SHORT PAPER

Effect of captopril on TNF-α and IL-10 in the livers of Bile Duct ligated Rats

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ABSTRACT

Background: The renin-angiotensin system has an important role in hepatic inflammation and fibrosis. Renin-angiotensin system blockade by angiotensin-converting enzyme (ACE) inhibitors provides some protective effects against hepatic fibrogenesis. Captopril as an ACE inhibitor can decrease inflammatory mediators and attenuate hepatic fibrosis in the livers of bile duct ligated (BDL) rats. **Objective:** The present study was conducted to investigate the effects of captopril on cytokine production in hepatic fibrosis induced by a bile duct ligation model in rats. **Methods:** Male rats were divided into four groups including; control, sham operated, BDL, and BDL plus captopril (10 mg/kg/day, orally). After 28 days of treatment, the livers were removed for cytokine analysis. Hepatic interleukin (IL)-10 and tumor necrosis factor (TNF)-α levels were measured. **Results:** Captopril treatment decreased the hepatic content of the proinflammatory cytokine TNF-α and increased the anti-inflammatory cytokine IL-10. **Conclusion:** the present study suggests that the protective effect of captopril on hepatic fibrosis is likely to be mediated by cytokine production.

Key words: Captopril, Hepatic fibrosis, IL-10, TNF-α

INTRODUCTION

Angiotensin II plays an important role in liver inflammation and fibrosis. It mediates the expression of proinflammatory cytokines and the infiltration of inflammatory cells (1). These inflammatory responses occur during the course of hepatic fibrosis (2).

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Renin-angiotensin system blockade, either by ACE inhibition or angiotensin receptor 1 (AT1) blockade, has protective effects against the inflammatory response in liver fibrogenesis (1). Anti-inflammatory effects of certain ACE inhibitors have been shown both *in vitro* and *in vivo*. It has been demonstrated that captopril inhibits lipopolysaccharide (LPS)-induced production of proinflammatory cytokines such as TNF and IL-1β (3). Angiotensin-converting enzyme inhibitors improve hepatic steatosis by reducing TNF-α and IL-6 levels in the circulation and the liver (4). Interleukin-10 is a potent anti-inflammatory cytokine. It has been demonstrated that IL-10, as an immunosuppressive mediator, has a protective effect in some hepatic diseases (5). IL-10 treatment of patients with chronic hepatitis C reduces hepatic inflammation and fibrosis. IL-10 inhibits the production of TNF-α, nuclear factor kB and superoxide anions (6).
It has been reported that captopril inhibits the progression of hepatic fibrosis induced by CCl_4 in rats (7). In our previous study, we demonstrated that angiotensin blockade with captopril leads to the inhibition of hepatic fibrosis in BDL cirrhotic rat model (8). Progression of liver fibrosis during chronic inflammation may be stimulated by the production of proinflammatory cytokines that ultimately leads to cell death. The purpose of this study was to investigate the effects of captopril, an ACE inhibitor, on cytokine production in hepatic fibrosis induced in rat by bile duct ligation.

MATERIAL AND METHODS

Animals: Male Albino Wistar rats, weighing 150–200 g were obtained from the department of pharmacology, Tehran University of Medical Sciences, Tehran, Iran. Every 4 animals were housed in a cage under a 12–12 h light–dark cycle at 22-25°C. They were allowed to have free access to ad libitum food and tap water throughout the 4-week experiment. All experiments were carried out in accordance with institutional guidelines for laboratory animal care and use.
Groups and training protocol: Biliary hepatic fibrosis was induced by common bile duct ligation (BDL). Rats were anesthetized with ketamine and xylazine. After midline abdominal incision, the common bile duct was doubly ligated with a silk thread. Sham operated animals underwent an identical procedure and manipulation of the common bile duct without ligation. Four weeks after operation, BDL rats showed signs of liver failure, ascites and jaundice. Rats were divided into four experimental groups (n=4 in each group) as follows: (1) Control group did not undergo any operation or treatment; (2) Sham operated group; (3) BDL group without treatment and (4) BDL plus captopril (10 mg/kg/day, orally for 28 days) starting on the first day after BDL operation (8). On day 28 rats were euthanized and livers were removed for cytokine analysis.
Cytokine measurement: Liver tissue samples were removed from rats and snap frozen in liquid nitrogen. The samples (100mg) were homogenized in 800µl Tris–HCl buffer. All homogenized samples were centrifuged at 16,000g for 30min at 4 °C and the supernatants were taken and stored at −80 °C (9). The supernatant samples were used for the measurement of the cytokine concentrations. Hepatic IL-10 and TNF-α levels were measured by using specific ELISA kits (Bender MedSystems GmbH) and the results were expressed as pg cytokine/mg wet tissue.

Statistical analysis: Data are presented as means ± SEM. Statistical analysis was performed using one way ANOVA followed by TUKEY post-hoc test. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

To evaluate the mechanism of the protective effects of captopril in hepatic fibrosis, we measured the levels of TNF-α and IL-10 in supernatants of liver tissues from each study group. As shown in figure 1A, bile duct ligation caused remarkable elevation ($p < 0.001$) in the hepatic level of TNF-α as compared with those of the control and the sham operated groups. Treatment of rats with captopril reduced BDL-induced production of TNF-α compared with the BDL group ($p < 0.001$). There was no significant difference among the control, sham and captopril plus BDL groups. Figure 1B shows that IL-10 cytokine levels tend to decrease in BDL rats. However, the decreasing effect of BDL on hepatic level of IL-10 was not statistically significant when compared with the control and the sham operated groups. Administration of captopril produced a significant increase ($p < 0.05$) in the hepatic level of IL-10 compared with the BDL group. No significant difference was observed between captopril-treated and sham operated groups in this study.

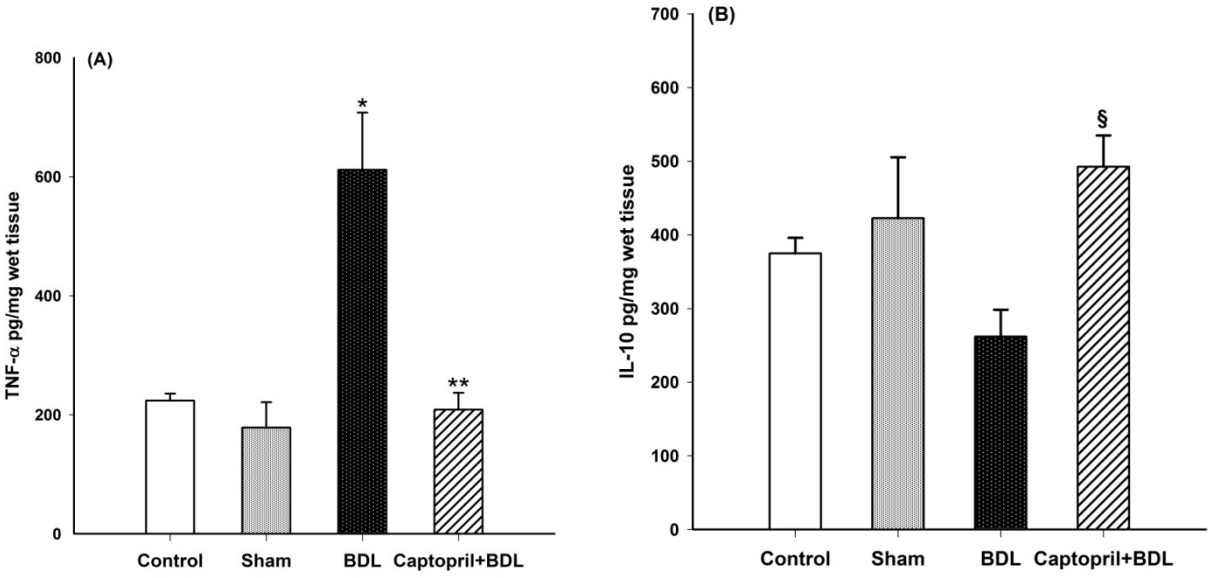

Figure 1. Effect of captopril (10 mg/kg/day) on hepatic levels of TNF-α and IL-10 in four experimental groups. Captopril prevented the BDL-induced increase in TNF-α (**A**), while captopril treatment increased the level of IL-10 (**B**). Data are expressed as mean ± SEM. *$P < 0.001$ compared with the sham group; **$P < 0.001$ compared with BDL group. §$P < 0.05$ compared with BDL group.

Liver fibrogenesis occurs as a result of hepatic cell injury which leads to recruitment of inflammatory cells, followed by the release of cytokines (5). Several reports suggest that angiotensin II plays an important role in hepatic fibrosis by inducing the expression

of potent cytokines (10,11). Cytokines have an important function in the development of hepatic inflammation and fibrosis (2). There is an imbalance between anti-inflammatory and proinflammatory cytokines during liver fibrosis. It has been demonstrated that hepatic concentration of TNF-α increases following BDL in mice (1). Chronic BDL significantly augments plasma and hepatic levels of proinflammatory cytokines such as TNF-α and IL-1β (12). Captopril has a potent inhibitory effect on the LPS-induced production of TNF in *vitro* (13). In another study it is shown that pretreatment with captopril inhibits the expression of TNF-α in rabbits (14).

Some studies have suggested a protective role for the anti-inflammatory cytokine, IL-10, on hepatic fibrogenesis. IL-10 inhibits the synthesis of proinflammatory and profibrogenic cytokines including TNF-α, IL-6 and TGF-β (2,15). IL-10 decreases the levels of some transmitters such as endothelin, angiotensin II and prostacyclin. These transmitters have a significant role in rat hepatic fibrosis induced by CCl_4 (6). It has been shown that IL-10 decreases the damage and the inflammation in a rat model of hepatic ischemia and reperfusion (16). The ACE inhibitors enalapril and captopril increase IL-10 production by splenocytes in mice (17).

In our previous study we showed that captopril increases liver glutathione (GSH) content, diminishes lipid peroxidation and the hepatic content of hydroxyproline, and reduces hepatic inflammation (8). Our present study shows that captopril treatment is able to reduce BDL-induced production of the proinflammatory cytokine, TNF-α. This observation is important, because TNF-α plays a major role in the development of hepatic fibrosis. In addition, we demonstrated that captopril treatment increases the hepatic content of IL-10 in BDL rats. Interleukin-10 is an anti-inflammatory cytokine and has an important role in the prevention of hepatic inflammation and fibrosis. Altogether, the results of current study suggest that the protective effect of captopril in hepatic fibrosis is likely to be mediated by cytokine production.

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REFERENCES

1 Yang L, Bataller R, Dulyx J, Coffman TM, Ginés P, Rippe RA, et al. Attenuated hepatic inflammation and fibrosis in angiotensin type 1a receptor deficient mice. J Hepatol. 2005; 43:317-23.
2 Zhang LJ, Yu JP, Li D, Huang YH, Chen ZX, Wang XZ. Effects of cytokines on carbon tetrachloride-induced hepatic fibrogenesis in rats. World J Gastroenterol. 2004; 10:77-81.
3 Schindler R, Dinarello CA, Koch KM. Angiotensin-converting-enzyme inhibitors suppress synthesis of tumour necrosis factor and interleukin 1 by human peripheral blood mononuclear cells. Cytokine. 1995; 7:526-33.
4 Zhang X, Li ZZ, Liu DF, Xu X, Mei ZC, Shen W. Angiotensin-converting enzyme inhibitors improve hepatic steatosis by modulating expression of tumour necrosis factor-alpha, interleukin-6 and adiponectin receptor-2 in rats with type 2 diabetes. Clin Exp Pharmacol Physiol. 2009; 36:631-6.
5 Muriel P. NF-kappaB in liver diseases: a target for drug therapy. J Appl Toxicol. 2009; 29:91-100.
6 Wang XZ, Zhang LJ, Li D, Huang YH, Chen ZX, Li B. Effects of transmitters and interleukin-10 on rat hepatic fibrosis induced by CCl4. World J Gastroenterol. 2003; 9:539-43.
7 Wei YH, Jun L, Qiang CJ. Effect of losartan, an angiotensin II antagonist, on hepatic fibrosis induced by CCl4 in rats. Dig Dis Sci. 2004; 49:1589-94.
8 Karimian G, Mohammadi-Karakani A, Sotoudeh M, Ghazi-Khansari M, Ghobadi G, Shakiba B. Attenuation of hepatic fibrosis through captopril and enalapril in the livers of bile duct ligated rats. Biomed Pharmacother. 2008; 62:312-6.

9 Amirshahrokhi K, Dehpour AR, Hadjati J, Sotoudeh M, Ghazi-Khansari M. Methadone ameliorates multiple-low-dose streptozotocin-induced type 1 diabetes in mice. Toxicol Appl Pharmacol. 2008; 232:119-24.
10 Kim MY, Baik SK, Park DH, Jang YO, Suk KT, Yea CJ, et al. Angiotensin receptor blockers are superior to angiotensin-converting enzyme inhibitors in the suppression of hepatic fibrosis in a bile duct-ligated rat model. J Gastroenterol. 2008; 43:889-96.
11 Toblli JE, Muñoz MC, Cao G, Mella J, Pereyra L, Mastai R. ACE inhibition and AT1 receptor blockade prevent fatty liver and fibrosis in obese Zucker rats. Obesity(Silver Spring). 2008; 16:770-6.
12 Fernández-Martínez E, Pérez-Alvarez V, Tsutsumi V, Shibayama M, Muriel P. Chronic bile duct obstruction induces changes in plasma and hepatic levels of cytokines and nitric oxide in the rat. Exp Toxicol Pathol. 2006; 58:49-58.
13 Peeters AC, Netea MG, Kullberg BJ, Thien T, van der Meer JW. The effect of renin-angiotensin system inhibitors on pro- and anti-inflammatory cytokine production. Immunology. 1998; 94:376-9.
14 Zagariya A, Bhat R, Navale S, Chari G, Vidyasagar D. Inhibition of meconium-induced cytokine expression and cell apoptosis by pretreatment with captopril. Pediatrics. 2006; 117:1722-7.
15 Breitkopf K, Haas S, Wiercinska E, Singer MV, Dooley S. Anti-TGF-beta strategies for the treatment of chronic liver disease. Alcohol Clin Exp Res. 2005; 29:121S-131S.
16 Dinant S, Veteläinen RL, Florquin S, van Vliet AK, van Gulik TM. IL-10 attenuates hepatic I/R injury and promotes hepatocyte proliferation. J Surg Res. 2007; 141:176-82.
17 Albuquerque D, Nihei J, Cardillo F, Singh R. The ACE inhibitors enalapril and captopril modulate cytokine responses in Balb/c and C57Bl/6 normal mice and increase CD4 (+) CD103 (+) CD25 (negative) splenic T cell numbers. Cell Immunol. 2010; 260:92-7.