American Journal of Biology and Life Sciences
2015; 3(6): 228-231
Published online October 22, 2015 (http://www.openscienceonline.com/journal/ajbls)

Open Science

Effect of Lisinopril, an Angiotensin-Converting Enzyme Inhibitor, on Fibrotic Liver Regeneration

Aysha Ambreen*, Samina Jalali, Sarwat Jahan, Naushaba Memom, Riffat Gilani

Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

Email address

adeel.aysha@gmail.com (A. Ambreen)

To cite this article

Aysha Ambreen, Samina Jalali, Sarwat Jahan, Naushaba Memom, Riffat Gilani. Effect of Lisinopril, an Angiotensin-Converting Enzyme Inhibitor, on Fibrotic Liver Regeneration. *American Journal of Biology and Life Sciences*. Vol. 3, No. 6, 2015, pp. 228-231.

Abstract

Hepatic fibrosis resulted in defective liver regeneration following partial hepatectomy. Angiotensin converting enzyme (ACE) inhibitor, lisinopril enhances liver regeneration and reduces fibrosis. Present study is conducted to evaluate the efficacy of ACE inhibitor, lisinopril on the fibrotic liver regeneration. Six weeks old female Sprague-Dawley rats were made fibrotic by intraperitoneal administration of carbon tetrachloride at the dose of 1.5 ml/kg for seven weeks while vehicle received olive oil at the same dose for the same duration. Vehicle and fibrotic control group was given saline (1ml) while treated group received lisinopril (2.5mg/kg) orally for one week followed by two-third partial hepatectomy. Liver regeneration rate, serum functional markers, alanine aminotransferase (ALT), asparate aminotransferase (AST) and bilirubin levels were determined 24 hours post-surgery. The result indicated that lisinopril administration increased liver regeneration rate and reduced ALT, AST and bilirubin levels of fibrotic rats following partial hepatectomy. Histopathological liver evaluation showed that hepatic cell plate width was decreased, sinusoids were widened, and fibrosis was reduced by lisinopril treatment in regenerating fibrotic livers. In conclusion ACE-Inhibitor, lisinopril affected the regeneration of fibrotic liver with improved functional capability after partial hepatectomy.

Keywords

Partial Hepatectomy, Hepatic Fibrosis, Alanine Aminotransferase, Asparate Aminotransferase, Bilirubin

1. Introduction

Liver has a remarkable capacity to regenerate following loss of hepatic tissue with tremendous sequential changes in gene expression, growth factors and morphologic structure [1]. In contrast to normal liver fibrotic and cirrhotic liver has impaired and slow liver regeneration ([2], [3]). Impaired regeneration and dysfunction of cirrhotic liver following partial hepatectomy (PHx) are the most serious risk factor for postoperative liver failure [4]. Several substances have been identified that potentiate liver regeneration following PHx, angiotensin converting enzyme (ACE) inhibitor, lisinopril is one of them ([5-7]). ACE inhibitor, lisinopril through augmentation of hepatocyte growth factor (HGF) and activation of B2 receptors enhances liver regeneration [6].

In addition to effect liver regeneration, ACE inhibitors also play an important role in hepatic fibrogenesis [8]. These drugs inhibit the progression of liver fibrosis and fatty liver [9]. Studies regarding therapeutic benefits of ACE inhibitors in hepatic fibrosis treatment have also been conducted ([10], [11]). Present study has been conducted to evaluate the efficacy of ACE inhibitor, lisinopril on the fibrotic liver regeneration.

2. Material and Methods

2.1. Animals

Six-week old female Sprague-Dawley rats weighing about 180-200g were obtained from National Institute of Health (NIH), Islamabad. Animals were acclimated for one week before dosing under controlled environmental conditions at 25°C with a 12 hour light/dark cycle.

2.2. Induction of Fibrosis and Experimental Procedure

The already established carbon tetrachloride (CCl_4) induced fibrotic rat model [12] was used for scheduling the CCl_4 dose regimen. Briefly, 1.5 ml/kg of CCl_4 diluted in olive oil (1:7

dilution) was intraperitoneally administered to rats while vehicle received olive oil at the same dose for the same duration. Vehicle and fibrotic control animals received 1ml saline while treated group received oral dose of 2.5mg/kg lisinopril for one week.

2.3. Partial Hepatectomy

After establishment of fibrosis all animals were subjected to two-third partial hepatectomy [13]. Briefly, animals were anesthetized with an intramuscular injection of ketamine (100mg/kg). A midline incision was made; median and left lateral lobes were ligated by silk suture and resected. The peritoneum was then reapproximated with catgut followed by closure of the skin with silk sutures.

2.4. Sampling

Animals were dissected out 24 hours post-surgery; the liver remnants were removed and weighed for liver regeneration rate determination. Blood was collected by cardiac puncture and was centrifuged for 20 minutes at the rate of 3000 rpm to separate serum. Serum was stored at -20°C for biochemical analysis. For histopathological examination, parts of the excised livers were processed for histology.

2.5. Liver Regeneration Rate

Liver regenerative capacity following lisinopril pretreatment was determined by estimating liver regeneration rate (LRR). LRR was calculated by the following formula [14]:

$$LRR\ (\%) = 100\times \{(C-(A-B)\}/A$$

Where A is the estimated liver weight at surgery; B is the excised liver weight at surgery; and C is the remnant liver weight at dissection. Estimated liver weight was calculated by the equation A = B/0.67

2.6. Biochemical Analysis

To evaluate liver functional capability under lisinopril pretreatment, serum biochemical analysis for alanine aminotransferase (ALT), aspartate transaminase (AST) and total bilirubin were done spectrophotometrically by using commercially available AMP Diagnostics kits. All the reactions were performed at 37°C.

2.7. Histopathological Examination

Liver specimens were fixed in 4% paraformaldehyde followed by dehydration in ascending grades of alcohol, clearing in xylene and embedding in paraffin. Thin sections of 5µm were cut out and stained with hematoxylin and eosin (H&E) for microscopic histopathological examination.

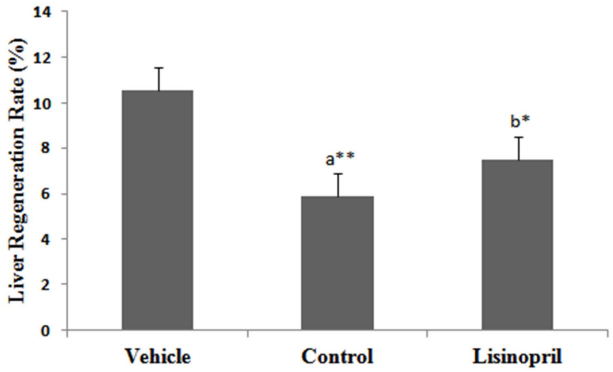
2.8. Statistical Analysis

Data were presented as mean ± SEM and was analyzed by one way analysis of variance (ANOVA) with Tukey post hoc test. Statistical significance was taken at P< 0.05.

3. Results

3.1. Liver Regeneration Rate

Fibrotic rats had significantly less regeneration rate as compared to vehicle rats. Lisinopril pretreatment had significantly increased the regeneration rate of fibrotic rats 24 hours following PHx (Fig 1)



*P<0.05, **P<0.001, [a]Vehicle vs Control, [b]Control vs Lisinopril

Fig. 1. *Effect of Lisinopril treatment on liver regeneration rate following partial hepatectomy.*

3.2. Biochemical Analysis

Serum biochemical analysis showed that fibrotic rats had significantly elevated levels of ALT, AST and total bilirubin 24 hours following PHx compared to the vehicle and that lisinopril treatment had significantly decreased the levels of above mentioned parameters (Table 1).

Table 1. *Effect of Lisinopril treatment on ALT, AST and total bilirubin levels following PHx.*

Treatment Groups	ALT (U/L)	AST (U/L)	Total Bilirubin (mg/dL)
Vehicle	537.4 ± 16	540.9 ± 15	0.39 ± 0.01
Control	$810.4 \pm 20^{a***}$	$852.5 \pm 28^{a**}$	$1.27 \pm 0.05^{a***}$
Lisinopril	$701.0 \pm 27^{b*}$	$723.9 \pm 20^{b*}$	$0.90 \pm 0.05^{b**}$

*p<0.05, **p<0.01, ***p<0.001
ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase
[a]Vehicle vs Control, [b]Control vs Lisinopril

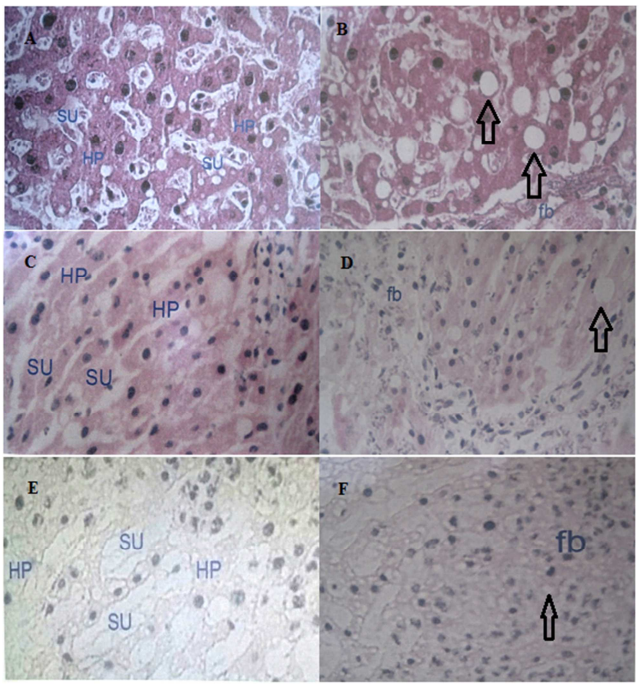
3.3. Histopathological Liver Examination

Histological liver examination of all partially hepatectomized rats showed lipid droplets 24 hours post PHx, a characteristic feature of partial hepatectomy. In vehicle rats irregular hepatic plates with widened sinusoids and slight collagen fibers had been seen. In contrast fibrotic control rats showed increased collagenous tissue along with apoptotic nuclei during histopathological liver evaluation. These rats had narrow liver sinusoidal spaces which were considerably widened by lisinopril pretreatment. Hepatic plate width was significantly reduced in lisinopril treated rats compared to control. (Table 2) (Fig 2)

Table 2. *Effect of Lisinopril treatment on hepatic cell plate and sinusoidal width following PHx.*

Treatment Groups	HCPW (µm)	SW (µm)
Vehicle	11.26 ± 0.37	9.46 ± 0.77
Control	11.94 ± 0.39	6.02 ± 0.61[a**]
Lisinopril	10.04 ± 0.41[b*]	9.23 ± 0.73[b*]

HCPW: Hepatic Cell Plate Width; SW: Sinusoidal Width
*p<0.05, **p<0.001
[a]Vehicle vs Control, [b]Control vs Lisinopril



SU = Sinusoidal spaces, HP = Hepatic plate, fb= fibrosis while Arrow head showed lipid droplets

Fig. 2. *Effect of lisinopril pretreatment on histopathology of rat liver 24 hours following partial hepatectomy. Vehicle rats showed irregular hepatic plates with widened sinusoids and lipid droplets (Panel A &D. No histopathological liver changes were seen in vehicle rats. Fibrotic livers in contrast showed collagen fibers along with apoptotic nuclei and other degenerative changes. These rats had narrow liver sinusoid spaces (Panel B&E). Lisinopril pretreatment had increased the liver sinusoidal spaces however hepatic plate width was reduced in treated rats compared to control (Panel C&F).*

4. Discussion

In this study the efficacy of ACE inhibitor in improving the fibrotic liver regeneration had been evaluated. Fibrotic and cirrhotic livers reported to have impaired and slow regeneration as compared to normal liver ([2], [3]). Significantly less liver regeneration rate of fibrotic rats compared to vehicle rats had been observed in the present study. ACE inhibitor lisinopril, which proved to enhance normal liver regeneration ([5], [6]) when administered orally for one week before PHx had significantly improved the LRR of fibrotic liver 24 hours post after PHx. That improvement of liver regeneration rate may be due to potential stimulating effect of lisinopril on hepatocyte growth factor ([15-17]), the most potent growth factor of hepatocytes [18].

Liver function enzymes i.e. ALT, AST and bilirubin were significantly elevated in fibrotic rats than that of normal liver after PHx as was previously reported by [19]. Lisinopril was successful in improving all the studied liver functional parameters of regenerating fibrotic liver following 24 hours PHx. ACE-inhibitors already have been reported to improve the functional capability of regeneration livers [7]. Lisinopril pretreatment significantly decrease the hepatic Ischemia/reperfusion induced increase in ALT, AST, and total bilirubin levels [20]. Moreover, angiotensin II blockers have been shown to reduce the above mentioned liver function markers in several studies ([21], [22]).

A characteristic feature of lipid accumulation after PHx [23] has been seen in all groups in the study. Anti-fibrogenic effect of lisinopril had been observed by histopathological examination. Lisinopril has been reported to reduce carbon tetrachloride-induced hepatic fibrosis in rats [24]. Hepatic cell plate width was reduced in lisinopril treated rats. That reduction might be the result of increased sinusoidal pressure due to the widening of liver sinusoidal spaces after lisinopril pretreatment. The widened sinusoids could be due to vasodilation effect of ACE inhibitors ([25, 26]).

In conclusion angiotensin converting-enzyme inhibitor, lisinopril affected fibrotic liver regeneration by increasing the liver regeneration rate and improving the hepatic functional capability with specific effects on liver histology.

Acknowledgements

We are thankful to Uzma Zulfiqar, Shumaila Irum, Amna Nazeer, Saher Shahid and Sumaira Hassan for their technical and moral assistance.

References

[1] Michalopoulos GK, DeFrances MC. Liver Regeneration. Science 1997; 276: 60-66.

[2] Andiran F, Ayhan A, Tanyel FC, Abbasoğlu O, Sayek I. Regenerative capacities of normal and cirrhotic livers following 70% hepatectomy in rats and the effect of α-tocopherol on cirrhotic regeneration. J. Surg. Res. 2000; 89: 184-188.

[3] Hashimoto M, Watanabe G. Functional capacity of the cirrhotic liver after partial hepatectomy in the rat. Surgery 1999; 126: 541-547.

[4] Belghiti J, Hiramatsu K, Benoist S, Massault P, Sauvanet A, Farges O. Seven hundred forty-seven hepatectomies in the 1990s: an update to evaluate the actual risk of liver resection. J Am Coll Surg. 2000; 191: 38-46.

[5] Ramalho FS, Ramalho LNZ, Castro-e-Silva Júnior O, Zucoloto S, Corrêa FMA. Angiotensin-converting enzyme inhibition by lisinopril enhances liver regeneration in rats. Brazilian Journal of Medical and Biological Research 2001; 34: 125-127.

[6] Yayama K, Sugiyama K, Miyagi R, Okamoto H. Angiotensin-converting enzyme inhibitor enhances liver regeneration following partial hepatectomy: involvement of bradykinin B2 and angiotensin AT1 receptors. Biological and Pharmaceutical Bulletin 2007; 30: 591-594.

[7] Koh SL, Ager E, Malcontenti-Wilson C, Muralidharan V, Christophi C. Blockade of the renin–angiotensin system improves the early stages of liver regeneration and liver function. Journal of Surgical Research 2013; 179: 66-71.

[8] Tuncer I, Ozbek H, Ugras H, Ugras S, Bayram I. Anti-fibrogenic effects of captopril and candesartan cilexetil on the hepatic fibrosis development in rat: The effect of AT1-R blocker on the hepatic fibrosis. Experimental and Toxicologic Pathology 2003; 55: 159-166.

[9] Toblli JE, Muñoz MC, Cao G, Mella J, Pereyra L, Mastai R. ACE inhibition and AT1 receptor blockade prevent fatty liver and fibrosis in obese Zucker rats. Obesity 2008; 16: 770-776.

[10] Huang ML, Li X, Meng Y, Xiao B, Ma Q, Ying SS, Wu PS, Zhang ZS. Upregulation of angiotensin-converting enzyme (ACE) 2 in hepatic fibrosis by ACE inhibitors. Clinical and Experimental Pharmacology and Physiology 2010; 37: e1-e6.

[11] Reese LJ, Tider DS, Stivala AC, Fishbein DA. Effects of Angiotensin Converting Enzyme Inhibitors on Liver Fibrosis in HIV and Hepatitis C Coinfection. AIDS research and treatment 2012; doi:10.1155/2012/978790.

[12] Özbek H, Uğraş S, Dülger H, Bayram I, Tuncer I, Öztürk G, Öztürk A. Hepatoprotective effect of Foeniculum vulgare essential oil. Fitoterapia 2003; 74:317-319.

[13] Higgins GM, Anderson RM. Experimental pathology of the liver-Restoration of the liver of the white rat following partial surgical removal. Arch. Pathol. 1931; 12: 186-202.

[14] Fishback, FC. A morphologic study of regeneration of the liver after partial removal. Arch. Pathol. 1929; 7: 955-977.

[15] Nakano N, Moriguchi A, Morishita R, Kida I, Tomita N, Matsumoto K, Nakamura T, Higaki J, Ogihara T. Role of angiotensin II in the regulation of a novel vascular modulator, hepatocyte growth factor (HGF), in experimental hypertensive rats. Hypertension 1997; 30: 1448-1454.

[16] Matsumoto K, Morishita R, Moriguchi A, Tomita N, Yo Y, Nishii T, Matsumoto K, Nakamura T, Higaki J, Ogihara T. Prevention of renal damage by angiotensin II blockade accompanied by increased renal hepatocyte growth factor in experimental hypertensive rats. Hypertension 1999; 34: 279-284.

[17] Day RM, Thiel G, Lum J, Chévere RD, Yang Y, Stevens J, Sibert L, Fanburg BL. Hepatocyte growth factor regulates angiotensin converting enzyme expression. Journal of Biological Chemistry 2004; 279: 8792-8801.

[18] Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugimura A, Tashiro K, Shimizu S. Molecular cloning and expression of human hepatocyte growth factor. Nature 1989; 342: 440-443.

[19] Aly HF, Mohamad HE, Soliman AM, Shams SGE. Role of melatonin in management of partially hepatectomized and/or propagated-cirrhotic livers of rats. Int. J. Pharm. Sci. Rev. Res. 2014; 26: 11-31.

[20] Morsy MA. Protective Effect of Lisinopril on Hepatic Ischemia/reperfusion Injury in Rats. Indian Journal of Pharmacology 2011; 43: 652-655.

[21] Wei HS, Lu HM, Li DG, Zhan YT, Wang ZR, Huang X, Cheng JL, Xu QF. The regulatory role of AT 1 receptor on activated HSCs in hepatic fibrogenesis: effects of RAS inhibitors on hepatic fibrosis induced by CCl_4. World Journal of Gastroenterology 2000; 6: 824-828.

[22] Abd-Allah OM, Sharaf EI, Din AA. Evaluation of antifibrotic and antioxidant effects of olmesartan medoximil, a new angiotensin II blocker, on experimentally-induced liver fibrosis in rats. Journal of the Egyptian Society of Pharmacology and Experimental therapeutics 2008; 29: 553-577.

[23] Newberry EP, Kennedy SM, Xie Y, Luo J, Stanley SE, Semenkovich CF, Crooke RM, Graham MJ, Davidson NO. Altered hepatic triglyceride content after partial hepatectomy without impaired liver regeneration in multiple murine genetic models. Hepatology 2008; 48: 1097-1105.

[24] Ohishi T, Saito H, Tsusaka K, Toda K, Inagaki H, Hamada Y, Kumagai N, Atsukawa K, Ishii, H. Anti-fibrogenic effect of an angiotensin converting enzyme inhibitor on chronic carbon tetrachloride-induced hepatic fibrosis in rats. Hepatology research 2001; 21: 147-158.

[25] Auch-Schwelk W, Bossaller C, Claus M, Graf K, Gräfe M, Fleck E. Local potentiation of bradykinin-induced vasodilation by converting-enzyme inhibition in isolated coronary arteries. Journal of cardiovascular pharmacology 1992; 20: S62-S67.

[26] Witherow FN, Helmy A, Webb DJ, Fox KA, Newby DE. Bradykinin contributes to the vasodilator effects of chronic angiotensin-converting enzyme inhibition in patients with heart failure. *Circulation* 2001; *104*: 2177-2181.