process is combined with ineffective regeneration and repair, there is increasing distortion of the normal liver architecture, and the end result is cirrhosis. Emerging anti-fibrotic therapies are aimed at inhibiting the accumulation of fibrogenic cells and/or preventing the deposition of extracellular matrix proteins. Although many therapeutic interventions are effective in experimental models of liver fibrosis, the mechanisms underlying the anti-fibrotic effect on liver fibrosis remain unclear. The aim of the present study was to investigate the underlying mechanisms of anti-fibrotic effect of angiotensin converting enzyme inhibitor (ACEI) on experimental liver fibrosis induced by carbon tetrachloride (CCl<sub>4</sub>). Thirty five white albino male rats of 150-200g average weight were randomly divided into three groups, **Group I:** The control group (n = 10), **Group II:** CCl<sub>4</sub> injected rats without any treatment (n = 10) and **Group III:** CCl<sub>4</sub> injected rats that received an ACEI, perinodopril, dissolved in distilled water, 6mg/kg/day for 4 weeks (n = 15). Venous blood was collected from retro orbital vein for serum separation for assessment of liver functions. Liver tissue was subdivided into three portions for: pathological examination, estimation of transforming growth factor- $\beta_1$  (TGF- $\beta_1$ ) and matrix metalloproteinase-9 (MMP-9) by ELISA and expression of nuclear factor- $\kappa$ B (NF- $\kappa$ B) in a trial to understand the mechanism underlying the anti-fibrotic effect of ACEI. Tissue TGF- $\beta_1$ , MMP-9 and NF- $\kappa$ B were significantly higher in CCl<sub>4</sub> group (group II) compared with control group (group I) and they were decreased significantly with administration of ACEI with CCl<sub>4</sub> (group III). In conclusion, ACEI attenuates the progression of hepatic fibrosis induced by CCl<sub>4</sub> by reducing expression of NF- $\kappa$ B, TGF- $\beta_1$ , and MMP-9.

Key words: liver fibrosis, TGF-  $\beta_1$ , MMP-9 and NF- $\kappa$ B

## Introduction:

Chronic liver injury is caused by a variety of insults, including viral hepatitis, alcohol, drug abuse and autoimmune hepatitis. It is associated with increased inflammatory cell infiltration and may involve the interplay of different inflammatory mediators, which is a common stage in most of chronic liver diseases. If treated properly in that stage, hepatic fibrosis can be reversed and its progression to irreversible cirrhosis, which leads to lethal complications and high mortality, may be prevented. Currently, there is no established therapy to delay or reverse the progression of liver fibrosis <sup>(1)</sup>. Once cirrhosis develops, liver transplantation is the only therapy to avoid a fatal condition <sup>(2)</sup>. Therefore, an accurate assessment of liver fibrosis is very important in order to predict the prognosis and start the appropriate prophylactic therapy to prevent disease progression. Liver biopsy (LB) is still the gold-standard method for assessing liver fibrosis <sup>(3, 4, 5)</sup>. However, it is difficult to perform LB for all patients who need to be assessed repeatedly due to its invasiveness and prohibitive cost. In addition, biopsy samples are usually too small to diagnose the disease accurately and diagnostic opinions often differ among pathologists <sup>(6, 7)</sup>. As a result, a pathological

examination does not always provide an accurate diagnosis. Recent studies work hard to assess a noninvasive method for the diagnosis and evaluation of the prognosis of liver fibrosis<sup>(8)</sup>.

Recently, several lines of evidence have suggested that the rennin-angiotensin system (RAS) plays an important role in the pathogenesis of organ fibrosis<sup>(9)</sup>. Angiotensin II (Ang II) plays a central role in the regulation of systemic blood pressure and fluid homeostasis. Its action is mediated mainly by two subtypes of receptors, Ang II type 1(AT<sub>1</sub>) and Ang II type 2 (AT<sub>2</sub>) receptors<sup>(10)</sup>.

Moreover, the expression of TGF- $\beta$ <sub>1</sub>, the key cytokine in the development of organ fibrosis, is increased by Ang II and the blockade of the (RAS) by ACEI or by AT<sub>1</sub> antagonists has been shown to reduce the progression of organ fibrosis. However, the mechanisms underlying the anti-fibrotic effect of ACEI on liver fibrosis remain unclear<sup>(11)</sup>.

It should be noted that, upon liver injury, hepatic stellate cells (HSCs) undergo morphological trans-differentiation to myofibroblast-like cells having a proliferative, fibrogenic and contractile phenotype; this process is known as activation. Activated HSCs express smooth muscle actin (SMA) and produce an excess of collagen and other ECM components<sup>(2)</sup>.

In addition, activated HSCs secrete transforming growth factor- beta1 (TGF- $\beta$ <sub>1</sub>), which is a key mediator in human fibrogenesis leading to a fibrotic response and proliferation of connective tissue. Furthermore, HSCs disturb the balance between matrix metalloproteinases (MMPs) and the naturally occurring tissue inhibitors of metalloproteinases (TIMP 1 and 2), leading to matrix breakdown and replacement by connective tissue-secreted matrix<sup>(12)</sup>.

Nuclear factor- $\kappa$ B (NF- $\kappa$ B) is a transcriptional factor, which binds to specific DNA motifs to regulate transcriptional activity of its target genes involved in HSCs activation<sup>(13)</sup> and releasing of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6), which are involved in the process of fibrogenesis<sup>(14)</sup>.

Therefore, the aim of the present work is to further evaluate the anti-fibrotic effect of ACEI in rat liver fibrosis induced by CCl<sub>4</sub>, and to study its relationship with the expression of NF- $\kappa$ B, TGF- $\beta$ <sub>1</sub> and MMP-9.

## **Materials and methods:**

Thirty five white albino male rats of 150-200g average weight were included in the study .They were bred in the animal house of kasr Al-Aini Hospital, Cairo University. Rats were randomly divided into three groups:

**Group I:** (n = 10) control group, fed with regular rat chaw.

**Group II:**( n = 10)fibrotic model, they received 0.2 ml CCl<sub>4</sub> subcutaneously twice weekly for 6 weeks.

**Group III:** (n = 15) model of liver fibrosis that received an ACE inhibitor, perinodopril, dissolved in distilled water, 6mg/kg/day for 4 weeks by oral feeding.

**Sample collection and preparation:** At the time of sacrificing the rats, venous blood was collected from retro orbital vein for serum separation for assessment of liver functions. Liver tissue was subdivided into three portions: The first portion was stored in formaldehyde for pathological examination. The second portion was stored at -70°C in lysis buffer that contains guanidinium thiocyanate and B-mercaptoethanol for RNA extraction. The third portion, also, was stored at -70°C in phosphate buffer saline for TGF- $\beta_1$  and MMP-9 assay by ELISA.

**Liver functions:**

Serum ALT and albumin were estimated by using conventional laboratory methods.

**Measurement of TGF-  $\beta_1$  and MMP-9:**

About 19 mg of liver tissue was homogenized in one ml lysis buffer for protein extraction which contained 0.06 mol/l Tris buffer (pH 6.8), 2% SDS (sodium dodecyl sulphate), 100 mmol/l sodium fluoride and one mmol/l phenyl methyl sulfonyl fluoride (PMSF), supplied by sigma, St Louis, USA. After cell lysis, the homogenate was centrifuged at 10,000 rpm for 20 minutes at 4°C and the supernatant was examined for detection of TGF- $\beta_1$  and MMP-9 using the quantitative sandwich enzyme linked immuno-sorbent assay (ELISA). The kits were supplied by Ray Biotch, Inc.

**Detection of NF- $\kappa$ B by RT-PCR:**

**RNA extraction:**

Total RNA was extracted from liver tissue samples using the SV total RNA isolation system kit (Promega, Madison, USA) according to the manufacturer's recommendations.

**RT-PCR:**

cDNA was generated from 5mg of total RNA extracted with 20 pmol oligo dt primer and was denaturated at 70°C for 2min. then a transcription mixture containing 50 mmol KCl, dNTPs and 200 units of MMLV reverse transcriptase were added, the reaction was placed at 42°C for one hour then at 95°C for 5 min. to stop the reaction. Then PCR reaction was performed by adding the PCR mix which contained 10mmol /l tris HCL pH 8.3, 2.3 units of taq polymerase, 100 mmol of NTPs and 100 mmol of each specific NF- $\kappa$ B primer with the following sequence,

forward: **CAGCCTCTTGAGTAGCTGGG**. Reverse: **GTCAGGAGTTCGAGACCAGC**.

Then the reaction mixture was subjected to 40 cycles of 95°C for one min., 55°C for one min. and 72°C for 2min. after the last cycle a final extension at 72°C for 10 min was done.

### **Agarose gel electrophoresis:**

All PCR products were electrophoresed on 2% agarose stained with ethidium bromide and visualized by UV Trans-illuminator. Fragment sizes were 113 pb.

### **Gel documentation:**

The PCR products were semi-quantitated using the gel documentation system (BioDO, Analyser) supplied by Biometra.

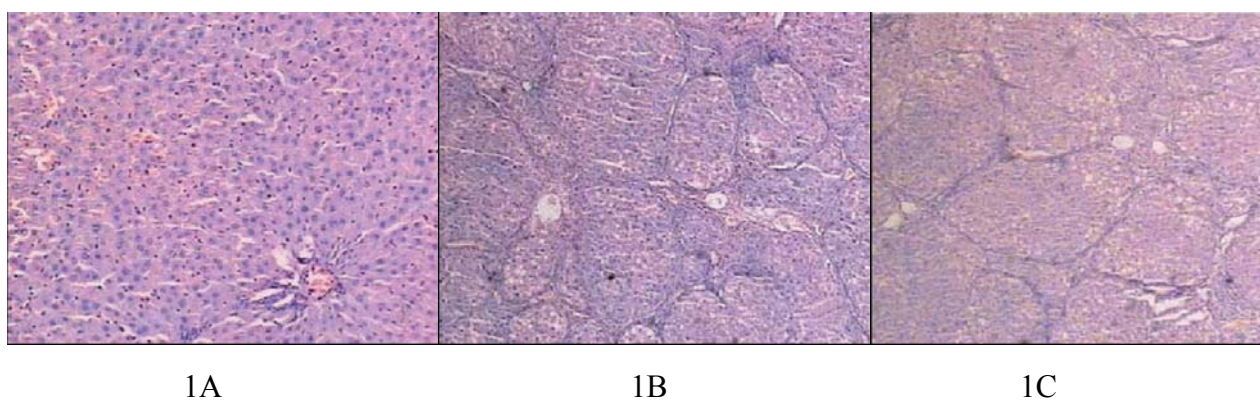
### **Statistical analysis:**

Numerical data was presented as mean  $\pm$  SD. Differences between the three group parameters were compared by one way ANOVA. To study the relationship between the variables, Pearsons correlation coefficient was calculated. The results were considered statistically significant at  $P \leq 0.05$ .

### **Results:**

#### **Pathological assay :**

Liver tissue samples from control rats (group I) showed normal lobular architecture with central veins and radiating hepatic cords (**Figure 1A**). Liver tissue samples from model group (group II) showed that more fibrous tissues were formed extending into the hepatic lobules to separate them completely. A large number of inflammatory cells infiltrated the intra-lobular and inter-lobular regions. The liver structure was disordered and there were more necrotic and fatty degenerated liver cells compared with the controls (**Figure 1B**). In the ACEI treated group (group III), hepatocyte degeneration, necrosis and infiltration of inflammatory cells were all apparently ameliorated and collagen deposition was, also, markedly reduced (**Figure 1C**).



**Figure( 1 )** Light microscopy showing normal liver tissue in control group (1A) (HE  $\times$  100), degenerated and necrotic liver cells associated with inflammatory cells in model group (1B) (HE  $\times$  40), attenuated necrosis and infiltration of inflammatory cells after ACEI treatment (1C) (HE  $\times$  40).

#### **Liver function (table 1):**

- 1- Serum albumin level is significantly lower in CCl<sub>4</sub> group (group II) compared with control group (group I) ( $p < 0.05$ ) and it increased significantly when treated with ACEI (group III).

2- Serum ALT increased significantly in CCl<sub>4</sub> group (group II) in comparison to control group (group I) (p< 0.05) and it decreased significantly when treated with ACEI (group III).

**Table (1): Mean values and ± SD of serum Albumin and ALT**

Parameters Group	Control n = (10) I	CCl <sub>4</sub> group n = (10) II	CCl <sub>4</sub> + ACEI n = (15) III
Serum Albumin (g/ml)	5.003± 0.556 <sup>b</sup>	2.500 ± 0.549 <sup>a</sup>	3.3987± 0.408 <sup>ab</sup>
Serum ALT (U/l)	62.610 ± 10.76 <sup>b</sup>	90.270 ± 21.214 <sup>a</sup>	74.193± 11.910 <sup>b</sup>

a: significant difference in comparison to control group.

b: significant difference in comparison to CCl<sub>4</sub> group.

**TGF- β<sub>1</sub> and MMP-9 assay (table 2):**

Tissue TGF- β<sub>1</sub> is significantly higher in CCl<sub>4</sub> group (group II) compared with control group (group I) and it decreased significantly with administration of ACEI with CCl<sub>4</sub> (group III) (**Figure 2**).

Tissue MMP-9 increased significantly in CCl<sub>4</sub> (group II) when compared with control group (group I). It decreased significantly with administration of ACEI with CCl<sub>4</sub> (group III) (**Figure3**).

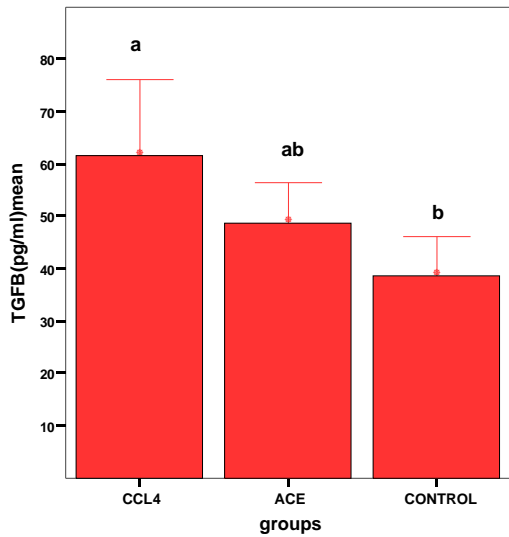
**Table (2): Mean values and ± SD of Tissue TGF- β<sub>1</sub> and MMP-9**

Parameters Group	Control n = (10) I	CCl <sub>4</sub> group n = (10) II	CCl <sub>4</sub> + ACEI n = (15) III
Tissue TGF- β <sub>1</sub> (pg/ml)	38.518 ± 7.637 <sup>b</sup>	61.490 ± 14.525 <sup>a</sup>	49.053± 7.617 <sup>ab</sup>
Tissue MMP-9 (ng/ml)	200.950 ± 14.452 <sup>b</sup>	291.860±41.110 <sup>a</sup>	233.513± 28.976 <sup>ab</sup>

a: significant difference in comparison to control group.

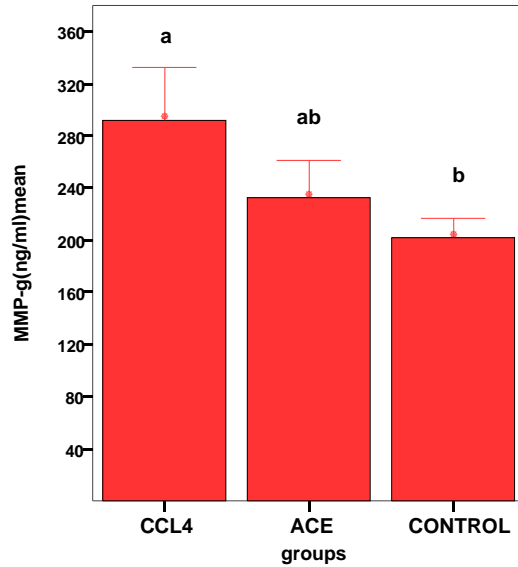
b: significant difference in comparison to CCl<sub>4</sub> group.

TGFB ( pg/ml ) mean among the studied groups



a means significant difference from control  
b means significant difference from CCL4

MMP-g ( ng/ml ) mean among the studied groups

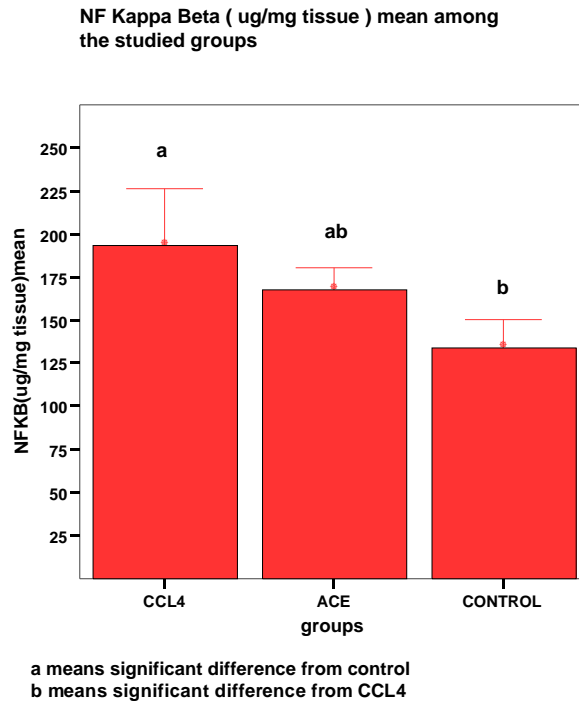


a means significant difference from control  
b means significant difference from CCL4

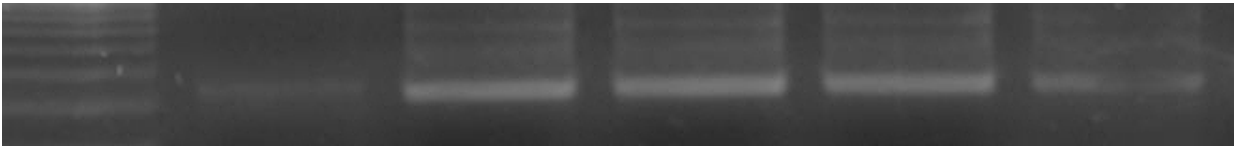
**Figure (2) Tissue TGF- $\beta_1$  (pg/ml) mean among the studied groups**  
**Figure (3) Tissue MMP-9 (ng/ml) mean among the studied groups**

**NF- $\kappa$ B expression:**

Tissue NF- $\kappa$ B increased significantly in CCl<sub>4</sub> group (group II) compared with control (group I) and it decreased significantly with administration of ACEI with CCl<sub>4</sub> (group III) (**Figures 4, 5**).



**Figure(4)Tissue NF-κB (ug/mg tissue) mean among the studied groups**



**Figure (5): Agarose gel electrophoresis of PCR product of NF kappa beta gene.100 bp molecular weight marker(Lane1).PCR product of NF kappa beta (113 bp) in control group (Lane 2), second rat group (Lanes 3,4&5),third rat group (Lane 6).**

**Discussion:**

Liver fibrosis is characterized by the excessive accumulation of extra cellular matrix proteins, including collagen, which occurs in most types of chronic liver diseases. The knowledge about the cellular and molecular mechanisms of liver fibrosis has been greatly advanced. Activated hepatic stellate cells, portal fibroblasts and myofibroblasts of bone marrow origin have been identified as major collagen-producing cells in the injured liver. These cells are activated by NF- κB and fibrogenic cytokines such as TGF- β<sub>1</sub> , TNF-α and IL-6<sup>(13, 15, 16)</sup>.

CCl<sub>4</sub>-induced liver cirrhosis in rats exhibits several features of human liver diseases, including excessive fibrosis with uniform micronodular transformation, portal hypertension, ascites, and hyperdynamic circulation. Liver cirrhosis has been extensively investigated in terms of the biochemical and molecular mechanisms of its development and complications<sup>(17)</sup>.

As regards liver function tests, the current study showed increased serum activity of ALT and decreased serum level of albumin in fibrotic model (group II), compared to controls (group I), indicating liver damage. But, after 6 weeks of treatment with perinodopril, (group III), there was less liver damage indicated by lower ALT activity and higher albumin levels, suggesting that ACEI may attenuate CCl<sub>4</sub>-induced liver fibrosis by down regulation of the inflammatory response in the liver<sup>(18)</sup>.

Histological examination, also, demonstrated a significant correlation with the liver function assessment indicated by the infiltration of a large number of inflammatory cells in the intra-lobular and interlobular regions, formation of more fibrous tissue, also, the liver margin was uneven in the fibrotic model group (II) compared with the control group. In contrast, perinodopril (group III) had obviously attenuated the extent of necrosis and reduced the immigration of inflammatory cells compared with the fibrotic model group.

In the present work, TGF- $\beta_1$  levels were increased significantly in the fibrotic model group ( $p < 0.05$ ) and were decreased significantly ( $p < 0.05$ ) after treatment with ACEI, but they did not return to the values of the control group.

ACEI (perinodopril) could prevent hepatic fibrosis by down regulation of TGF-  $\beta_1$  which leads to suppression of the activation of hepatic stellate cells<sup>(19)</sup>. These results agreed with those of Powell et al.<sup>(20)</sup> who reported that the inheritance of polymorphisms in the TGF- $\beta_1$  and angiotensinogen genes and the influence of genotypes on the stage of hepatic fibrosis in patients with chronic hepatitis C. Patients who inherit neither of the pro-fibrogenic genotypes have no or only minimal fibrosis. The documentation raises the novel suggestion that Ang-II may be a mediator of fibrosis in the liver, and since a strong interaction between Ang II and TGF- $\beta_1$  has been clearly demonstrated, these results will open several ways for the use of ACEI in anti-fibrotic strategies in the future.

Matrix metalloproteinases (MMPs) are Zn-dependant endopeptidases. They are capable of degrading all kinds of extracellular matrix proteins, but, also, can process a number of bioactive molecules<sup>(21)</sup>.

MMPs are initially synthesized as inactive zymogens with a pro-peptide domain that must be removed before the enzyme is active. The pro-peptide domain contains a conserved cysteine residue which interacts with the zinc in the active site and prevents binding and cleavage of the substrate keeping the enzyme in an inactive form. MMPs are activated by other MMPs, plasmin as well as other factors<sup>(22)</sup>.

The activities of MMPs are inhibited by TIMPs<sup>(23)</sup>. They are capable of inhibiting the activities of all known MMPs and play a key role in maintaining the balance between ECM deposition and degradation in different physiological processes, including liver fibrosis development<sup>(24)</sup>.

In the present study, the level of MMP-9 was measured and was found to be increased significantly in the fibrotic model group (group II) ( $p < 0.05$ ) and decreased significantly ( $p < 0.05$ ) with administration of ACEI (group III). These data are in accordance with those of Senties et al.<sup>(25)</sup> who explained the suppressive effect of ACEI by its interaction with zinc at the active site of MMP-9 leading to its inactivation and thus relief fibrosis.



The associated increase in the levels of MMP-9 and TGF- $\beta_1$  in fibrotic model group (group II) and the decrease in their levels after treatment with ACEI (group III) could explain the partial role of TGF- $\beta_1$  in increasing the level of MMP-9 that lead to deposition of an excess of extra cellular matrix proteins in liver fibrosis <sup>(26)</sup>.

Nuclear factor –Kappa Beta (NF- $\kappa$ B) is involved in the control of a large number of normal cellular processes such as inflammatory and immune responses, developmental processes, cell growth and apoptosis. Also, it is involved in several pathological conditions like arthritis, asthma, neuro-degenerative diseases, heart diseases, cancer and liver fibrosis <sup>(27)</sup>. NF- $\kappa$ B, also, regulates expression and activation of matrix metalloproteinases (MMPs) <sup>(28)</sup>.

In the current study NF- $\kappa$ B increased significantly in fibrotic model group ( $p < 0.05$ ) and decreased significantly in ACEI treated group ( $p < 0.05$ ) but did not reach the control group levels. These results are in agreement with those of Jonsson et al. <sup>(29)</sup>, who reported that the dual effects of ACEI to inhibit the activity of NF- $\kappa$ B and MMP-2/9, in addition to reducing protein expression of TGF- $\beta_1$ , are likely to contribute to the anti-fibrotic actions of these agents.

#### **Conclusion:**

The present study demonstrated a significant relationship between angiotensin II and liver fibrosis. ACEI attenuates the progression of hepatic fibrosis induced by CCl<sub>4</sub> by reducing expression of TGF- $\beta_1$ , NF- $\kappa$ B and MMP-9.

These results suggest that NF- $\kappa$ B, TGF- $\beta_1$  and MMP-9 may be helpful as fibrosis markers but further studies are needed to discriminate accurately the stage of fibrosis as accurately as liver biopsy. This can limit the number of liver biopsies to patients whose clinical findings conflict with the biomarker results.

#### **References:**

- 1- **Friedman SL. (2003):** Liver fibrosis from bench to bedside. *J. Hepatol.*, 38 (Suppl.1): S38 – 53.
- 2- **Everson GT. (2005):** Management of cirrhosis due to chronic hepatitis. *C. J. Hepatol.*, 42 (Suppl.): S65-S74.
- 3- **Bataller R and Brenner DA. (2005):** Liver fibrosis. *J. Clin. Invest.*, 115: 209-218.
- 4- **Pinzani M, Rombouts K, Colagrande S. (2005):** Fibrosis in chronic liver diseases: diagnosis and management. *J. Hepatol.*, 42(Suppl.): S22-36.
- 5- **Dienstag JL. (2002):** The role of liver biopsy in chronic hepatitis C. *Hepatology*, 36: S152-160.
- 6- **Bedossa P, Dargere D, Paradis V. (2003):** Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology*, 38: 1449-1457.

- 7- **Rousselet MC, Michalak S, Dupre F, Croue A, Bedossa P, Saint-Andre JP, Cales P. (2005):** Scoring of chronic viral hepatitis. *Hepatology*, 41: 257-264.
- 8- **Afdhal NH and Nunes D. (2004):** Evaluation of liver fibrosis: A concise review. *Am. J. Gastroenterol.*, 99: 1160-1174.
- 9- **Croquet V, Moal F, Veal N, Wang J, Oberti F, Roux J, Vuillemin E, Gallois Y, Douay O, Chappard D, Cales P. (2002):** Hemodynamic and antifibrotic effects of losartan in rats with liver fibrosis and/ or portal hypertension. *J. Hepatol.*, 37: 773-780.
- 10- **Paizis G, Gilbert RE, Cooper ME, Murthi P, Schembri JM, Wu LL, Rumble JR, Kelly DJ, Tikellis C, Cox A, Smallwood RA, Angus PW. (2001):** Effect of angiotensin II type 1 receptor blockade on experimental hepatic fibrogenesis. *J. Hepatol.*, 35:376-385.
- 11- **Campbell SE and Katwa LC. (1997):** Angiotensin II stimulated expression of transforming growth factor-beta1 in cardiac fibroblasts and myofibroblasts. *J. Mol. Cell Cardiol.*, 29:1947-1958.
- 12- **Iredale JP. (2003):** Cirrhosis: new research provides a basis for rational and targeted treatments. *B.M.J.*, 327:143-7.
- 13- **Karin M, and Ben-Neriah Y. (2000):** The control of NF- $\kappa$ B activity. *Annu. Rev. Immunol.*, 18: 621-663.
- 14- **Hellerbrand C, Jobin C, Licato LL, Sartor RB, Brenner DA. (1998):** Cytokines induce NF- $\kappa$ B in activated but not in quiescent rat hepatic cells. *Am. J. Physiol.*, 275: G269 -278.
- 15- **Forbes SJ, Russo FP, Rey V, Burra P, Rugge M, Wright MA, Alison MR. (2004):** A significant proportion of myofibroblasts are of bone marrow origin in human liver fibrosis. *Gastroenterology*, 126: 955-963
- 16- **Gressner AM, Weiskirchen R, Breitkopf K, Dooley S. (2002):** Roles of TGF-beta in hepatic fibrosis. *Front. Biosci.*, d793-d807.
- 17- **Proctor E and Chatamra K. (1982):** High yield micronodular cirrhosis in the rat. *Gastroenterology*, 83:1183-190.
- 18- **Yoshiji H, Yoshii J, Ikenaka Y, Noguchi R, Tsujinoue H, Nakatani T, Imazu H, Yanase K, Kuriyama S, Fukui H. (2002):** Inhibition of renin-angiotensin system attenuates liver enzyme-altered preneoplastic lesions and fibrosis development in rats. *J. Hepatol.*, 37: 22-30.
- 19- **Marra f. (1999):** Hepatic stellate cells and the regulation of liver in inflammation *J. Hepatol.*, 31:1120-1130.
- 20- **Powell EE, Edwards – smith CJ, Hay JL. (2000):** Host genetic factors influence disease progression in chronic hepatitis C. *Hepatology*, 31:828-833.
- 21- **Arthur MJ. (2000):** Fibrogenesis II. metalloproteinases and their inhibitors in liver fibrosis. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 279: G245-G249.

- 22- Pei D, Kang T, Qi H (2000):** "Cysteine array matrix metalloproteinase (CA-MMP)/MMP-23 is a type II transmembrane matrix metalloproteinase regulated by a single cleavage for both secretion and activation". *J. Biol. Chem.*, 43: 33988-97.
- 23- Comez DE, Alonso DF, Yoshiji H, Thorgeirsson UP. (1997):** Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *Eur. J. Cell Biol.*, 74: 111-122
- 24- Benyon RC, Iredale JP, Goddard S, Winwood PJ, Arthur MJ. (1996):** Expression of tissue inhibitors of metalloproteinases 1 and 2 is increased in fibrotic human liver. *Gastroenterology*, 110: 821-831.
- 25- Senties-Gomez MD, Galvez-Gastelum FJ, Meza-Garcia E, Armendariz-Borunda J. (2005):** Hepatic fibrosis: role of matrix metalloproteases and TGFbeta: *Gac. Med. Mex.*, 141(4):315-22.
- 26- Xu Li, Ying Meng, Xi-Shan Yang, Ling-Fei Mi, Shao-Xi Cai. (2005):** ACEI attenuates the progression of CCl<sub>4</sub> -induced rat hepatic fibrogenesis by inhibiting TGF-B1, PDGF-BB, NF-kB and MMP-2, 9. *Gastroenterology*, 31:4807-4811.
- 27- Rippe RA, Schrum LW, stefanovic B, Solis-Herruzo JA, Brenner DA. (1999):** NF- kappa Beta inhibits expression of the alphas(I) collagen gene. *DNA Cell Biol.*, 18:751-761.
- 28- Boya P, Larrea E, Sola I, Majano PL, Jiménez C, Civeira MP, Prieto J. (2001):** Nuclear factor-kappa Beta in the liver of patients with chronic hepatitis C: decreased RelA expression is associated with enhanced fibrosis progression. *Hepatology*, 34: 1041- 1048.
- 29- Jonsson JR, Clouston AD, Ando Y, Kelemen LI, Horn MJ, Adamson MD, Purdie DM, Powell EE. (2001):** Angiotensin – converting enzyme inhibition attenuates the progression of rat hepatic fibrosis. *Gastroenterology*, 121: 148-155

## الملخص العربي

بيرينودوبريل- مثبط الانزيم المحول للأنجيوتنسين- يخفف من التهاب النسيج الليفي

### الكبدى التجريبي

هبة شوقى •، سمر مرزوق ••، ايمان عبية ••

وأميرة حسونة ••

قسمى الفسيولوجيا والكيمياء الحيوية الطبية ••

كلية الطب ، جامعة القاهرة

يتضمن التهاب النسيج الليفي الكبدى العديد من التغيرات الخلوية والجزيئية التي تؤدي الى ايداع الكثير من البروتينات في المنطقة خارج الخلية .

وعندما لا تصاحب هذه التغيرات عملية تجديد وإصلاح فعالة سيؤدي ذلك في النهاية الى تليف الكبد . ويهدف علاج التهاب النسيج الليفي إلى تثبيط الخلايا المسببة للتليف بالإضافة الى منع إيداع البروتينات في المنطقة خارج الخلايا إلا أن الآليات التي يتم بها منع التليف لا تزال غير معروفة .

تهدف هذه الدراسة بحث الآليات التي يمنع بها مثبط الأنزيم المحول للأنجيوتنسين حدوث التليف . وقد اشتملت الدراسة على عدد ٣٥ من الجرذان البيضاء من نوع الرات ، تتراوح أوزانها بين ١٥٠، ٢٠٠ جرام وقد قسمت الى ثلاث مجموعات :

\*المجموعة الأولى الضابطة : تحتوى على عدد ١٠ جرذان .

\*المجموعة الثانية : تحتوى على عدد ١٠ جرذان تم حقنها برابع كلوريد الكربون فقط .

\*المجموعة الثالثة : تحتوى على عدد ١٥ من الجرذان تم حقنها برابع كلوريد الكربون والتي تم علاجها بمثبط الانزيم المحول للأنجيوتنسين – بيرينودوبريل – ( ٦مج / كج / يوم لمدة ٤ أسابيع ) وقد تم جمع عينات الدم لتحليل وظائف الكبد ، كما تم تقسيم عينات نسيج الكبد الى ثلاثة أقسام للتحليل الباثولوجي للأنسجة والتعبير الجيني للعامل النووي كابا بيتا وقياس عامل النمو المحول بيتا – ١ وما تريكس ميتالوبروتينيز - ٩ .

• أوضحت نتائج الدراسة أن هناك زيادة ذات دلالة إحصائية في التعبير الجيني للعامل النووي كابا بيتا وايضا في مستويات عامل النمو المحول بيتا وماتريكس ميتالوبروتينيز -٩ في أنسجة المجموعة الثانية مقارنة بالمجموعة الأولى الضابطة كما وجد نقصا ذو دلالة احصائية في هذه المستويات عند قياسهم في المجموعة الثالثة التي استخدم فيها المثبط كعلاج .

• ونستخلص من هذه النتائج أن مثبط الانزيم المحول للأنجيوتنسين يخفف من التهاب النسيج الليفي الكبدى عن طريق تقليل التعبير الجيني للعامل النووي كابا بيتا ومستويات عامل النمو المحول بيتا -١ وماتريكس ميتالوبروتينيز - ٩ .