Evaluation of Chronic Losartan Treatment Effect on Cardiac Chronotropic Dysfunction in Biliary Cirrhotic Rats

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Abstract- Cirrhosis is associated with cardiac chronotropic and inotropic dysfunction which is known as cirrhotic cardiomyopathy. Cardiac responsiveness to adrenergic stimulation is impaired in cirrhosis. Moreover, there is vagal nerve dysfunction which is related to neuromodulatory dysfunction of the angiotensin II in the cirrhosis. This study was aimed to explore the hypothesis that administration of Losartan-angiotensin II receptor antagonist increases cardiac chronotropic response to isoproterenol in cirrhotic rats; and if so, whether this is associated with altered cardiac TGF-β receptor expression. Cirrhosis was induced by surgical ligation of the bile duct (BDL) in male Wister rats. Half of the BDL-group and control group were treated with losartan for four weeks. Four weeks after bile duct ligation or sham surgery the atria were isolated and spontaneously beating rate and chronotropic responsiveness to β-adrenergic stimulation was assessed using standard organ bath. The pathological assessment was done on the atria. Moreover, the expression of TGF-β has assessed the atria using quantitative RT-PCR. Bile duct ligation could induce a significant hypo-responsiveness to adrenergic stimulation. In cirrhotic rats, the chronotropic responses increased after chronic treatment with losartan, but it was not significant. The pathological study showed that losartan could not decrease fibrosis in atria in losartan treated cirrhotic group. TGF-β expression is markedly increased in cirrhotic rats which are significantly decreased in atria following administration of losartan. These results might be considered as angiotensin II role in cirrhotic cardiomyopathy, but further studies are required to elaborate the mechanism as well as the possible advantage of losartan. We conclude that cirrhosis in rats is associated with altered expression of TGF-β in the atrium which losartan can ameliorate it.

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Keywords: Cardiomyopathy; Cirrhosis; Losartan; TGF-β

Introduction

Cirrhotic patients suffer from a host of cardiovascular abnormalities which are collectively known as "cirrhotic cardiomyopathy." The symptoms include impaired cardiac responses with a significant attenuation of both chronotropic and inotropic changes after physiological or pharmacological stimulation compared with healthy subjects (1). The systemic circulation in cirrhosis is hyperdynamic showing increased heart rate and cardiac output (CO) and decreased systemic vascular resistance with low normal or decreased arterial blood pressure (5). Recent studies have shown that cardiac chronotropic dysfunction is mainly caused by increased cardiac nitric oxide synthesis.

Another feature of cirrhosis is electrophysiological changes characterized by prolonged repolarization and impaired cardiac excitation-contraction coupling. Cirrhosis is associated with histological abnormalities in cardiomyocyte which are as follows: edema, mild diffuse fibrosis, exudation, nuclear and cytoplasmic vacuolation, unusual pigmentation, and ventricular dilatation and hypertrophy (3,4).

Over-activity of the sympathetic nervous system in cirrhosis is followed by increased frequency and circulating catecholamines; which are in direct relation to the severity of the disease. Long period sympathetic

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over-activity with increased cellular exposure to noradrenaline is known to cause myocardial injury and impaired β adrenergic function (5,8). The decreased lymphocyte beta-adrenoceptor density mirrored a similar downregulation of b-receptors in the heart of cirrhotic patients. Gerbes *et al.*, found decreased b2-adrenergic receptor density in blood lymphocytes from patients with cirrhosis.

The dilated arterioles in cirrhosis cause a relatively arterial under-filling which activate the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system (SNS). The active hormone, AII exert many important actions on the heart and liver. The receptor mediating ANG II actions is AT1-R. Among the ANG ll myocardial effects is chronotropic action that is produced by AT1 receptors through sinoatrial and atrioventricular nodes. ANG II also directly stimulates ventricular remodeling and growth via AT1 by bioactivation of growth factors (platelet-derived growth factor and TGF-β1). A large number of studies suggest that the renin-angiotensin system (RAS) is involved in hepatic fibrogenesis (10). TGF-β signaling pathway mediates dilative ventricular remodeling by inducing interstitial fibrosis (9). Blockade of TGF-β mediated action can prevent liver fibrosis in cirrhosis. Losartan administration (1×10-8-1×10-5 M) reduced TGF-β1 levels in culture supernatants of KCs stimulated with Ang II, indicating that the inhibitory effects of losartan on hepatic fibrosis might be related to its inhibitory action of the binding of Ang II to its receptor in KCs, thereby decreasing the generation of TGF-β1 (10). In this study, we tried to investigate the possible roles of angiotensin pathway in cirrhotic cardiomyopathy and examined the effect of chronic losartan administration on attenuation of cirrhotic complications.

Materials and Methods

Reagents and materials

All materials were purchased from Sigma (Pool, UK) unless noted otherwise**.**

Animals

Male Wistar albino rats (body weight 250-280) were obtained from the Department Pharmacology Comparative Biology Unit (Tehran University of Medical Sciences). Animals were given free access to standard rodent chow and water, with a light/dark cycle of 12 h; at a temperature of 22° C. All animal procedures were in accordance with 'Guide for the Care and Use of Laboratory Animals' (NIH US publication No 85-23, revised 1985) recommendations. The animals (n=60) were randomly divided into two groups, namely; sham-operated group (sham) and bile duct ligated cirrhotic group (BDL). Sham-operated animals were subdivided into Losartan-treated (sham/losartan) and saline-treated (sham/saline) groups. Similarly, BDL animals were subdivided into Losartan-treated (BDL/losartan) and saline-treated (BDL/ saline) groups. For in vitro study, 6-7 rats were used in each experimental group (25 rats in total). For real-time PCR study, 5-7 rats were used in each experimental group (23 animals in total) and finally 3 rats were used for histopathological study in each experimental group performed at day 21 post-operation. (12 rats in total).

Induction of cirrhosis

Under general anesthesia (ketamine 100 mg/kg and xylazine 8 mg/kg administered intraperitoneally), all animals underwent bile duct ligation to induce biliary cirrhosis or sham operation as previously described (2). The bile duct was isolated and doubly ligated, using the method of Cameron and Oakley. Sham-operation consisted of laparotomy and bile duct identification and manipulation without ligation. All studies were performed at day 28 post-operation. To determine the effect of chronic losartan administration, losartan (6 mg/kg/day) or saline was administered orally in two groups of BDL or sham-operated rats from day 3 to 28 post-operation.

On day 28 post-operation the hearts were excised under deep anesthesia and used for either in vitro study.

Preparations of isolated atria

Isolated spontaneously beating atria were used to study cardiac chronotropic response to adrenergic stimulation. The rat atria were isolated in cold oxygenated physiological salt solution. The atria were suspended in a 20-mL organ bath chamber under isometric tension of 1 g-force. The temperature of the bathing solution was 37.0±1° C, and pH was 7.4. The composition of physiological salt solution in millimolars was as follows: NaCl, 112; KCl, 5; $CaCl_2$, 1.8; $MgCl_2$, 1; NaH_2PO_4, 0.5; KH_2PO_4, 0.5; $NaHCO_3$, 25; glucose, 10; and EDTA, 0.004. The chamber solution was oxygenated with a gas mixture of 95% O_2 and 5% CO_2. As the right atrium contains the sinoatrial node, it was used for recording the spontaneous atrial beating. (Power lab system, AD Instrument®, Australia). The atria were stimulated by cumulative concentrations of isoproterenol from 10^{-10} to 10^{-5} M.

Histologic assessment of atria

Isolated atria were fixed in 10% neutral buffered formalin overnight at room temperature (n=9, 3 rats in each group). Following the initial fixation, tissues were progressively dehydrated with increasing concentrations of ethanol and embedded in paraffin. Four-micrometer sections were stained with hematoxylin and eosin (H and E) for histomorphological characterization or with Masson's trichrome for visualizing collagenous and elastic tissue. Stained sections were examined by two pathologists using a light microscope under blind conditions.

Real-time RT-PCR

Quantitative RT-PCR was conducted on day 29 post-operation for evaluation of TGF-β expression in atria in sham-operated and BDL rats in response to chronic losartan challenge. Rat atria were isolated as previously described and immersed in liquid nitrogen. Total RNA was extracted using RNeasy Fibrous Tissue Mini Kit (QIAGEN, Germany) based on manufacturer′s protocol. Genomic DNA was eliminated by DNase (Promega). The first-stranded cDNA was generated using deoxyribonuclease-treated RNA, random hexamer primer (p(dN) 6) and ribonuclease-free water, and then heated at 70° C for 5 min and placed on ice. Ribonuclease inhibitor, reverse transcriptase, and deoxynucleoside triphosphates were added and incubated at 42° C for 1 h. Oligonucleotide primers used for PCR amplification of rat TGF-β receptor and rat 18 S ribosomal RNA were as follows (10,11): Rat TGF-β, Forward: 5_GCAACAATTCCTGGCGTTAC-3_; Reverse:5_-GTATTCCGTCTCCTTGGTTCAG-3_.
Rat-18 ribosomal RNA, forward: 5_-ATCACCTTTCGATGGTAGTCG-3_; Reverse: 3_TCCTTGATGTGGTAGCC.

Real-time PCR comprised of 1 μL of cDNA template, 10 pmol each of forward and reverse oligonucleotide primers, 10 μL of optimized PCR Master Mix in a total reaction volume of 20 μL performed with a Rotor-Gene machine. We used 18S rRNA as an internal control gene for normalization of real-time PCR data. Thermal cycling protocol that we used was as follows: initial denaturation step (5 min at 94° C), followed by 38 cycles of annealing step (45 s at 58° C) and extension/elongation step (1 min at 72° C). For analyzing data, we used the competitive critical threshold (ΔΔCT) method in which the TGFβ RNA was adjusted to RNA of 18srRNA (11).

Calculation of sample size

N=(Zα+Zβ)2 ×252/(0.96+0.84)2=d2×2(8)2/(13)2=6
β=0.2, A=0.05, d=13, S=8

Six animals are included in each group study according to similar previous studies.

Statistical analysis

All results were expressed as mean±SEM. ANOVA was used for evaluation of the effects of two variables (cirrhosis vs. sham-operated and type of treatment). Bonferroni post-test was then performed for multiple comparisons. *P* less than 0.05 were considered statistically significant. Statistical analyses were carried out using GraphPad Prism 5.0 (GraphPad Software, Inc., SanDiego, CA, USA).

Results

BDL rats revealed manifestations of biliary cirrhosis such as jaundice, dark urine, and ascites. To confirm the development of cirrhosis, we visually inspected stiffness of the liver and measured spleen weight in all experimental animals. Bile duct ligation was associated with a significant increase in spleen weight (1.70±0.05 vs. 3.05±0.11 g in Sham and BDL rats, respectively, *P*<0.01), which is consistent with the development of portal hypertension.

Heart rate was 252±6 beats/ min in anesthetized sham-operated animals. Heart rate in BDL rats was 295±30 beats/min.

In vitro study

Data showed a significant impairment in chronotropic responses of isolated atria to isoproterenol following chronic bile duct ligation (F BDL vs. Sham=19.25 *P*<0.0001)

The maximum response (Rmax) and chronotropic responsiveness to isoproterenol were lower in BDL group in comparison with sham-operated group (Figure 1). However, there was no significant difference in EC50 of isoproterenol between BDL (log EC50=8.50±0.31 and sham-operated groups (log-EC50=8.95±0.19 .As shown in Figure 1A, losartan could increase the chronotropic responsiveness of isoproterenol in BDL group (Saline vs. Los=2.58, *P*=0.1427. Although Rmax was markedly changed after losartan treatment it was non-significant in BDL operated rats (Figure 1). Increasing the sample size may make these results statistically significant.

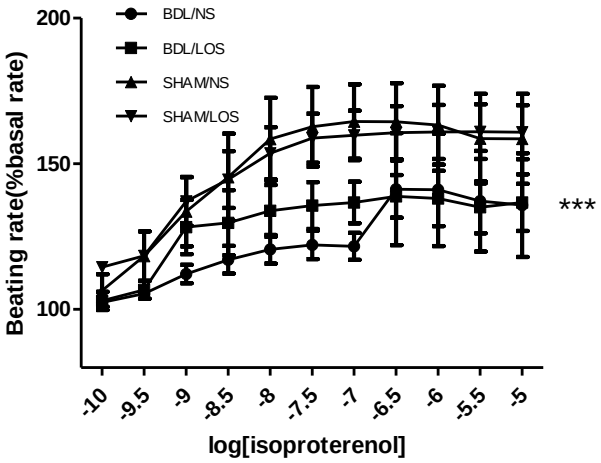
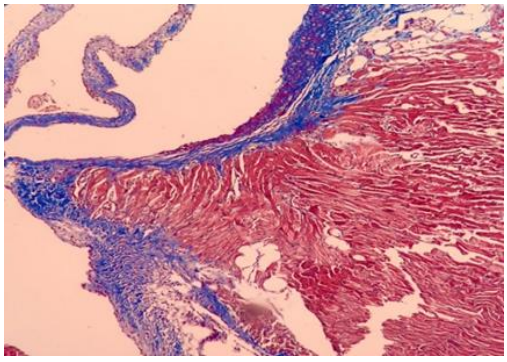


Figure 1. Chronotropic responsiveness to β-adrenergic stimulation

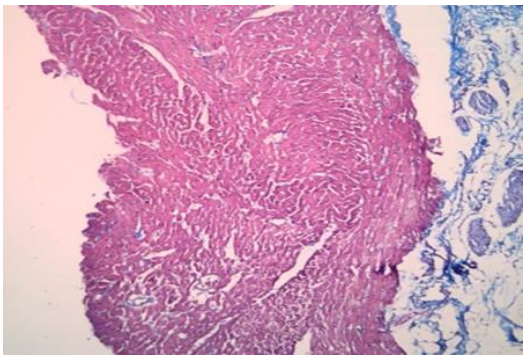
Concentration-dependent responsiveness of isolated atria to isoproterenol in sham-operated (Sham) or cirrhotic (BDL) rats treated with saline or losartan. Chronotropic studies were obtained on spontaneous beating isolated atrium. Data are shown as mean ± SEM. 6-7 rats were used in groups. **P* < 0.05 and ****P* < 0.001 in comparison with BDL/Saline group (two-way ANOVA). ***P* < 0.01 compared with BDL/Saline group in that concentration (Bonferroni post-test)

BDL operation developed fibrosis in the atria

The pathological study showed that losartan did not decrease fibrosis in atria in the losartan-treated cirrhotic group (Figure 2).



a) BDL group showing gross fibrosis



b) Control group showing no fibrosis

Figure 2. Histopathological staining for fibrosis in rat atria. Photomicrographs are shown in sections of the atria isolated from cirrhotic (BDL, Figure 2a) or control (Sham, Figure 2b) rats with or without losartan treatment. 3 rats were used in each group

Table 1. BDL/LOS group

Group	Interstitial inflammation	Interstitial fibrosis	Myocytes size variation	Myocyte vacuolization	Nuclear size variation	Nuclear vacuolization	Pigment deposition	Subepicardial fibrosis
1	Mild focal	Mild to moderate focal	Minimal	-	Minimal	Minimal focal	-	Moderate focal
2	Mild	-	Mild to moderate	Minimal focal	Mild	-	-	Minimal focal

Table 2. BDL/NS group

Group	Interstitial inflammation	Interstitial fibrosis	Myocyte size variation	Myocyte vacuolization	Nuclear size variation	Nuclear vacuolization	Pigment deposition	Subepicardial fibrosis
1	Minimal focal	Minimal focal	Minimal	Minimal focal	Minimal	-	-	-
2	Minimal focal	Minimal focal	Minimal focal	Minimal focal	Minimal	Minimal focal	-	Mild focal

TGF-β mRNA expression

TGF-β expression is markedly increased in cirrhotic rats which are significantly decreased in atria following administration of losartan (Figure 3).

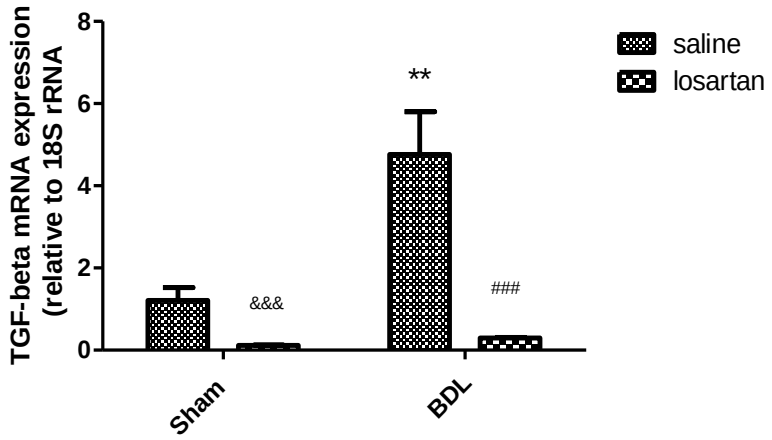


Figure 3. TGF-β mRNA expression in rat atria in Sham-operated (Sham) or cirrhotic (BDL) group

Animals were challenged with either saline or losartan .The atria were isolated for quantitative RT-PCR. TGF-β mRNA levels in atria of sham-operated and cirrhotic animals are shown after normalization to ribosomal 18s RNA. Data are shown as mean ± SEM. $^{**}P < 0.01$, $^{\&\&\&}P<0.001$ compared with Sham/Saline group. $^{\#\#\#}P < 0.001$ compared with BDL/Saline group. 5-7 rats were used in groups.

Error bars for sham/los and BDL/los group are too small.

SEM sham/los=0.00228) (SEM BDL/los=0.006939

Discussion

Bile duct ligation could induce hypo-responsiveness to adrenergic stimulation. This abnormality is a part of cardiovascular derangements known as ‘cirrhotic cardiomyopathy’ which can be ameliorated by chronic losartan treatment. Chronic losartan can also reduce atrial fibrosis and TGF-β expression in cirrhotic rats.

Cirrhotic patients suffer from host of cardiovascular abnormalities which are collectively known as "cirrhotic cardiomyopathy." The symptoms include impaired cardiac responses with a significant attenuation of both chronotropic and inotropic changes after physiological or pharmacological stimulation compared with healthy subjects (1). The systemic circulation in cirrhosis is hyperdynamic showing increased heart rate and cardiac output (CO) and decreased systemic vascular resistance with low normal or decreased arterial blood pressure (5).

A study conducted by Gaskari *et al*., have shown there is a marked decrease in isolated papillary muscle contractility in response to adrenergic stimulation in BDL rats (2,3). The improper chronotropic response may be due to problems with the baroreflex arc such as vagal denervation or impaired β adrenergic function (4,5).

Recent studies have shown that cardiac chronotropic dysfunction is mainly caused by increased cardiac nitric oxide synthesis. Nitric oxide modifies cardiac function through guanylyl cyclase dependent and independent mechanisms. NO attenuates cardiac pacemaker activity through cyclic guanosine monophosphate (cGMP) pathway. It also mediates nitration or S-nitrosation of tyrosine residues in cardiac proteins by reactive nitrogen species such as peroxynitrite or nitryl chloride [formed by reaction of NO with superoxide anion (O2_)], and increase the activity of myeloperoxidase, which catalyzes the synthesis of hypochlorous acid, finally causing myocardial oxidative stress and increased formation of reactive oxygen species (2). Ljubuncicetal reported an enhanced formation of lipid peroxidation products in the cardiac tissue of rats with biliary cirrhosis compared with controls (1).

Electrophysiological changes observed in cirrhotic patients include prolonged repolarization manifested by a prolonged QT interval and impaired cardiac excitation-contraction coupling which can be associated with an increased risk of certain ventricular arrhythmias, particularly the 'torsade de pointes' type of ventricular tachycardia (5,9). Evidence show alterations of ion channel activity in cardiomyocyte plasma membranes may play a role in repolarization prolongation. Ward and colleagues reported an abnormal function of two types of potassium channels in ventricles of rats with biliary cirrhosis. The degree of QT prolongation is correlated with the severity of liver disease (9). Studies show that the prolonged QT is partly normalized after oral β blocker administration (5).

Cirrhosis is associated with histological abnormalities in cardiomyocyte which are as follows: edema, mild diffuse fibrosis, exudation, nuclear and cytoplasmic vacuolation, unusual pigmentation, and ventricular dilatation and hypertrophy (3,4).

Demolition of the hepatic structure during cirrhosis increases intrahepatic blood flow resistance, resulting in hepatic insufficiency and portal hypertension which is the main complication of cirrhosis (6). Portal hypertension is characterized by the development of peripheral arterial vasodilatation and hyperdynamic circulation (10).

The dilated arterioles cause a relatively arterial under-filling which activate the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system (SNS). Aldosterone enhances sodium reabsorption in the distal nephron, and norepinephrine increases sodium reabsorption in the proximal tubule. Thereafter, sodium retention and plasma volume expansion required for the full expression of hyperdynamic circulation occur. Raises in portal pressure through an increase in hepatic vascular resistance and vasoconstriction are mediated by the interaction between angiotensin II and the AT1 receptor on activated stellate cells. It is shown that 1-week losartan administration decreases portal pressure, improved the systemic and splanchnic hemodynamics, decreased plasma aldosterone and norepinephrine levels, reduced vascular resistance and suppressed sodium retention and plasma volume expansion in BDL rats. (7,16,18).

Overactivity of the sympathetic nervous system in cirrhosis is followed by increased frequency and circulating catecholamines which are in direct relation to the severity of the disease. The major triggers of the sympathetic hyperactivity are: baroreceptor mediated stimulation, owing to the low arterial blood pressure and hepatic dysfunction, and a volume receptor-mediated stimulation, owing to reduced central and arterial blood volume (5).

Long period sympathetic overactivity with increased cellular exposure to noradrenaline is known to cause myocardial injury and impaired β adrenergic function (5,8). The heart expresses three types of adrenergic receptors: beta 1, beta 2, beta 3. Beta 1 is predominant receptor in the heart and induces cAMP formation and PKA activation which then phosphorylates downstream target proteins, causing calcium channel opening (13,14).

As mentioned earlier, sympathetic nervous system activity and circulating catecholamines are upregulated during cirrhosis. Increased beta 1 stimulation causes enhanced cAMP production, but myocyte has negative feedback mechanism that protects against excessive cAMP synthesis. Long-term exposure of b-adrenergic receptors to catecholamines leads to receptor internalization, sequestration, and downregulation resulting in a decrease of b-adrenergic receptor density on the plasma membrane. This can prevent overproduction of cAMP by beta receptor (13,14).

As there is a good correlation between lymphocyte and myocardial β-receptors, the decreased lymphocyte beta-adrenoceptor density mirrored a similar

downregulation of b-receptors in the heart of cirrhotic patients. Gerbes *et al.,* found decreased b2-adrenergic receptor density in blood lymphocytes from patients with cirrhosis. Studies in the biliary cirrhotic rat model demonstrated a reduced cardiomyocyte plasma membrane b-adrenergic receptor density without changes in receptor binding affinity. It was also observed that b-adrenergic receptors were desensitized in vivo (4,5).

Diminished inotropic and chronotropic cardiac contractile responses to agonist stimulation are observed in cirrhosis. Ramond *et al.,* conducted a parallel study in human and rats. He demonstrated that when cirrhotic patients were given isoproterenol, the dose required for heart rate to increase 25 beats/min (CD25) was significantly higher in cirrhotic patients (mean 4.47 mg) than the control subjects (mean, 1.34 mg). In an animal study, he infused isoproterenol into bile duct ligated cirrhotic rats and observed that the doses required for the heart rate to increase by 50 beats/min in cirrhosis were four times higher than that observed in the sham-operated controls. The maximal chronotropic response in cirrhotic rats was 30% lower than in the controls (4).

In the liver, the renin-angiotensin system (RAS) is frequently activated in patients with chronic liver diseases and there is an increase in plasma renin activity. The active hormone, AII, has many important actions in the central and peripheral nervous system, the adrenal, kidney, intestine, and heart (Peach 1977) (15). The receptor mediating ANG II actions is AT1-R. Their binding results in increasing intracellular Ca2+ concentration, cell proliferation, and reactive oxygen species production.

Renin and angiotensinogen mRNAs are found in all four chambers of the heart which are highly concentrated in the right atrium in the adult heart (Lindpaintner and Ganten 1991). The physiologic effects of the cardiac RAS are divided into two parts: those concerning the coronary circulation and those affecting the myocardium. These effects can be further subdivided into effects that occur immediately, such as changes in coronary blood flow and mechanical activity of the myocardium, or effects that are long-term events, such as those associated with growth and remodeling.

Among the ANG ll myocardial effects is chronotropic action that is produced by AI1 receptors through sinoatrial and atrioventricular nodes, since this specialized conduction tissue has a large number of angiotensin-binding sites (Saito *et al*., 1987). (15). Angiotensin affects the heart rate through a number of mechanisms: increase in the synthesis and release of neurotransmitter from presynaptic sympathetic nerve terminals, decreased uptake of neurotransmitter by the nerve terminal and sensitization of postjunctional structures (15).

ANG II directly stimulates ventricular remodeling and growth via AI1 by bioactivation of growth factors (platelet-derived growth factor and TGF-β1) in vascular smooth muscle, mesangial cell, renal proximal tubule, cardiac fibroblast, and vascular endothelial cells. It also increases the synthesis and reduces the degradation of pathological extracellular matrix and increases the transcriptions of types I and III collagen, procollagen α1 (I) and α1 (III), and fibronectin in cardiac fibroblasts.

A large number of studies suggest that the renin-angiotensin system (RAS) is involved in hepatic fibrogenesis and chronic liver disease progression. 10 Myocardial fibrosis is a dynamic process which affects the myocardial wall stiffness and causes impaired left ventricular filling and diastolic dysfunction. 5 Etiology of hepatic fibrosis is said to be excessive accumulation of extracellular matrix caused by both increased synthesis and decreased or unbalanced degradation of extracellular matrix. The central mediator of hepatic fibrosis is hepatic stellate cell (HSC) (6,10).

Hepatic stellate cells are pericytes found in the perisinusoidal space of the liver and are the major cell type involved in liver fibrosis, which is the formation of scar tissue in response to liver damage. HSC activation is influenced by paracrine cytokines (tumor necrosis factor-α [TNF-α], transforming growth factor-β [TGF-β], platelet-derived growth factor [PDGF]) which are produced by hepatic Kupffer cells (KCs). Activated HSCs undergo a morphological trans-differentiation that change their phenotype from retinoid-storing quiescent cells to extracellular matrix-producing myofibroblasts. These cells are characterized by a proliferative, fibrogenic, contractile phenotype. They produce extracellular matrix and secrete pro-inflammatory cytokines which contribute to portal hypertension by contracting the intrahepatic vasculature via activation of the myeloid differentiation factor 88 (MyD88) and nuclear factor-kB (NF-kB). In vitro, exposure of HSC soon after culture to conditioned medium from cultures of KCs accelerated the activation process and increased cell proliferation and fibrogenesis (11).

Activated HSCs express variety of receptors for vasoconstrictor substances such as ANG II on their surface. AT-II activates a series of signal transduction pathways in the activated HSC (Ac-HSC) through binding to AT-II type 1 receptor (AT1-R) to stimulate DNA synthesis, contraction, cell proliferation, collagen

synthesis and excessive extracellular matrix accumulation in HSCs all of which leads to liver fibrosis (6.10). More AT1-R and AT1-R mRNA are expressed on activated HSCs in G1 than the quiescent HSCs in G2–G5. It means that local RAS was markedly activated in G1 and that RAS inhibition with angiotensin blockades attenuated the progression of fibrosis. Also, the results of real-time RT-PCR and Western blotting showed that angiotensin receptors were markedly expressed in G1, and the expression of AT1-R was inhibited in G2-G5, in which animals were administered an angiotensin blockade. Immunohistochemical staining findings also showed significant reductions in α-SMA-positive cells (indicating HSC activation) in G2-G5, in which animals were administered an angiotensin blockade. 6

AT-II directly stimulated TLR4 mRNA expression in the Ac-HSC by activating NF-kB of the mesangial cells, leading to inflammatory responses. Activation of TLR4 signaling enhances TGF-β signaling in the HSC. Authors found that the liver fibrosis was significantly suppressed by treatment with ARB along with suppression of the Ac-HSC and TLR4MyD88-NF-kB signaling cascade in the liver.

We revealed that cirrhosis is associated with adrenergic hypo-responsiveness and increased atrial fibrosis and TGF-β expression. Our results show that chronic losartan administration can improve chronotropic responses, and reduce atrial fibrosis and TGF-β expression, meaning that renin-angiotensin-aldosterone system plays an important role in cirrhosis and can affect TGF-β expression.

As mentioned earlier, ANG II enhanced TGF-β1 transcription. TGF-β is a potent profibrogenic mediator produced by a number of non-parenchymal cells, like activated HSCs (hematopoietic stem cells), KCs (Kupffer cells), and endothelial cells .It has important roles in liver fibrosis namely increase in the production of type I, III, and IV collagen, fibronectin and proteoglycan, matrix accumulation by inhibiting the production of interstitial collagenase and stromelysin and promoting the production of the potent collagenase inhibitor, TIMP-1, and plasminogen activator inhibitor-1. In addition, TGF-β signaling pathway mediated dilative ventricular remodeling by inducing interstitial fibrosis (9).

After 48 h of coronary artery ligation, amount of TGF-β1 expression increased in immunostaining of cardiomyocytes surrounding the infarcted area, suggesting an important role for this factor in the repair process (Casscells *et al*., 1990) (15). The expressions of the mRNAs of TGF-β1, procollagen, and collagen were decreased in angiotensin-blocked groups, meaning that ANG II effectively influence TGF-β1 action and hepatic fibrosis by inducing the expression of potent cytokines (6).

The hepatic level of TGF-β, which is mainly produced in the Ac-HSC, and total collagen content in the liver were suppressed by treatment with ARB at a similar magnitude (11). Studies show that blockers of angiotensin II action (Ang II), such as angiotensin-converting enzyme inhibitors (ACEI) and Ang II receptor antagonists, induced regression or prevented the development of hepatic fibrosis in some animal models (11).

However, ARBs (losartan, irbesartan) are more potent than ACEIs (captopril, ramipril) in the prevention of hepatic fibrosis. Reasons for this difference are as follows: First, although ACEIs initially decrease levels of circulating ANG II, continuous administration of ACEIs leads to a dose-dependent compensatory rise in levels of circulating active renin and blood ANG I, which is still partially converted to ANG II even during peak inhibition of the angiotensin-converting enzyme (ACE). Increasing the dose of the ACEI may provide more ACE inhibition; it probably does not result in a better blockade of the RAS. Second, bradykinin accumulation secondary to ACE inhibition by an ACEI plays an important role in the progression of fibrosis, stimulating the proliferation of mesangial cells such as HSCs and activating TGF-β1, leading to accumulation of extracellular matrix proteins.6

Recent studies revealed that AT1 receptor is also expressed in hepatic KCs, and Ang II can stimulate mRNA expression of TGF-β and fibronectin in hepatic KCs. But the HSC has a more important role than KC in TLR4-mediated fibrogenic response.

Increased TGF-β1 secretion from KCs promotes the proliferation and collagen synthesis of cultured HSCs. So, blockage of TGF-β1 synthesis may be an alternative for treatment of fibrosis. In the study by Leung *et al.*, Ang II stimulated the expression of TGF-β in KCs through its receptor located in KCs.It also increased the secretion of TGF-β1 from KCs (10).

Losartan administration (1×10-8-1×10-5 M) reduced TGF-β1 levels in culture supernatants of KCs stimulated with Ang II, indicating that the inhibitory effects of losartan on hepatic fibrosis might be related to its inhibitory action of the binding of Ang II to its receptor in KCs, thereby decreasing the generation of TGF-β1 (10). In vitro experiments showed that losartan (1×10-7-1×10-5 M) significantly reduced TNF-α levels in culture

supernatants of KCs stimulated with Ang II, meaning that the inhibitory action of losartan on hepatic fibrosis could be associated with its inhibiting TNF-α release from activated KCs (10).

NO shows an important role in the modulation of the vascular effects of ANGII. In non-portal hypertensive animals, ANG II stimulates NO release from endothelial cells mostly due to an upregulation of eNOS protein expression and activity. Blockade of the AT1 receptors also remarkably inhibit ANGII-mediated NO release. Studies confirm that plasma NOx levels decreased in CBL rats receiving losartan. Additionally, losartan blunted the dose-response vasopressor effect to l-NAME in CBL rats. This hypothesis is further supported by the Western blot analysis which showed a decreased aortic eNOS protein expression in cirrhotic rats receiving losartan. It's demonstrated that 1-week administration of losartan in cirrhotic rats might down-regulate the aortic eNOS expression (7). Decreased eNOS protein expression by losartan may act through both the AT1 receptor blockade related down-regulation of eNOS expression and the amelioration of shear stress related NO production.

Marked hypo-responsiveness to ANGII in portal hypertensive rats was blunted following NOS inhibition. So, losartan-induced down-regulation of eNOS protein expression may lead to an increased vascular reactivity to endogenous vasoconstrictors like ANGII that also contributes to the mechanism of action of losartan in portal hypertension (7).

In order to decide drug dosing in liver failure, 3 important factors need to be considered (1) pharmacokinetic alterations of drugs, (2) pharmacodynamic alteration of drugs, and (3) increased the susceptibility of patients to adverse events particularly hepatotoxicity. Though there is no predictable test which can be used to determine drug dosage in patients with decompensated liver cirrhosis, drugs with first pass metabolism require reduction in oral dosages, for high clearance drugs both loading and maintenance dosages need adjustment, for low clearance drugs maintenance dose needs adjustment, whenever possible measuring drug level in the blood and monitoring of adverse events frequently should be done.

All ACEI are prodrugs, like enalapril, and are converted to the active agents by hydrolysis, primarily in the liver. All of the ACE inhibitors except fosinopril and moexipril are eliminated primarily by the kidneys; doses of these drugs should be reduced in patients with renal insufficiency. ACE inhibitors and ARBs in particular, were frequently used despite being not recommended for patients with cirrhosis & accounted for nearly 1/3 of all antihypertensive medications prescribed for cirrhosis. For patients with the severe liver disease, lisinopril and captopril are not prodrugs (e.g., do not require hepatic activation), and lisinopril has almost solely renal elimination. Enalaprilat, the intravenous formulation of enalapril, is the only intravenously available ACE inhibitor and can be given to patients with severe liver dysfunction as it is also not a prodrug.

Five percent of these are mostly due to hyperkalemia (especially when combined with potassium-sparing diuretics such as spironolactone), urinary system disorders, including kidney injury and worsening liver function and acute renal failure (particularly in patients with bilateral renal artery stenosis or stenosis of the renal artery of a solitary kidney).

The use of statins, ACE inhibitors, and ARBs all are associated with reduced mortality and morbidity in chronic liver disease, according to a nationwide cohort study in Sweden. A great deal of evidence supporting the role of the RAS in hepatic fibrosis has come from animal studies using ACE inhibitors and angiotensin receptor blockers (ARB). Numerous studies using a variety of animal models have demonstrated antifibrotic effects of these drugs. In our study also, it was demonstrated that ARB's namely losartan could improve adrenergic responsiveness and reduce atrial TGF-β expression and fibrosis. So, use of ARB's in liver failure like cirrhosis should be explored in clinical setting.

This study has some limitations. First, we did not measure the serum levels of angiotensin II which might explain the effectiveness of ARBs. Second, only the BDL model was used as cirrhotic rat model. Additional study using another model such as the CCl4-induced cirrhotic rat model can assure that ACE upregulation in the BDL model did not interfere with the results (6).

The current study tried to highlight the role of angiotensin II in cirrhotic cardiomyopathy, but further studies are required to elaborate the mechanism as well as the possible advantage of losartan. We conclude that cirrhosis in rats is associated with enhanced expression of TGF-β in the atrium, attenuated chronotropic responses to vasopressor agents and gross fibrosis in hepatic cells. Chronic losartan treatment could improve adrenergic responsiveness and reduce atrial TGF-β expression, but its effect on atrial fibrosis was not significant. The reason may be much more prolonged losartan treatment is needed to reduce atrial fibrosis. Also, cirrhotic cardiomyopathy is a multifactorial phenomenon, and losartan treatment alone cannot improve all of its signs so that a multi-lateral study is

required to fulfill the purpose of the study.

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