Original Article
Effect of aldosterone and its antagonist on the expression of PAI-1 and TGF-β1 in rat hepatic stellate cells

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Abstract: Background: Aldosterone has been implicated in a variety of organ fibroses, but its role and mechanism in liver fibrosis remain unclear. Methods: Rat primary hepatic stellate cells (HSCs) were isolated, cultured, and characterized. HSCs were incubated with aldosterone (10^{-6} M) for 4 h, 8 h, 12 h, 24 h, and 48 h, after which TGF-β1 (transforming growth factor beta 1) expression was measured by real-time PCR. Rat HSCs were treated with different concentrations of aldosterone (10^{-6} M, 10^{-7} M, 10^{-8} M, and 10^{-9} M), and the expressions of PAI-1 (plasminogen activator inhibitor-1) and TGF-β1 were determined by measuring mRNA and protein. HSCs were incubated in groups containing aldosterone (10^{-6} M), spironolactone (10^{-5} M), both aldosterone and spironolactone, or neither aldosterone nor spironolactone (control), after which mRNA and protein expression of PAI-1 and TGF-β1 were measured. Collagen I expression was detected by immunohistochemical analysis of supernatants of the aldosterone (10^{-6} M), TGF-β1, and aldosterone plus TGF-β1 groups. SMAD expression was detected in rat HSC control, HSC plus aldosterone (10^{-6} M), HSC plus TGF-β1, and HSC plus aldosterone plus TGF-β1 groups. Results: HSCs were incubated with aldosterone for 4 h, 8 h, 12 h, 24 h, and 48 h after which TGF-β1 expression was measured. We found that TGF-β1 expression increased in a time dependent manner and reached a peak at 24 h. The expression of TGF-β1 in groups treated with aldosterone for 4 h, 8 h, 12 h, and 24 h was significantly different from the control group ($P < 0.01$). No significant difference was seen in TGF-β1 expression between the groups treated with aldosterone for 24 h and 48 h ($P > 0.05$). Compared with the control group, TGF-β1 expression was significantly increased after incubation with different concentrations of aldosterone (10^{-6} M, 10^{-7} M, 10^{-8} M, and 10^{-9} M) ($P < 0.01$). There were significant differences in the expression of TGF-β1 between 10^{-6} M and 10^{-7} M aldosterone treatment groups ($P < 0.01$). Compared with the control group, the expression of PAI-1 was significantly increased after incubation with different concentrations of aldosterone (10^{-6} M, 10^{-7} M, 10^{-8} M, and 10^{-9} M) ($P < 0.01$). PAI-1 expression was increased in the aldosterone, spironolactone, and aldosterone plus spironolactone groups. The expression of PAI-1 was significantly enhanced in the aldosterone and aldosterone plus spironolactone groups compared with the control group ($P < 0.01$). There was a marked enhancement of collagen I expression in the aldosterone, TGF-β1, and aldosterone plus TGF-β1 groups ($P < 0.05$). Collagen I expressions in the aldosterone and TGF-β1 groups were significantly different from the aldosterone plus TGF-β1 group ($P < 0.01$). Compared with the control group, SMAD expression was markedly elevated in the aldosterone, TGF-β1, and aldosterone plus TGF-β1 groups ($P < 0.05$). The expression of SMAD was significantly increased in the aldosterone plus TGF-β1 group compared with the aldosterone group ($P < 0.01$). Conclusion: This study demonstrated that aldosterone promoted HSC activation and the expression of TGF-β1, PAI-1, and collagen in hepatic fibrosis progression and that spironolactone administration partially reversed the effects. The aldosterone promotional effect on hepatic fibrosis was partially mediated by TGF-β1.

Keywords: Aldosterone, hepatic stellate cells, PAI-1, TGF-β1, spironolactone

Introduction

Liver fibrosis is the pathological outcome of chronic liver injury and contributes to portal hypertension and liver dysfunction. It is also a risk factor for hepatocellular carcinoma. Hepatic stellate cells (HSCs) have been recognized as the primary collagen-producing cells during liver injury processes and are a key factor in the development of liver fibrosis. The
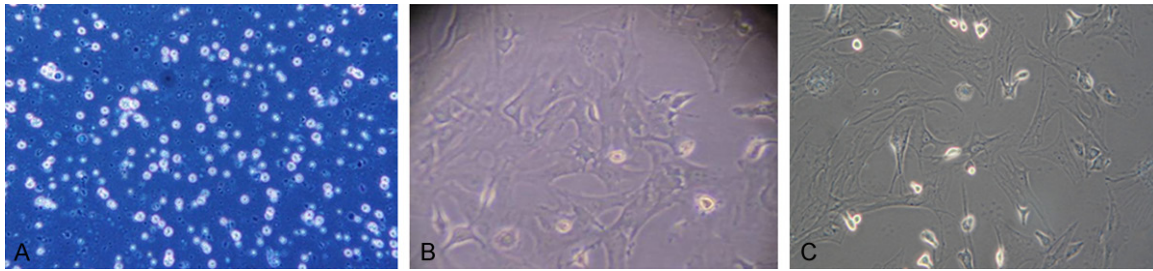
Figure 1. A. Measurement of hepatic stellate cell (HSC) viability with typan blue test (× 100); B. Three days of HSCs (× 200); C. Seven days of HSCs (× 200).

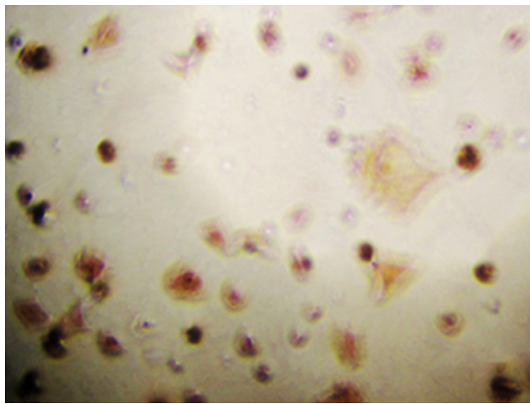**Figure 2.** Desmin immunohistochemistry of hepatic stellate cells (× 100).

onset and development of fibrosis depends on multiple interactive promotional and inhibitory factors.

Aldosterone is an important mineralocorticoid that is released upon stimulation by angiotensin II, potassium, and ACTH. Aldosterone promotes sodium and water reabsorption and potassium excretion, thereby regulating the balance of fluids and electrolytes [1]. Studies have reported that aldosterone has additional effects on epithelial tissue and non-epithelial tissue that are associated with inflammation and organ fibrosis [2]. Aldosterone is an important member of the renin-angiotensin system (RAAS), and increasing attention has been given to the role of aldosterone in the pathogenesis of liver disease. In cirrhosis, the renin-angiotensin-aldosterone system is activated, and angiotensin II (AngII) stimulates the synthesis and secretion of aldosterone, which participates in a variety of biological effects. Aldosterone binds to specific mineralocorticoid receptors (MR) that are located in the cytosol of target epithelial cells. This steroid-receptor

complex then translocates to the cell nucleus where it modulates gene expression, electrolyte balance, and water-sodium retention, which ultimately leads to high blood pressure [3]. Aldosterone can activate NADPH oxidase and promote inflammation by increasing reactive oxygen species (ROS) [4, 5]. Aldosterone also can contribute to the accumulation of extracellular matrix (ECM) by stimulating increases in the expression levels of collagen types I and IV, transforming growth factor [5, 6], connective tissue growth factor (CTGF) [7, 8], and PAI-1 (Plasminogen activator inhibitor-1) [9]. Aldosterone plays an important role in the pathogenesis of fibrosis [10]. Aldosterone is a strong stimulator of collagen synthesis and mitosis [11], and it promotes fibrosis of the heart, lung, kidney, and other organs [12]. However, little is known about aldosterone's specific role or mechanism of action as an independent stimulating factor in liver fibrosis.

Recently, researchers have investigated the role of plasminogen inhibitor-1 in fibrosis and confirmed that the expression of PAI-1 was increased in $CC1_4$ (carbon tetrachloride)-induced liver fibrosis in rats [13]. It has been reported that activation of plasminogen leads to degradation of ECM, which is related to the cell membrane receptor urokinase-type plasminogen activator (uPA) [14]. As fibrosis progressed, increased PAI-1 expression inhibited the activity of the fibrinolytic system and MMPs, thereby further accelerating development of liver fibrosis [15]. Studies have shown that plasminogen activator inhibitor-1 (PAI-1) plays an important role in liver matrix reconstruction after liver injury and fiber degradation in liver cirrhosis [16-19]. Researchers demonstrated that activation of plasminogen into plasmin by uPA is critical for TGF-β1 (Transforming growth factor beta 1) release and activation [20, 21].

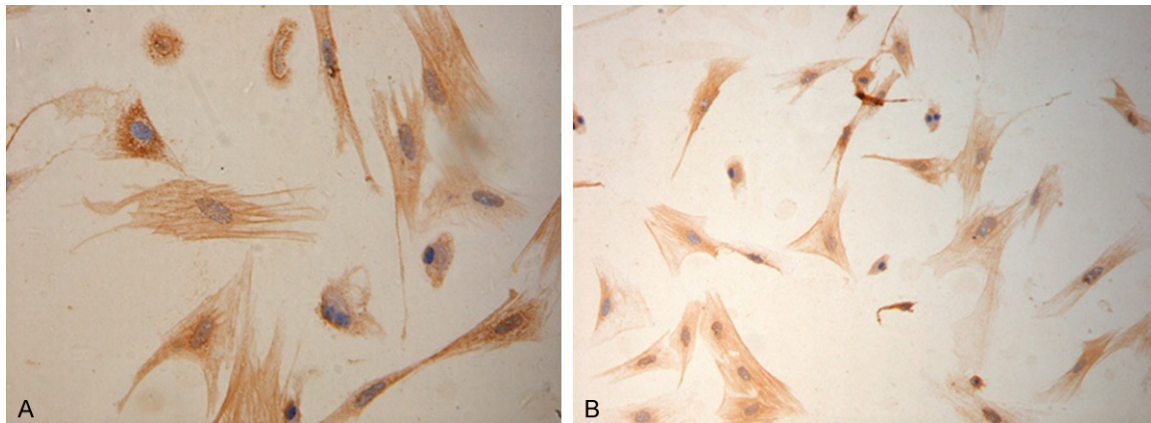**Figure 3.** Detection of α-SMA in hepatic stellate cells by immunohistochemistry. A. (× 400). B. (× 200).

Activated HSCs can produce uPA and PAI-I, but less is known about the role of aldosterone and PAI-1 in the development of liver fibrosis.

Spironolactone is a widely used aldosterone receptor antagonist. It binds competitively to the aldosterone receptor because it has a chemical structure similar to aldosterone. Its interaction with the aldosterone receptor interferes with aldosterone-MR complex formation and ultimately hinders aldosterone-induced protein synthesis, thereby antagonizing the biological effects of aldosterone.

Materials and methods

Primary HSC isolation, culture, and characterization

Adult male Sprague Dawley (SD) rat liver was digested using pronase-collagenase perfusion. HSCs were isolated with density gradient centrifugation. The morphology of HSCs was observed under a light microscope. Cells were counted in a hemocytometer with optical microscope, and cell viability was determined by trypan blue staining. HSCs were identified with immunocytochemical detection of desmin. Activated HSCs were identified by immunohistochemistry detection of α-SMA expression after a 48 h incubation with aldosterone.

Measurement of mRNA expression of PAI-1 and TGF-β1 by real-time PCR

Monolayer cultured cells were lysed with TRIzol and agitated vigorously with chloroform for 15 s. After setting at room temperature for 3 min the lysate was centrifuged at 12,000 × g for 15 min at 4°C. RNA in the aqueous phase was precipitated with isopropanol, and the upper aqueous phase was transferred to a new microcentrifuge tube. RNA was precipitated by adding 0.75% ethanol, after which the microcentrifuge tube and centrifuged at 12,000 × g at 4°C for no more than 5 min. The supernatant was removed and the RNA was dried at room temperature for 5-10 min. The mRNA expression level of PAI-1 and TGF-β1 was determined by real-time PCR.

Determination of protein expression of PAI-1 and TGF-β1 by Western blot

HSCs were harvested and total protein was separated with SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). After electrophoresis, protein was transferred to a polyvinylidene difluoride (PVDF) membrane and incubated in 5% milk overnight to block the membrane. The primary antibody was added and incubated with the membrane overnight at 4°C, after which a secondary antibody was added to the membrane and incubated at room temperature for 2 h. Protein bands were visualized using an enhanced chemiluminescence kit and exposure to X-ray film.

Quantification of collagen type I content with immunohistochemistry

Cells were seeded into 96-well culture plates at a density of 1 × 10^6 cells/L. Samples were dewaxed in xylene and dehydrated in graded alcohol. Antigens were retrieved in citrate buffer, and peroxidase was blocked with 5% hydrogen peroxide in deionized water. Samples were
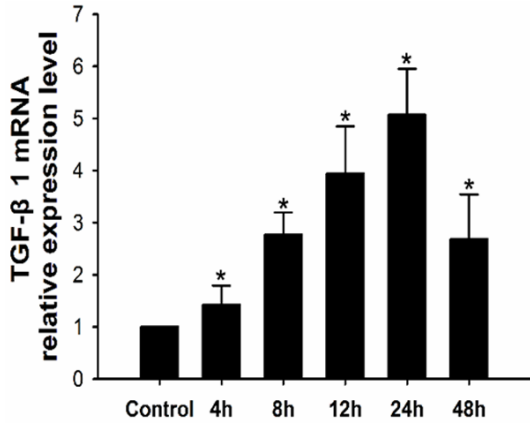
Figure 4. Effect of different times of aldosterone incubation on the expression TGF-β1. Compared with control group: *$P < 0.05$.
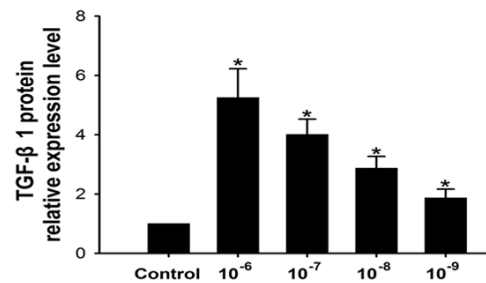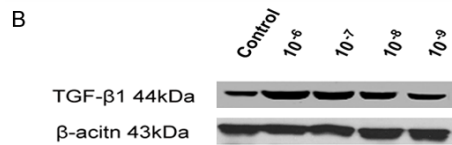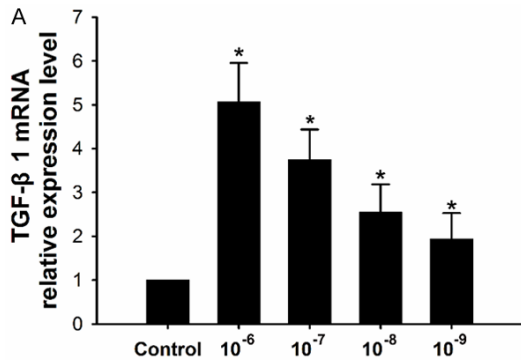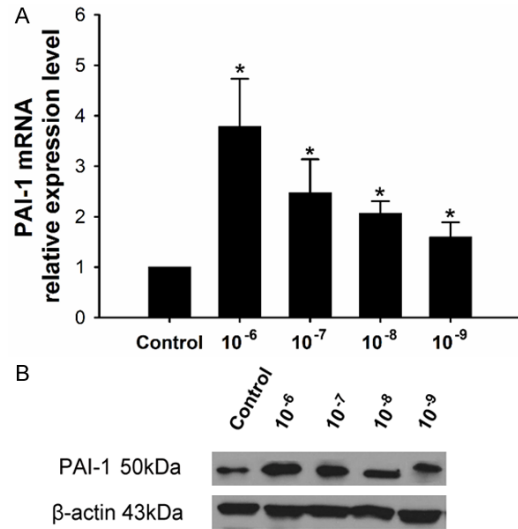

Figure 5. A. Effect of different concentrations of aldosterone on TGF-β1 mRNA expression. B. Effect of different concentrations of aldosterone on TGF-β1 protein expression. *: Compared with control group; #: comparison between 10^{-6} M and 10^{-7} M groups. *$P < 0.05$; **$P < 0.01$, ***$P < 0.001$.

Figure 6. A. Effect of different concentrations of aldosterone on PAI-1 mRNA expression. B. Effect of different concentrations of aldosterone on PAI-1 protein expression. *: Compared with control group; #: comparison between 10^{-6} M and 10^{-7} M groups. *$P < 0.05$; **$P < 0.01$, ***$P < 0.001$.

37°C, samples were examined with diaminobenzidine (DAB) under a microscope. All slides were counterstained and fixed with hematoxylin.

Statistical analysis

Data are presented as the mean ± SD and subjected to statistics analysis by SPSS 17.0. Comparison between groups was made using the Student's t-test and ANOVA analysis. Statistical significance was accepted as $P < 0.05$.

Results

Identification of HSC viability, purity, and morphology

Cell yield was determined in a cell-counting chamber as follows: cell number = (total num-

pre-incubated with non-immune goat serum for 20 min at 37°C to block non-specific antigenic sites and then incubated with primary antibody (1:100) overnight. After incubation with biotin-labeled second antibody (1:100) for 30 min at
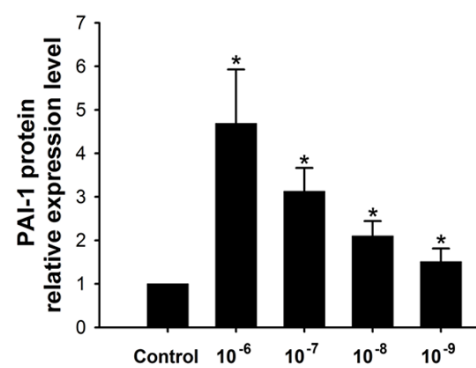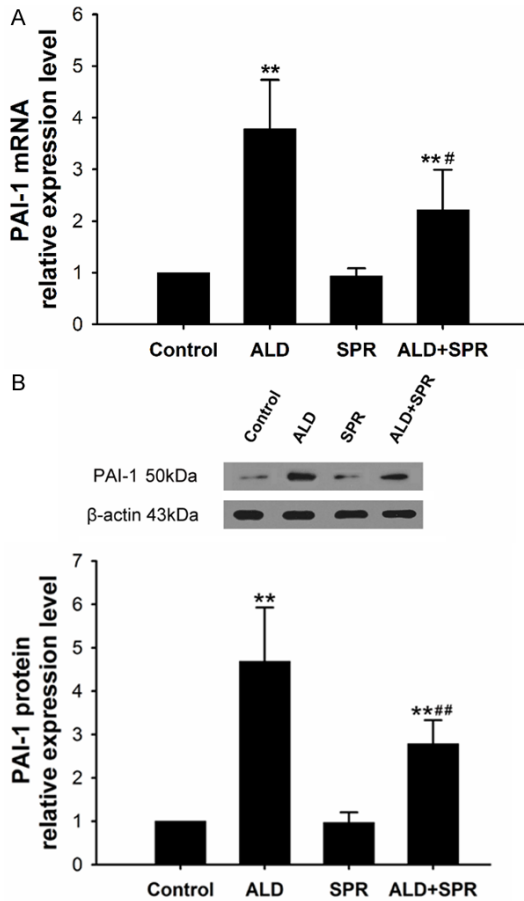
Figure 7. A. Effect of aldosterone on PAI-1 mRNA expression. B. Effect of aldosterone on PAI-1 protein expression. *: Compared with control group; #: Comparison between aldosterone group and aldosterone plus spironolactone group. *P < 0.05; **P < 0.01.

Figure 8. A. Effect of aldosterone and its antagonist on TGF-β1 mRNA expression. B. Effect of aldosterone and its antagonist on TGF-β1 protein expression. *: Compared with control group; #: Comparison between aldosterone group and aldosterone plus spironolactone group. *P < 0.05; **P < 0.01, ##P < 0.01.

ber of cells in 4 grids /4) × 10^4 × (volume of cell suspension in ml). Values were obtained from two separate experiments and were expressed as mean ± SD. Cell viability was determined with 0.4% trypan blue staining. Briefly, 10 μL 0.4% trypan blue solution was added to 90-μL cell suspension, and the living cells were not visualized using optical microscopy. The viability was calculated with the equation HSC (%) = number of living cells/total cell number × 100%. The cell yield was about 4 × 10^7 as determined with a blood cell counting plate. The trypan blue test showed that the viability was about 95.0 ± 1.2%. Freshly isolated HSCs were non-adherent, small, and rounded, with lipid droplets in the cytoplasm. Strong refractivity was observed with inverted phase-contrast microscopy. After 24 h in culture, cells began to adhere and stretch, and they displayed an oval appearance with lessened translucency. Most
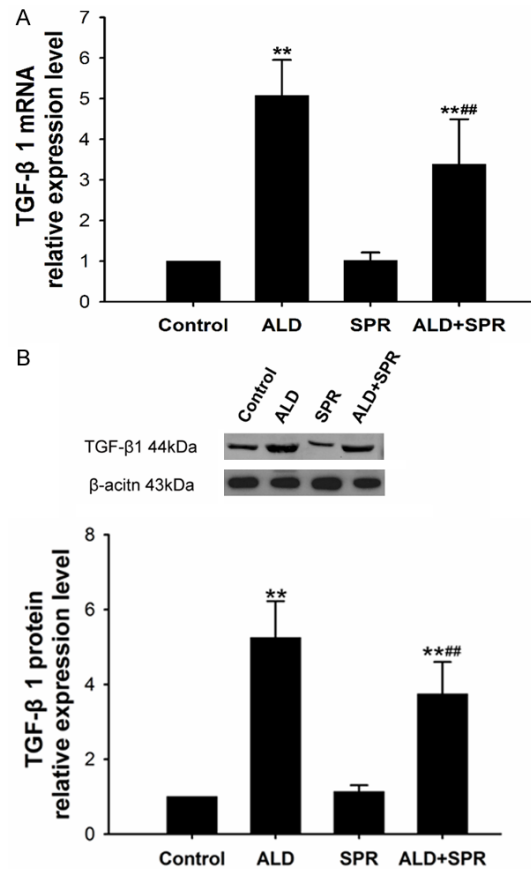
of the adherent cells had stretched into pseudopodia after 48 h. HSCs were star-shaped, irregular, and larger in volume, and they displayed a focal growth pattern as shown in **Figure 1**.

Identification of s by immunohistochemistry

HSCs were identified with desmin immunohistochemistry. As shown in **Figure 2**, the positive cells were brown with blue nuclei. The purity was greater than 90%.

Identification of activated HSCs after aldosterone stimulation

The expression of α-SMA in HSCs was detected by immunohistochemistry. The results indicated that the expression of α-SMA was increased after aldosterone stimulation. Many thin fila-
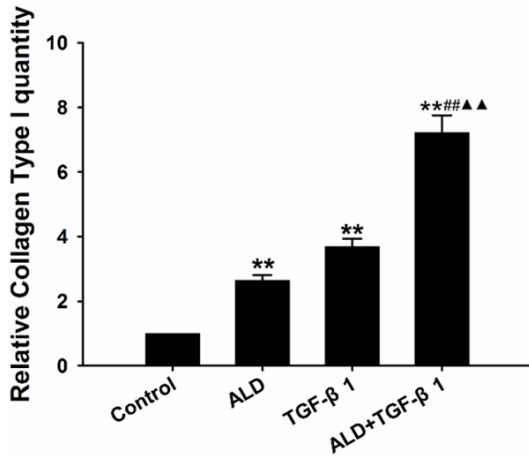
Figure 9. Effect of aldosterone on collagen I expression in hepatic stellate cells. *: Compared with control group; #: Comparison between aldosterone group and aldosterone plus TGF-β1 group; ▲: Comparison between TGF-β1 group and aldosterone plus TGF-β1 group *P < 0.05, **P < 0.01, ***P < 0.001.

ments were observed in HSCs as shown in **Figure 3**.

Effect of different times of aldosterone incubation on the expression TGF-β1 in HSCs

HSCs were incubated with aldosterone for 4 h, 8 h, 12 h, 24 h, and 48 h, and the expression of TGF-β1 was detected with real-time PCR. The results showed that TGF-β1 expression increased in a time dependent manner and reached a peak at 24 h. TGF-β1 expression in aldosterone-treated groups was significantly different from that in the control group ($P < 0.05$). Further, there were significant differences in the expression of TGF-β1 among groups treated with aldosterone for 4 h, 8 h, 12 h, and 24 h ($P < 0.01$) as shown in **Figure 4**.

Effect of different concentrations of aldosterone on TGF-β1 expression

HSCs were incubated with different concentrations of aldosterone. The mRNA expression of TGF-β1 was measured with real-time PCR after aldosterone incubation for 24 h. Western blot was used to measure the protein expression of TGF-β1 after aldosterone treatment for 36 h. Compared with the control group, the TGF-β1 expression was significantly increased after incubation with different concentrations of aldosterone (10^{-6} M, 10^{-7} M, 10^{-8} M, and 10^{-9} M) ($P < 0.01$). Also, there was a significant difference in the expression of TGF-β1 between the
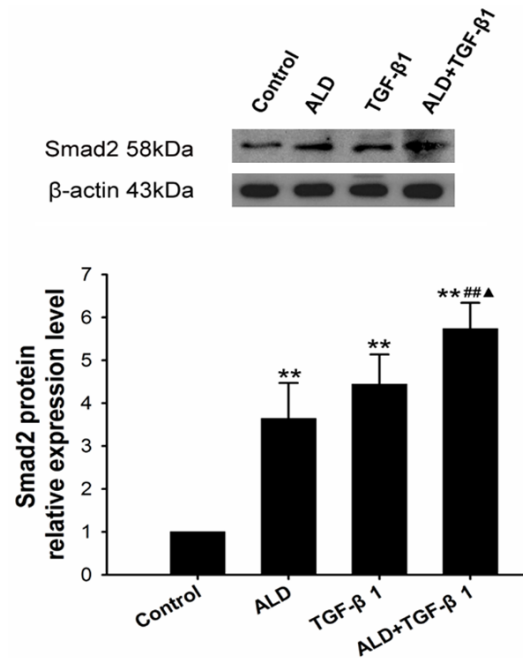

Figure 10. Effect of aldosterone on SMAD expression in hepatic stellate cells. *: Compared with control group; #: Comparison between aldosterone group and aldosterone plus TGF-β1 group; ▲: Comparison between TGF-β1 group and aldosterone plus TGF-β1 group. *P < 0.05, **P < 0.01, *** P < 0.001.

groups of 10^{-6} M and 10^{-7} M aldosterone treatment as shown in **Figure 5**.

Effect of different concentrations of aldosterone on PAI-1 expression

HSCs were incubated with different concentrations of aldosterone. The mRNA expression of PAI-1 was measured with real-time PCR after incubation for 24 h. Western blot was used to measure the protein expression of PAI-1 after incubation for 36 h. Compared with the control group, the PAI-1 expression was significantly increased after incubation with different concentrations of aldosterone (10^{-6} M, 10^{-7} M, 10^{-8} M, and 10^{-9} M) ($P < 0.05$). There was a significant difference in PAI-1 expression between the 10^{-6} M and 10^{-7} M groups. No differences were observed between the 10^{-8} M and 10^{-9} M groups as shown in **Figure 6**.

Effect of aldosterone and spironolactone on PAI-1 expression

HSCs were incubated in four different groups: control, aldosterone (10^{-6} M), spironolactone (10^{-5} M), and aldosterone plus spironolactone. Compared with the control group, PAI-1 expres-

sion was significantly different in the three other groups ($P < 0.01$). The mRNA and protein expression of PAI-1 in the aldosterone plus spironolactone group was significantly decreased compared with the aldosterone group ($P < 0.05$) as shown in **Figure 7**.

Effect of aldosterone and spironolactone on TGF-β1 expression

HSCs were incubated in four different groups: control, aldosterone (10^{-6} M), spironolactone (10^{-5} M), and aldosterone plus spironolactone. Compared with the control group, TGF-β1 expression was significantly different in the three other groups ($P < 0.01$). There was significant difference in the expression of TGF-β1 between the aldosterone and the aldosterone plus spironolactone groups ($P < 0.01$) as shown in **Figure 8**.

Effect of aldosterone on collagen I expression in HSCs

Compared with the control group, collagen I expression was significantly increased in the aldosterone (10^{-6} M), TGF-β1 (5 ng/ml), and aldosterone (10^{-6} M) plus TGF-β1 (5 ng/ml) groups ($P < 0.01$). There was a significant difference in the expression of collagen I between the aldosterone and aldosterone plus TGF-β1 groups ($P < 0.01$). Collagen I expression in the TGF-β1 group was significantly different from the aldosterone plus TGF-β1 group ($P < 0.01$) as shown in **Figure 9**.

Effect of aldosterone on SMAD expression in HSCs

Compared with the control group, the expression of SMAD was significantly increased in the aldosterone (10^{-6} M), TGF-β1 (5 ng/ml), and aldosterone (10^{-6} M) plus TGF-β1 (5 ng/ml) groups ($P < 0.05$). There was a significant difference in SMAD expression between the aldosterone and aldosterone plus TGF-β1 groups ($P < 0.01$). SMAD expression in the TGF-β1 group was significantly different from the aldosterone plus TGF-β1 group ($P < 0.05$) as shown in **Figure 10**.

Discussion

HSCs are the most important cells in regulating liver ECM deposition. HSCs maintain the balance between ECM synthesis and degradation through differential expression of uPA and PAI and regulates plasminogen and MMP activities [22]. In this study, we found that aldosterone stimulated expression of PAI-1 in HSCs. The highest expression level of PAI-1 was observed with an aldosterone concentration of 10^{-6} M. Compared with the control group, PAI-1 expression was significantly increased by stimulation with 10^{-8} M and 10^{-9} M aldosterone ($P < 0.01$). However, a significant difference in PAI-1 expression was not observed between these two groups ($P > 0.05$). These results clearly showed that aldosterone stimulation directly induced the overexpression of PAI-1 and that the effect could be partially reversed by spironolactone. The effect of aldosterone on HSCs is similar to that produced by fibrosis matrix protein metabolism. Since PAI-1 is considered a key factor of fibrinolysis and ECM accumulation, it might be involved in aldosterone-induced degradation of extracellular matrix. In addition to the physiological effects of aldosterone on the maintenance of renal extracellular salt, potassium, and water homeostasis, our results proved that aldosterone induced liver fibrosis by reducing the degradation of extracellular matrix and matrix degradation, probably through PAI-1 mediation. The latest study [23] demonstrated that the kidney collagen content and PAI-1 mRNA expression were significantly increased in aldosterone/salt-treated mice. However, the glomerulus hypertrophy was obviously alleviated by PAI-1 deficiency. Application of an MR blocker decreased PAI-1 expression and ameliorated glomerular sclerosis in rats with nephritis or diabetes [24, 25]. In agreement with the previous study, our research demonstrated that treatment with aldosterone led to increased expression of PAI-1 and TGF-β1, which was reversed by the administration of SPR.

A number of studies indicated that *in vitro* aldosterone treatment enhanced TGF-β1 secretion, but the mRNA level of TGF-β1 expression was rarely reported [26-28]. Because aldosterone affected HSCs independently of the renin-angiotensin system, it remains unclear whether aldosterone stimulation of HSCs increases TGF-β1 expression. The present *in vitro* study demonstrated that aldosterone stimulation significantly enhanced TGF-β1 expression in a time dependent manner relative to a control ($P < 0.05$). Expression of TGF-β1 in the aldosterone plus spironolactone group was significant-

ly reduced compared with the aldosterone group ($P < 0.05$). The increased expression of PAI-1 in hepatic fibrosis is probably due to the stimulation of TGF-β1 [29]. TGF-β1 plays a critical role in hepatic fibrosis, and its expression is regulated by many molecules, especially RASS [30, 31]. In liver fibrosis, the increased secretion of TGF-β1 by mesenchymal cells promotes PAI expression in sinus endothelial cells, thereby reducing plasminogen content and ECM degradation [32]. In our study, the expression of SMAD in HSCs was dramatically increased in the aldosterone-treated and aldosterone plus TGF-β1-treated groups ($P < 0.01$). Moreover, SMAD expression in the aldosterone plus TGF-β1-treated group was remarkably higher than in the group treated with aldosterone alone. It is well know that SMAD is an important intracellular effector molecule in the TGF β signaling pathway. Without the SMAD complex, TGF-β1 is unable to induce HSC transition and secretion of collagen and other ECMs [33].

Our study demonstrated that aldosterone stimulation resulted in increased expression of PAI-1 in HSCs. Therefore, aldosterone contributed to matrix accumulation by enhancing ECM synthesis and reducing its degradation.

In summary, our study demonstrated that aldosterone promoted fibrosis progression by increasing the expression of TGF-β1, PAI-1, and collagen in HSCs and decreasing ECM degradation, which was partially mediated by the TGF-SMAD signaling pathway. SMAD expression was markedly elevated after aldosterone treatment. Our study showed that aldosterone-induced hepatic fibrosis was partially mediated by the TGF-SMAD pathway. The effects of aldosterone were partially reversed by spironolactone treatment.

Acknowledgements

Disclosure of conflict of interest

None.

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References

[1] Funder JW. The nongenomic actions of aldosterone. Endocr Rev 2005; 26: 313-321.

[2] Funder JW. Non-genomic action of aldosterone: role in hypertension. Curr Opin Nephrol Hypertens 2001; 10: 227-230.

[3] Connell JM, Davies E. The new biology of aldosterone. J Endocrinol 2005; 186: 1-20.

[4] Takebayashi K, Matsumoto S, Aso Y, Inukai T. Aldosterone blockade attenuates urinary monocyte chemoattractant protein-1 and oxidative stress in patients with type 2 diabetes complicated by diabetic nephropathy. J Clin Endocrinol Metab 2006; 91: 2214-7.

[5] Shibata S, Nagase M, Yoshida S, Kawachi H, Fujita T. Podocyte as the target for aldosterone: roles of oxidative stress and Sgk1. Hypertension 2007; 49: 355-64.

[6] Mathew JT, Patni H, Chaudhary AN, Liang W, Gupta A, Chander PN, Ding G, Singhal PC. Aldosterone induces mesangial cell apoptosis both in vivo and in vitro. Am J Physiol Renal Physiol 2008; 295: F73-81.

[7] Patni H, Mathew JT, Luan L, Franki N, Chander PN, Singhal PC. Aldosterone promotes proximal tubular cell apoptosis: role of oxidative stress. Am J Physiol Renal Physiol 2007; 293: F1065-71.

[8] Han JS, Choi BS, Yang CW, Kim YS. Aldosterone-induced TGF-beta1 expression is regulated by mitogen-activated protein kinases and activator protein-1 in mesangial cells. J Korean Med Sci 2009; 24 Suppl: S195-203.

[9] Lai L, Chen J, Hao CM, Lin S, Gu Y. Aldosterone promotes fibronectin production through a Smad2-dependent TGF-beta1 pathway in mesangial cells. Biochem Biophys Res Commun 2006; 348: 70-5.

[10] Juknevicius I, Segal Y, Kren S, Lee R, Hostetter TH. Effect of aldosterone on renal transforming growth factor-beta. Am J Physiol Renal Physiol 2004; 286: F1059-62.

[11] Hostetter TH, Rosenberg ME, Ibrahim HN, Juknevicius I. Aldosterone in renal disease. Curr Opin Nephrol Hypertens 2001; 10: 105-110.

[12] Zhou G, Kandala JC, Tyagi SC, Katwa LC, Weber KT. Effects of angiotensin II and aldosterone on collagen gene expression and protein turnover in cardiac fibroblasts. Mol Cell Biochem 1996; 154: 171-8.

[13] Gauer S, Segitz V, Goppelt-Struebe M. Aldosterone induces CTGF in mesangial cells by activation of the glucocorticoid receptor. Nephrol Dial Transplant 2007; 22: 3154-9.

[14] Yamahara H, Kishimoto N, Nakata M, Okazaki A, Kimura T, Sonomura K, Matsuoka E, Shiotsu Y, Adachi T, Matsubara H, Iwasaka T, Mori Y.

Direct aldosterone action as a profibrotic factor via ROS-mediated SGK1 in peritoneal fibroblasts. Kidney Blood Press Res 2009; 32: 185-93.

[15] McMahon EG. Recent studies with eplerenone, a novel selective aldosterone receptor antagonist. Curr Opin Pharmacol 2001; 1: 190-6.

[16] Mars WM, Liu ML, Kitson RP, Goldfarb RH, Gabauer MK, Michalopoulos GK. Immediate early detection of urokinase receptor after partial hepatectomy and its implications for initiation of liver regeneration. Hepatology 1995; 21: 1695-1701.

[17] Bezerra JA, Bugge TH, Melin-Aldana H, Sabla G, Kombrinck KW, Witte DP, Degen JL. Plasminogen deficiency leads to impaired remodeling after a toxic injury to the liver. Proc Natl Acad Sci U S A 1999; 96: 15143-15148.

[18] Salgado S, Garcia J, Vera J, Siller F, Bueno M, Miranda A, Segura A, Grijalva G, Segura J, Orozco H, Hernandez-Pando R, Fafutis M, Aguilar LK, Aguilar-Cordova E, Armendariz-Borunda J. Liver cirrhosis is reverted by urokinase plasminogen activator gene therapy. Mol Ther 2000; 2: 545-551.

[19] Fibbi G, Pucci M, Grappone C, Pellegrini G, Salzano R, Casini A, Milani S, Del Rosso M. Functions of the fibrinolytic system in human Ito cells and its control by basic fibroblast and pletelet-derived growth factor. Hepatology 1999; 29: 868-878.

[20] Okuno M, Moriwaki H, Imai S, Muto Y, Kawada N, Suzuki Y, Kojima S. Retinoids exacerbate rat liver fibrosis by inducing the activation of latent TGF-13 in liver stellate cells. Hepatology 1997; 26: 913-921.

[21] Okuno M, Sato T, Imai S, Kawada N, Suzuki Y, Yoshimura H, Moriwaki H, Onuki K, Masushige S, Muto Y, Friedman SL, Kato S, Kojima S. Increased 9, 13-cis-retinoic acid in rat hepatic fibrosis:implication for a potential link between retinoid loss and TGF-β mediated fibrogenesis in vivo. J Hepatol 1999; 30: 1073-1080.

[22] Friedman SL. Mechanisms of disease: mechanisms of hepatic fibrosis a therapeutic implications. Nat Clin Pract Gastroenterol Hepatol 2004; 1: 982105.

[23] Ma J, Weisberg A, Griffin JP, Vaughan DE, Fogo AB, Brown NJ. Plasminogen activator inhibitor-1 deficiency protects against aldosterone induced glomerular injury. Kidney Int 2006; 69: 1064-1072.

[24] Fujisawa G, Okada K, Muto S, Fujita N, Itabashi N, Kusano E, Ishibashi S. Spironolactone prevents early renal injury in streptozotocin-induced diabetic rats. Kidney Int 2004; 66: 1493-502.

[25] Gullulu M, Akdag I, Kahvecioglu S, Filiz G, Savci V. Aldosterone blockage in proliferative glomerulonephritis prevents not only fibrosis, but proliferation as well. Ren Fail 2006; 28: 509-14.

[26] Han KH, Kang YS, Han SY, Jee YH, Lee MH, Han JY, Kim HK, Kim YS, Cha DR. Spironolactone ameliorates renal injury and connective tissue growth factor expression in type II diabetic rats. Kidney Int 2006; 70: 111-20.

[27] Lai L, Chen J, Hao CM, Lin S, Gu Y. Aldosterone promotes fibronectin production through a Smad2-dependent TGF-beta1 pathway in mesangial cells. Biochem Biophys Res Commun 2006; 348: 70-5.

[28] Yuan J, Jia R, Bao Y. Aldosterone up-regulates production of plasminogen activator inhibitor-1 by renal mesangial cells. J Biochem Mol Biol 2007; 40: 180-8.

[29] Seki T, Watanabe K, Saito K. Purification and characterization of hepatic plasminogen activator in the conditioned medium of rat hepatocytes in primary culture. Biosci Biotech Biochem 1993; 57: 1369-1371.

[30] Border WA, Noble NA. Interactions of transforming growth factor-beta and angiotensin II in renal fibrosis. Hypertension 1998; 31: 181-8.

[31] Huang Y, Wongamorntham S, Kasting J, McQuillan D, Owens RT, Yu L, Noble NA, BorderW. Renin increases mesangial cell transforming growth factor-beta1 and matrix proteins through receptor-mediated, angiotensin II-independent mechanisms. Kidney Int 2006; 69: 105-13.

[32] McMahon EG. Recent studies with eplerenone, a novel selective aldosterone receptor antagonist. Curr Opin Pharmacol 2001; 1: 190-6.

[33] Paradis V, Dargere D, Bonvoust F, Vidaud M, Segarini P, Bedossa P. Effects and regulation of connective tissue growth factor on hepatic stellate cells. Lab Invest 2002; 82: 767-74.