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• CLINICAL RESEARCH •

Expression of local renin and angiotensinogen mRNA in cirrhotic portal hypertensive patient

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Abstract

AIM: To investigate the expression of local renin and angiotensinogen mRNA in cirrhotic portal hypertensive patients.

METHODS: The expression of local renin and angiotensinogen mRNA in the liver, splenic artery and vein of PH patients was detected by RT-PCR analysis.

RESULTS: Expression of local renin mRNA in the liver of control group was (0.19±0.11), significantly lower than that in splenic artery(0.45±0.17)or splenic vein(0.39±0.12) respectively, (P<0.05). Expression of local angiotensinogen mRNA in the liver was (0.64±0.21), significantly higher than that in splenic artery(0.41±0.15) or in splenic vein (0.35±0.18) respectively, (P<0.05). Expression of local renin mRNA in the liver, splenic artery and vein of PH group was (0.78±0.28), (0.86±0.35) and (0.81±0.22) respectively, significantly higher than that in the control group, (P<0.05). Expression of local angiotensinogen mRNA in the liver, splenic artery and vein of PH group was (0.96±0.25), (0.83±0.18) and (0.79±0.23) respectively, significantly higher than that in the control group, (P<0.05). There was no significant difference between the liver, splenic artery and vein in the expression of local renin or local angiotensinogen mRNA in PH group, (P<0.05).

CONCLUSION: In normal subjects the expression of local renin and angiotensinogen mRNA was organ specific, but with increase of the expression of LRAS, the organ-specificity became lost in cirrhotic patients. LRAS may contribute to increased resistance of portal vein with liver and formation of splanchnic vasculopathy.

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INTRODUCTION

The initial clues to the presence of an extrarenal or tissue RAS were suggested in the studies of hypertension. Biochemical and histologic evidences have been established for the existence of a tissue-based RAS within a variety of tissues such as blood vessels, liver, kidney, spleen. Many researchers documented that this RAS system was functionally independent of the endocrine system[1-3] and called tissue or local RAS (LRAS). Its activition includes both short and long-term regulatory roles in cardiovascular homeostasis and secondary structural changes, instead of short-term regulatory profile for endocrine RAS. Locally generated AngII plays a significant role not only in controlling the growth of vascular smooth muscle cells (VSMC), hepatocytes and hepatic satellite cells (HSC was one of the most important cells in liver fibrosis[4-6]), but also in regulating local vascular tone including hepatic microcirculation. Hyperdynamic circulation and splanchnic vasculopathy were the common pathological process and changes in cirrhotic portal hypertension[7-9], vascular and hepatic RAS may have great contribution to it. Through detection of expression of the two components of LRAS, the relationship between local renin and angiotensinogen and cirrhotic portal hypertensions was investigated.

MATERIALS AND METHODS

Materials

The liver tissues and a section of splenic artery and vein were obtained during the operation of esophagogastric devascularization and splenectomy in 20 cirrhotic portal hypertensive patients. The same samples were obtained during splenectomy and partial hepatectomy in 24 controls.

13 male and 7 female patients were included in this study with mean age of 49±21, mild or severe gastroesophageal varices and splenomagely were found in all patients.

16 male and 8 female subjects with mean age of 39±17 served as control, 12 of them underwent partial hepatectomy for hepatic trauma and 12 underwent splenectomy for splenic injury. Those with hepatitis or hypertension were excluded.

A portion of the resected tissues was routinely fixed with 10 % formalin and embedded in paraffin and cut into sections, another portion of tissues was stored in liquid nitrogens at -80 ℃ for use.

Methods

Immunohistochemical analysis To investigate splanchnic vascular changes in these patients, we took the splenic veins by using monoclonal anti-vascular smooth muscle cell α-cm-actin antibody, PCNA, and the splenic veins were stained with HE. Immunohistochemical analysis was performed according to routine methods as suggested by the manufacturer.

Preparation of specimens for electron microscopic observation Fresh vascular tissues were made into cubes of 1 mm^3 and prefixed for 2 h in 2.5 % glutaraldehyde, and then postfixed at 4 ℃ for 2 h in 1 % osmic acid. Alcohol of increasing concentrations and acetone were used for dehydration. The specimens were then embedded in epoxy resin EPON 812 and cut into ultrathin sections. Plumbum-double-staining was used to prepare the samples for ultrastructural observation under transmission electron

microscope (Opton EM 10C Model).

Reverse-transcription polymerse chain reaction (RT-PCR) Messenger RNA (mRNA) levels of local renin and angiotensinogen were assessed by RT-PCR analysis using β-actin as the house keeping gene. Total RNA was prepared from frozen tissues of the liver, splenic artery and vein. Diluted complementary DNA was cloned in a total volume of 50 μl containing DEPC 31.5 μl, buffer solution 5 μl, dNTP 4 μl, 2.5 mmol/l $mgcl_2$ 3 μl, primers 20 μmol*4 μl, cDNA 2.5 μl, and TAG 0.25 μl. The PCR conditions were at 94 ℃ for 4 min followed by 35 cycles at 90 ℃ for 30 sec, at 54 ℃ for 30 sec, and at 72 ℃ for 1 min. Total RNA was also analyzed for β-actin transcript expression and PCR for 28 cycles for β-actin.

Table 1 Primer sequences

MRNA		Sequence	Size of products
Renin	sense	5'-TCT CAG CCA GGA CAT CAT CA-3'	288bp
	antisense	5'-AGT GGA AAT TCC CTT CGT AA-3'	
Angiotensinogen			256bp
	sense	5'- TGT TGC TGC TGA GAA GAT TG-3'	
	antisense	5'-CCG AGA AGT TGT CCT GGA TG –3'	

RT-PCR products were visualized under ultraviolet and analyzed by computer which provided the data for analysis. Study values were normalized as a ratio to the β-actin signal in each sample, and each value was analyzed as a transcriptional index (transcript of the interest mRNA expression in samples of interest/β-actin mRNA expression in samples of interest).

Statistical analysis

P value <0.05 was considered statistically significant, and all variable data were summarized in terms of mean ±SD and analyzed by Student *t* test using SAS software.

RESULTS

Immunohistochemical staining

In these patients, such typical cirrhotic changes as hepatocyte degeneration, necrosis, pseudolobule formation and fibrosis were seen, and splenic vein wall was thickened and VSMC in media tunica was disorderly arranged (Figures 1,2). With α-cm-actin antibody staining method, VSMC could be seen in the intima of splenic vein, suggesting migration of VSMC from media to intima. Hyperplasia of VSMC could be seen in both splenic artery and vein by PCNA staining (Figures 3,4), there wes no obviously positive staining in the control group.

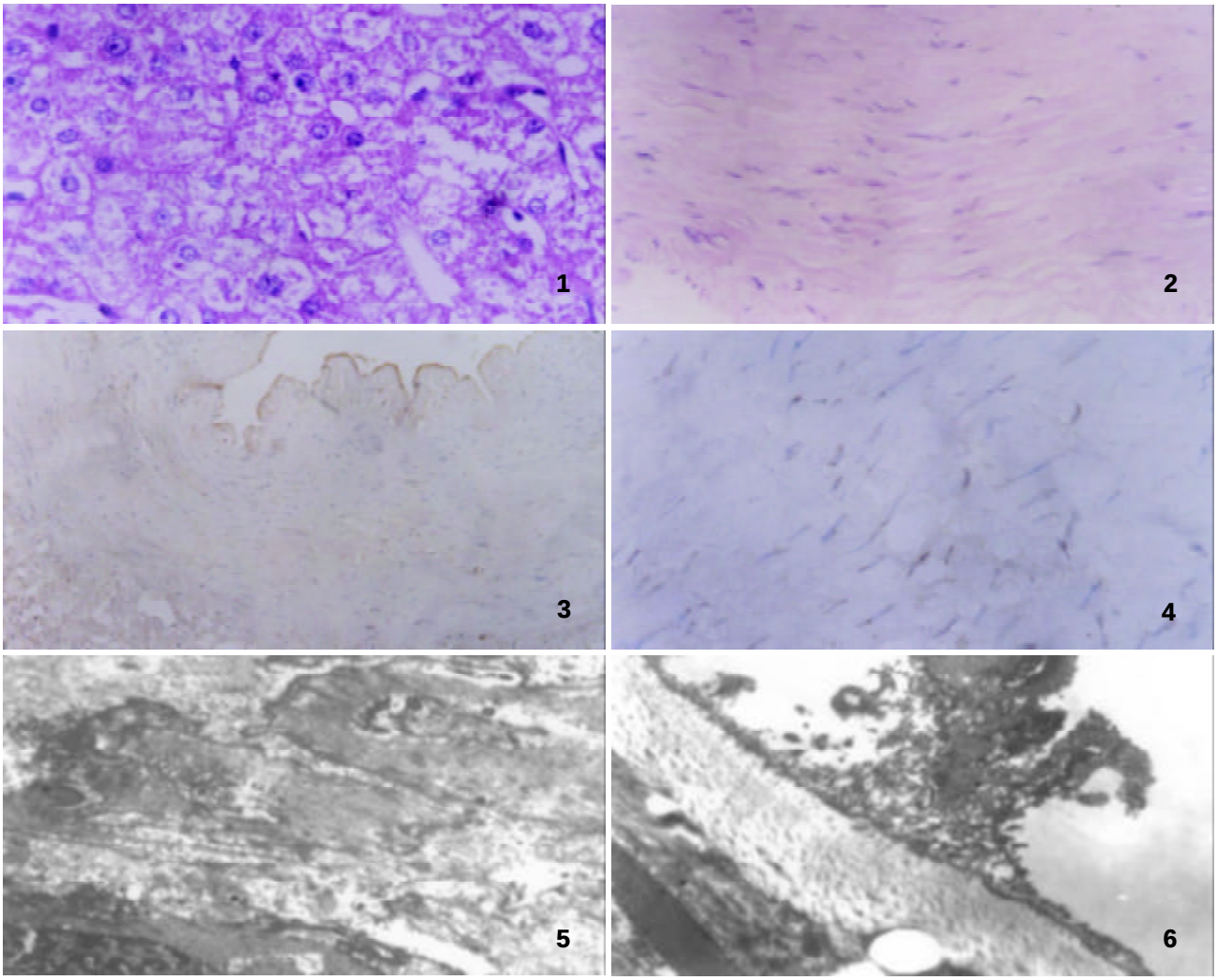

Figure 1 The liver tissue in portal hypertensive patients with HE staining × (400).
Figure 2 Splenic vein in portal hypertensive patients with HE staining method (×400).
Figure 3 Splenic vein in portal hypertensive patients by immunohistochemical staining with α-cm-actin antibody (×400).
Figure 4 Splenic vein in portal hypertensive patients by immunohistochemical staining with PCNA (×400).
Figure 5 Splenic vein of portal hypertensive patients under transmission electron microscope (×4000).
Figure 6 Splenic vein of portal hypertensive patients under transmission electron microscope (×6000).

Electron microscopic observation

In the splenic vein of these patients, endothelium was damaged with adhered thrombus, VSMC of media migrated into the subintima under transmission electron microscopy (Figures 5,6).

RT-PCR analysis

In the control group, the expression of local renin mRNA in the liver was significantly lower than that in the splenic artery or vein, $P<0.05$. Expression of local angiotensinogen mRNA in the liver was significantly higher than that in the splenic artery and vein, $P<0.05$. There was no significant difference in the expression of local renin or angiotensinogen mRNA between splenic artery and vein. ($P>0.05$, Figures 7,8,9).

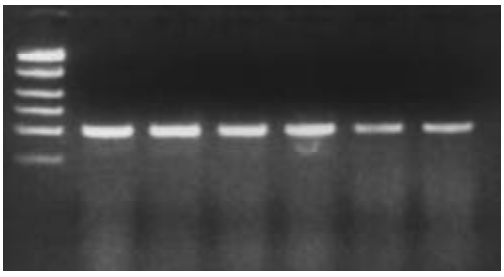

Figure 7 Expression of human β-actin mRNA (Note: from left to right there was PCR marker and lanes 1-6, figures 8,9 were the same).

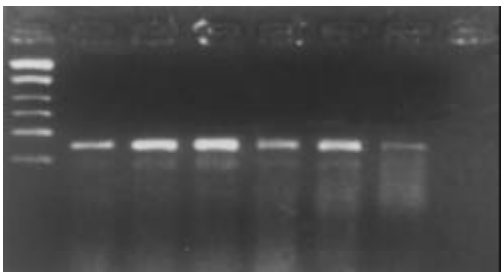

Figure 8 Expression of local renin mRNA in the liver, splenic artery and vein from the controls and cirrhotic portal hypertensive patients. Lanes 1,3,5 were splenic vein, artery and liver from controls, Lanes 2,4,6 were from patients.

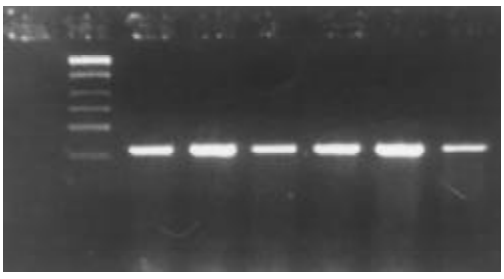

Figure 9 Expression of local angiotensinogen mRNA in the liver, splenic artery and vein from controls and portal hypertensive patients. Lanes 1,2,3 were splenic vein, artery and liver from controls, Lanes 4,5,6 were from portal hypertensive patients.

In cirrhotic portal hypertensive patient group, expression of local renin and angiotensinogen in liver, splenic artery and vein were all significantly higher than those in the controls respectively, $P<0.05$. Expression of local renin and angiotensinogen mRNA in the liver was not significantly different from those in the splenic artery or vein in PH group, $P>0.05$. The concrete data are listed in Table 2.

Table 2 Expression of local renin (Lr) and angiotensinogen (Lan) mRNA in the patient and control groups

	Control group (n=12)			Patient group (n=20)		
	Liver	Splenic artery	Splenic vein	Liver	Splenic artery	Splenic vein
Lr	0.19±0.11[a]	0.45±0.17	0.39±0.12	0.78±0.28[b]	0.86±0.35[b]	0.81±0.22[b]
Lan	0.64±0.21[a]	0.41±0.15	0.35±0.18	0.96±0.25[b]	0.83±0.18[b]	0.79±0.23[b]

The data were expressed as mean ±SD. Lr and Lan mRNA in the patient group versus those in the control group, [b]$P<0.05$. Within the same group, Lr and Lan mRNA in the liver versus those in splenic artery or vein respectively, [a]$P<0.05$.

DISCUSSION

The results demonstrated that expression of hepatic renin and angiotensinogen mRNA in cirrhotic portal hypertensive patients was significantly higher than that of the controls. Therefore the end product of LRAS, the synthesized local AngII increased and exerted a strong effect on hepatic microcirculation via its paracrine pathway.

Firstly, local AngII constricted blood vessels as well as sinusoids leading to increase of intrahepatic portal venous pressure through an increase in intracellular calcium in VSMC. Although VSMC was absent in the hepatic sinusoid, hepatic stellate cells (HSCs) expressed receptors for AngII, could also contract and increase the intrahepatic resistance[10-12].

Secondly, AngII has been shown to induce cell proliferation in various cell types, including hepatocytes and HSCs and was considered as a mediator of hepatic fibrogenesis[3,13]. Local angiotensinogen also induced increase of TGF-β mRNA expression which was an important growth promoting factor for HSC[14,15]. It was demonstrated that AngII could be a mitogenic factor for activated human HSCs through MAPK-dependent pathway[3,16]. Significant relationship was seen between high TGF-β and AngII production and the development of progressive hepatic fibrosis caused by hepatitis C virus[17,18]. AngII was also involved in the development of fibrosis in the heart and kidney through enhancement of TGF-β production[19]. *In vitro* study found that AngII could increase mRNA levels for collagen types I and III, procollagen α (I) and fibronectin in cardiac fibrosis[20]. Therefore it is possible that hepatic RAS plays an important role in the collagen synthesis, hepatic fibrosis and cirrhosis. In this study, the cirrhotic portal hypertension induced hepatic RAS activity which increased and accelerated the cirrhotic process and portal hypertension. By interrupting the vicious cycle, it was possible for medical treatment to prevent further progression of the disease. It has already been confirmed that catoprill could reduce the expression of procollagenI significantly and prevent liver fibrogenesis in a rat model of hepatic fibrosis[21-23].

The experimental data illustrated that expression of local renin and angiotensinogen mRNA in the splenic artery and vein of cirrhotic portal hypertensive patients was significantly higher than that of the controls and suggested that portal hypertension led to activation of LRAS of splenic vessels. The mechanism probably might be as follows: (1) Endothelial injury was caused by splanchnic hyperdynamic circulation and high blood flow shear, and the endothelial cells were the key site of LRAS metabolism[2]. (2) The splenic vessel wall was stretched by the increment of splanchnic blood flow. (3) Influence of other vasoactive substance.

When the expression of LRAS increased in splenic vessel, it could participate in many pathologic processes. Firstly, vascular RAS induced VSMC proliferation and enhanced progression of vascular remodeling. AngII induced hypertrophy, proliferation and migration of VSMC[24-26] with

modulation of expression of C-fos[22], C-jun, C-myc and synthesis of cytokines such as PDGF, b-FGf [23] etc. In this study, changes of VSMC in splenic vein of PH patients could be seen by HE stain, immunohistochemistry and electron microscopy. These suggested that vascular RAS was closely related with the structural alterations of the splenic vein. Response of VSMC to hypertension and injury of blood flow shear included: hypertrophy, proliferation and remodeling. As a result, the vascular RAS played an even more important role than endocrine RAS[27-30]. In addition, the matrix modulation was another key event in remodeling and vasculopathy[7,8]. Other studies reported that vascular RAS modulated the synthesis of vessel matrix via its effect on expression of PDGF and TGF-β etc[31,32]. Therefore, LRAS plays an important role in vascular remodeling and vasculopathy in portal hypertension.

Secondly, LRAS has vasoconstrictive functions. The response of splanchnic blood vessel to AngII decreased in advanced portal hypertension[33], it was due to decrease of AngII receptor on the blood vessel wall or due to the existence of post receptor dysfunction[34,35]. Furthermore, glucagon and other vasoactive substances could influence the effect of AngII on splanchnic vessel[36,37].

Thirdly, the imbalance of vasoconstrictors and vasodilators was existed in portal hypertension. Studies on vasculature showed that vascular RAS could change the response of blood vessel to other vasoactive substances[29] and *vice versa*[38]. For example, in the rabbit model of portal hypertension, the response degree of splanchnic vessel to AngII was improved by CO[33], the presence of ET could induce the local renin activity and increase sythesis of AngIIin rat mesentery artery[39], and LRAS could decrease the degradation of endogenous bradykin[30]. All the changes mentioned above could result in imbalance of vasodilators and vasoconstrictors. In this study, local renin and angiotensinogen mRNA expression in the liver was not significantly different from that in the splenic artery and vein in portal hypertensive patients, suggesting that the loss of expression of organ- specificity of LRAS components might be due to disordered metabolism of vasoactive substances.

In conclusion, increased LRAS activity in the hepatic and splenic vessels is due to cirrhotic portal hypertension, and the synthesis of local AngII increases, which contract the hepatic sinusoid, stimulate hyperplasia of HSC and proliferation of VSMC, and also interfere with the metabolism of other vasoactive substances. All these enhance the degree of cirrhosis and portal hypertension. By interruption of this vicious cycle, medical treatment may be able to improve the hemodynamic disturbance and ameliorate the splanchnic vasculopathy and to offer a new way for preventing the complications of portal hypertension[40].

REFERENCES

1 **Gricndling KK**, Murphy TJ, Alexander RW. Molecular biology of the renin-angiotensin system. *Circulation* 1993; **87**: 1816-1828
2 **Kelly MP**, Kahr O, Aalkjaer C, Cumin F, Samani NJ. Tissue expression of components of the renin-angiotensin system in experimental post-infarction heart failure in rats: effects of heart failure and angiotensin-converting enzyme inhibitor treatment. *Clin Sci* 1997; **92**: 455-465
3 **Bataller R**, Gines P, Nicolas JM, Gorbig MN, Garcia-Ramallo E, Gasull X, Bosch J, Arroyo V, Rodes J. AngiotensinII induces contraction and proliferation of human hepatic stellate cells. *Gastroenterology* 2000; **118**: 1149-1156
4 **Yao XX,** Tang YW, Yao DM, Xiu HM. Effects of Yigan Decoction on proliferation and apoptosis of hepatic stellate cells. *World J Gastroenterol* 2002; **8**: 511-514
5 **Liu XJ**, Yang L, Mao YQ, Wang Q, Huang MH, Wang YP, Wu HB. Effects of the tyrosine protein kinase inhibitor genistein on the proliferation, activation of cultured rat hepatic stellate cells. *World J Gastroenterol* 2002; **8**: 739-745
6 **Du WD,** Zhang YE, Zhai WR, Zhou XM. Dynamic changes of type I, III and IV collagen synthesis and distribution of collagen-producing cells in carbon tetracholoride-induced rat liver fibrosis. *World J Gastroenterol* 1999; **5**: 397-403
7 **Yang Z**, Zhang L, Li D, Qiu F. Pathological morphology alteration of the splanchnic vascular wall in portal hypertensive patients. *Chin Med J* 2002; **115**: 559-562
8 **Yang Z,** Liu RZ, Yang RG, Qui FZ. Pathology of endothelium, extracellular matrix and smooth muscle in gastric coronary vein of cirrhotic patients. *Zhonghua Waike Zazhi* 1996; **34**: 138-140
9 **Shi BM**, Yang Z. Vascular lesion and its mechanisms in spleen under statement of portal hypertension. *Zhonghua Yixue Zazhi* 2000; **80**: 196-198
10 **Bataller R**, Nicolas JM, Gines P, Esteve A, Nieves GM, Garcia RE, Pinzani M, Ros J,Jimenez W,Thomas AP,Arroyo V, Rodes J. Arginine vasopressin induces contraction and stimulates growth of cultured human hepatic stellate cells. *Gastroenterology* 1997; **113**: 615-624
11 **Pinzani M**, Failli P, Ruocco C, Casini A, Milani S, Baldi E, Giotti A, Gentilini P. Fat-storing cells as liver-specific pericytes. Spatial dynamics of agonist-stimulated intracellular calcium transients. *J Clin Invest* 1992; **90**: 642-646
12 **Don R**. The celluar pathogenesis of portal hypertension: stellate cell contractility, endothelin, and nitric oxide. *Hepatology* 1997; **25**: 2-5
13 **Wei HS**, Lu HM, Li DG, Zhan YT, Wang ZR, Huang X, Cheng JL, Xu QF. The regulatory role of AT1 receptor on activated HSCs in hepatic fibrogenesis: effects of RAS inhibitors on hepatic fibrosis induced by CCL(4). *World J Gastroenterol* 2000; **6**: 824-828
14 **Powell EE**, Edwards CJ, Hay JL, Andrew DC, Darrell HC, Claudia S, David MP, Julie RJ. Host genetic factors influence disease progression in chronic hepatitis C. *Hepatology* 2000; **31**: 828-833
15 **Dai WJ**, Jiang HC. Advances in gene therapy of liver cirrhosis: a review. *World J Gastroenterol* 2001; **7**: 1-8
16 **Marra F**, Grandaliano G, Valente AJ, Abboud HE. Thrombin stimulates proliferation of liver fat-storing cells and expression of monocyte chemotactic protein-1:potential role in liver injury. *Hepatology* 1995; **22**: 780-787
17 **Arved WS**, Johann F K, Christian P K. Effect of Losartan, an angiotensin II receptor antagonist, on portal pressure in cirrhosis. *Hepatology* 1999; **29**: 334-339
18 **Powell EE**, Edwards-Smith CJ, Hay JL, Clouston AD, Crawford DH, Shorthouse C, Purdie DM, Jonsson JR. Host genetic factors influence disease progression in chronic hepatitis C. *Hepatology* 2000; **31**: 828-833
19 **Lee LK**, Meyer TW, Pollock AS, Lovett DH. Endothelial cell injury intiates glomerular sclerosis in the rat remnant kidney. *J Clin Invest* 1995; **96**: 953-964
20 **Lijnen P**, Petrov V. Renin-angiotensin system, hypertrophy and gene expression in cardiac myocytes. *J Mol Cell Cardiol* 1999; **31**: 949-970
21 **Julie RJ**, Andrew DC, Yuichi A, Livia IK, Murray JH, Michael DA, David MP, Elizabeth EP. Angiotensin-converting enzyme inhibition attenuates the progression of rat hepatic fibrosis. *Gastroenterology* 2001; **121**: 148-155
22 **Wei HS**, Li DG, Lu HM, Zhan YT, Wang ZR, Huang X, Zhang J, Cheng JL, Xu QF. Effects of AT1 receptor antagonist, losartan, on rat hepatic fibrosis induced by CCL(4). *World J Gastroenterol* 2000; **6**: 540-545
23 **Yang YY**, Lin HC, Huang YT, Lee TY, Hou MC, Lee FY, Liu RS, Chang FY, Lee SD. Effect of 1-week losartan administration on bile duct-ligated cirrhotic rats with portal hypertension. *J Hepatol* 2002; **36**: 600-606
24 **Dzau VJ**. Local expression and pathophysiological role of renin-angiotensin in the blood vessels and heart. *Basic Res Cardiol* 1993; **88** (Suppl 1): 1-14
25 **Naftilan AJ**, Pratt RE, Dzau VJ. Induction of platelet-derived growth factor A-chain and c-myc gene expressions by angiotensin II in cultured rat vascular smooth muscle cells. *J Clin Invest* 1989; **83**: 1419-1423
26 **Ferns GA**, Raines EW, Sprugel KH, Motani AS, Reidy MA, Ross

R. Inhibition of neointimal smooth muscle accumulation after angioplasty by an antibody to PDGF. *Science* 1991; **253**: 1129-1132

27 **Taubman MB**, Berk BC, Izumo S, Tsuda T, Alexander RW, Nadal GB. Angiotensin II induces c-fos mRNA in aortic smooth muscle. Role of Ca^{2+} mobilization and protein kinase C activation. *J Biol Chem* 1989; **264**: 526-530

28 **Hiroshi I,** Masashi M, Richard EP, Gary HG, Victor JD. Multiple autocrine growth factors modulate vascular smooth muscle cell growth response to angiotensin II. *J Clin Invest* 1993; **91**: 2268-2274

29 **Holtz J**, Goetz RM. Vascular renin-angiotensin-system, endothelial function and atherosclerosis. *Basic Res cardiol* 1994; **89** (Suppl 1): 71-86

30 **Falkenhahn M**, Gohlke P, Paul M, Stoll M, Unger T. The renin-angiotensin system in the heart and vascular wall: new therapeutic aspects. *J Cardiovasc Pharmacol* 1994; **24** (Suppl 2):S6-13

31 **Hahn AW**, Resink TJ, Kern F, Buhler FR. Peptide vasoconstrictors, vessel structure,and vascular smooth-muscle proliferation. *J Cardiovasc Pharmacol* 1993; **22**(Suppl): S37-43

32 **Kato H**, Suzuki H, Tajima S, Ogata Y, Tominaga T, Sato A, Saruta T. Angiotensin IIstimulates collagen synthesis in cultured vascular smooth muscle cells. *J Hypertens* 1991; **9**: 17-22

33 **Sitzmann JV,** Li SS, Wu YP, Groszmann R, Bulkley GB. Decreased mesenteric vascular response to angiotensin II in portal hypertension. *J Surg Res* 1990; **48**: 341-344

34 **James VS**, Yuping Wu, Greti A, Paul AC, R Cartland B. Loss of angiotensin-II receptors in portal hypertensive rabbits. *Hepatology* 1995; **22**: 559-564

35 **Castro A**, Jimenez W, Claria J, Ros J, Martinez JM, Bosch M, Arroyo V, Piulats J, Rivera F, Rodes J. Impaired responsiveness to angiotensin II in experimental cirrhosis: role of nitric oxide. *Hepatology* 1993; **18**: 367-372

36 **Sitzmann JV**, Bulkley GB, Mitchell MC, Campbell K. Role of prostacyclin in the splanchnic hyperemia contributing to portal hypertension. *Ann Syrg* 1989; **209**: 322-327

37 **Benoit JN**, Barrowman JA, Harper SL, Kvietys PR, Granger DN. Role of humoral factors in the intestinal hyperemia associated with chronic portal hypertension. *Am J Physiol* 1984; **247**: G486-493

38 **Moller S**, Bendtsen F, Henriksen JH. Splanchnic and systemic hemodynamic derangement in decompensated cirrhosis. *Can J Gastroenterol* 2001; **15**: 94-106

39 **Rakugi H**, Tabuchi Y, Nakamaru M, Nagano M, Higashimori K, Mikami H, Ogihara T. Endothelin activates the vascular renin-angiotensin system in rat mesenteric arteries. *Biochem Int* 1990; **21**: 867-872

40 **Hulagu S**, Senturk O, Erdem A, Ozgur O, Celebi A, Karakaya AT, Seyhogullari M, Demirci A. Effects of losartan, somatostatin and losartan plus somatostatin on portal hemodynamics and renal functions in cirrhosis. *Hepatogastroenterology* 2002; **49**: 783-787

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