Aqueous Garlic Extract Alleviates Liver Fibrosis and Renal Dysfunction in Bile-Duct-Ligated Rats

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There is accumulating evidence that the renin-angiotensin system (RAS) is involved in hepatic inflammation and fibrogenesis. Garlic was found to lower the activity of the angiotensin converting enzyme (ACE) in the serum of rats in a diabetic model. We examined the effect of an aqueous garlic extract (AGE) on the ACE activity, cholestasis-induced liver fibrosis, and associated renal dysfunction in comparison with the effect of the standard drug enalapril. Both AGE and enalapril were administered orally for six weeks starting from the third day after bile duct ligation (BDL). BDL significantly increased the serum activities of liver enzymes, serum lactate dehydrogenase (LDH) activity, an indicator of liver cell death, serum total bilirubin (TB) level, liver myeloperoxidase (MPO) activity, and liver malondialdehyde (MDA) content. BDL was associated with elevation of serum urea and creatinine levels indicating renal dysfunction. BDL also caused an increase in the transcript levels of the genes coding for tumour necrosis factor alpha (TNF-α), transforming growth factor beta-1 (TGF-β1), and matrix metalloproteinase-13 (MMP-13), a collagenase, in liver tissues. A significant decrease in hepatic reduced glutathione (GSH) was observed in BDL rats, while serum ACE activity was increased.

Both AGE and enalapril counteracted all these deleterious changes, with the exception that only AGE reduced the MPO activity. These findings suggest that AGE possesses hepatoprotective properties, similar to enalapril, probably by modulating the levels of proteins such as TNF-α, TGF-β1 and MMP-13, and involving a reduction of ACE and of oxidative stress.

Key words: Bile Duct Ligation, Enalapril, Aqueous Garlic Extract

Introduction

Liver fibrosis accompanies most chronic liver disorders. It results from chronic damage to the liver in conjunction with the accumulation of extracellular matrix proteins which is characteristic of most types of chronic liver diseases (de Franchis et al., 2003). Fibrosis itself causes no symptoms, but can lead to portal hypertension because scarring distorts the blood flow through the liver (Neuschwander-Tetri and Caldwell, 2003).

There is accumulating evidence that the renin-angiotensin system (RAS) is involved in hepatic inflammation and fibrogenesis. RAS also has been implicated in the induction of oxidative stress which plays a major role in the molecular damage of proteins and informational errors in DNA and RNA molecules. It induces hepatic stellate cell (HSC) proliferation and up-regulates transforming growth factor beta-1 (TGF-β1) expression (Türkay et al., 2008). Blockade of RAS can attenuate the architectural injury of the liver (Bataller and Brenner, 2005). Inhibitors of the angiotensin converting enzyme (ACE), a peptidase which converts angiotensin I to angiotensin II, attenuate the effects of RAS. In consequence, angiotensin II synthesis is decreased, and the production of renin and angiotensin I, which favours the production of angiotensin (1 – 7) and other peptides, is increased (Huang et al., 2010). Angiotensin (1 – 7) has opposing actions to angiotensin II which gives RAS a dual influence over various tis-
sues. Angiotensin (1 – 7) has vasodilator and antiproliferative effects on blood vessels (Ferreira and Santos, 2005).

In previous studies, increased levels, or accelerated generation, of reactive oxygen species (ROS) and toxic degradation products of lipid peroxidation were found in the plasma of individuals with chronic liver disease and animal models of liver disease (Ljubuncic et al., 2000). The oxidative stress may be due to intra-organ generation of ROS. ROS production may be mediated by endotoxin and bile acids and causes lipid peroxidation and generation of lipid peroxides and/or malondialdehyde (MDA) in bile-duct-ligated rats (Tain et al., 2010).

Aqueous garlic extract (AGE), with its antioxidant and antifibrotic properties, may be of potential therapeutic value in protecting against liver fibrosis and oxidative injury due to biliary obstruction (Gedik et al., 2005). AGE might have value in lowering ACE activities and preventing some vascular complications of diabetes mellitus in diabetic animals (Hosseini et al., 2007). From this point of view, the aim of the present work was to study the effect of AGE on RAS in cholestasis-induced liver fibrosis. It also aimed to compare the effect of AGE with that of enalapril, a well-known ACE inhibitor, in this model of liver fibrosis.

**Materials and Methods**

**Animals**

Adult male albino rats, weighing 180 – 220 g, were obtained from the National Research Center, Cairo, Egypt. They were kept under constant environmental and nutritional conditions throughout the period of study. They were housed six per cage in plastic cages with wood shave bedding and received normal standard diet. They had free access to water and food. The protocol of the present study was approved by the Animal Care and Use Committee of the Faculty of Pharmacy, Zagazig University, Zagazig, Egypt and follows the Declaration of Helsinki. Every effort was done to minimize animals' suffering.

**Materials**

Enalapril was supplied by October Pharma Co. (Cairo, Egypt). The required dose was dissolved in drinking water.

**Preparation of aqueous garlic extract (AGE)**

Fresh peeled garlic cloves were obtained from cultivated plants (collected from the vicinity of Zagazig city, Sharquia Governate, Egypt, in April 2012). The identity of plant materials was confirmed by Dr. Assem El Shazly, Professor of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt. Fifty g of the edible portion was chopped and homogenized in 100 mL of distilled water in a mortar at room temperature. The homogenate was then filtered by passage through a 25-µm pore-size filter (Millipore, St. Quentin, France) and adjusted to give a crude aqueous extract of 500 g of garlic/L (Batirel et al., 1996). The obtained AGE was then dried by lyophilization and kept at −20 °C until required. The resulting powder was then reconstituted by dissolving in saline just before use.

**Experimental design**

The animals were randomly divided into 5 groups of 25 rats in group 3 and 20 in each other group. Group I received normal saline [2 mL/kg body weight (BW), orally] daily for 6 weeks and represented the normal control group. Group II underwent a midline incision and manipulation of the bile duct without ligation; it received saline (2 mL/kg BW, orally) for 6 weeks starting from the third day after surgery and served as sham group. Groups III – V were bile-duct-ligated and received the indicated treatment for 6 weeks starting from the third day after surgery. Group III received saline (2 mL/kg BW, orally) and served as diseased group. Group IV received enalapril [5 mg/kg BW, in drinking water, according to Karimian et al. (2008)]. Group V received AGE (250 mg/kg BW) as a single dose by orogastric tube daily at 09:00 a.m. throughout the period of study.

**Induction of liver fibrosis**

Fibrosis was induced by bile duct ligation under general anaesthesia with a mixture of ketamine hydrochloride (50 mg/kg BW) and diazepam (3 mg/kg BW) (Wei et al., 2001). The common bile duct was exposed, doubly ligated with 4-0 silk threads, and then excised between the ligatures to prevent regeneration. In the sham-operated group, the bile duct was exposed and left in situ without ligation. Meloxicam in a dose of 1 mg/kg BW was injected in the femoral muscle and was given for 3 d after surgery to reduce pain (Debaene
et al., 1990), and penicillin G (100,000 IU) (aq-pen vial) was administered by deep intramuscular injection for 3 d after surgery as prophylaxis against infection (Bourque et al., 2010).

Blood sampling and serum preparation

Blood was collected from the rats just before sacrifice. Samples were collected from the orbital sinus in clean dry centrifuge tubes. The tubes were left to clot for 15 min at room temperature. Serum was separated by centrifugation at 10,000 x g for 15 min, and divided into three aliquots which were frozen and kept at −20 °C. The first aliquot was used for the determination of the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH), as well as of urea, creatinine, and total bilirubin (TB). The second aliquot was used for the determination of ACE activity. The third aliquot was kept as reserve.

Tissue sampling

The isolated livers were perfused with phosphate-buffered saline (PBS) containing 0.16 mg heparin/mL. They were divided into three parts. All parts were immersed immediately in liquid nitrogen and kept at −80 °C. These parts were used to measure the contents of tumour necrosis factor alpha (TNF-α), transforming growth factor beta-1 (TGF-β1), matrix metalloproteinase-13 (MMP-13), myeloperoxidase (MPO), malondialdehyde (MDA), and reduced glutathione (GSH).

Biochemical analyses

Blood serum

ALT and AST activities were determined by a colourimetric method according to Reitman and Frankel (1957). Creatinine and urea levels were measured by kits supplied by Bio-Diagnostic Co. (Cairo, Egypt). LDH activity was determined by the kinetic method of Fasce and Rej (1970). TB level was measured colorimetrically as described by Gellis et al. (1963) and Ohkawa et al. (1979), respectively.

Liver tissues

Liver transcripts of TNF-α, TGF-β1, and MMP-13 were determined by semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). RNA was extracted, reverse transcribed into cDNA, and amplified by PCR, using the following primers: TNF-α: forward, 5'-ATTGGCAATGGGAAAAATGA-3'; reverse, 5'-TTATGACCTCTTTTGGTCTGA-3'; TGF-β1: forward, 5'-TTGAGTGTACCCACAGAGA-3'; reverse, 5'-TCCGACAGCCACACTTCTTC-3'; MMP-13: forward, 5'-GCTGGTCAGTCGCCCTTTT-3'; reverse, 5'-GCTAAGGAAGCAGAGGGATT-3'.

β-Actin transcripts were determined as an equalizing internal control, using the primers: forward, 5'-TCACCTGAAGTACCCCATGGAG-3'; reverse, 5'-TTGGGCCCTGGGGTTTCAGGGG-3'. Relative expression of each studied gene (R) was calculated following the formula: \( R = \frac{\text{densitometrical units of each studied gene}}{\text{densitometrical units of β-actin.}} \)

Liver MPO activities were determined by a colourimetric method using an MPO chlorination activity determination kit as described by Kettle and Winterbourn (1994).

Reduced GSH and MDA levels were determined in the liver homogenate by the colourimetric methods according to Beutler et al. (1963) and Oikawa et al. (1979), respectively.

Statistical analysis

Graph Pad Prism software version 5 was used for statistical analysis. Results were expressed as mean ± S.E.M. Comparison between different groups was carried out using one-way analysis of variance (ANOVA) followed by Tukey post hoc test. The statistical associations between functional parameters were assayed using Spearman nonparametric correlation analysis and \( p < 0.05 \) was considered significant.

Results

General observations

The rats started to show signs of jaundice on the fourth day after the bile duct ligation (BDL), with a higher mortality rate during the first two weeks after the operation (50%), which then decreased during the third and fourth weeks (20–30%) and again increased in the last two weeks (55%). The untreated BDL group had the highest mortality rate.
Serum activities of liver enzymes and total serum bilirubin

Table I shows that BDL significantly increased the serum activities of ALT and AST, and the serum TB level as compared to sham rats ($p < 0.05$). Both enalapril and AGE caused a significant ($p < 0.05$) decrease in the BDL-induced activities of ALT and AST, and the serum TB levels. Enalapril was more potent than AGE. There was a strong positive correlation between the elevation of serum TB level and the increase in enzyme activities ($r = +0.07$ and $+0.9$ for ALT and AST, respectively, at $p < 0.05$).

Liver cell death

BDL significantly increased the serum activities of LDH, an indicator of necrotic cell death, as compared to sham rats ($p < 0.05$). Both AGE and enalapril reduced the LDH activity as compared to the BDL group (Table I), enalapril being more potent. There was a strong positive correlation between serum TB level and LDH activity ($r = +0.9$ at $p < 0.05$).

Renal function

BDL led to significant increases in the serum urea and creatinine levels, as compared to sham rats ($p < 0.05$), which were partially reversed by AGE and enalapril (Table I).

Inflammation markers

MPO, an enzyme released from infiltrating inflammatory cells during inflammation, was increased significantly in BDL rats as compared to sham rats at $p < 0.05$. AGE, but not enalapril, caused a significant reduction in the MPO activity as compared to the BDL group (Table II). The TNF-α transcript level was increased in liver cells of the BDL group, as compared to the sham group, at $p < 0.05$. AGE and enalapril caused a comparable reduction in the TNF-α gene expression in the liver of BDL rats (Fig. 1A). There was a strong positive correlation between serum TB level on the one hand, and the MPO activity and TNF-α transcript levels on the other hand ($r = +0.9$ at $p < 0.05$).

ACE activity

BDL significantly increased the serum activity of ACE, as compared to sham rats ($p < 0.05$), and this effect was partially reversed by AGE and, more potently, enalapril (Table II). There was a strong positive correlation between serum TB and ACE activity ($r = 0.7$ at $p < 0.05$).

Table I. Effect of enalapril and aqueous garlic extract (AGE) on liver and renal functions in bile-duct-ligated rats. The indicated parameters were determined in the serum.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Sham</th>
<th>BDL</th>
<th>Enalapril</th>
<th>AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT [U/L]</td>
<td>24.29 ± 1.8</td>
<td>56.14 ± 2.13</td>
<td>1012* ± 6.38</td>
<td>103.2** ± 3.5</td>
<td>305.2** ± 4.06</td>
</tr>
<tr>
<td>AST [U/L]</td>
<td>126.3 ± 5.87</td>
<td>310.4 ± 5.73</td>
<td>1379* ± 11.03</td>
<td>728.3** ± 5.26</td>
<td>1467** ± 3.83</td>
</tr>
<tr>
<td>LDH [U/L]</td>
<td>929.7 ± 6.97</td>
<td>1637 ± 14.72</td>
<td>2607* ± 10.05</td>
<td>2224** ± 14.14</td>
<td>2389** ± 11.06</td>
</tr>
<tr>
<td>TB [mg/mL]</td>
<td>0.0002 ± 0.00002</td>
<td>0.0008 ± 0.0003</td>
<td>0.406* ± 0.001</td>
<td>0.037** ± 0.0002</td>
<td>0.023** ± 0.0007</td>
</tr>
<tr>
<td>Urea [mg/mL]</td>
<td>0.12 ± 0.0035</td>
<td>0.32 ± 0.008</td>
<td>3.28* ± 0.038</td>
<td>1.45** ± 0.034</td>
<td>1.97** ± 0.02</td>
</tr>
<tr>
<td>Creatinine [mg/mL]</td>
<td>0.0024 ± 0.0002</td>
<td>0.0072 ± 0.0005</td>
<td>0.027* ± 0.06</td>
<td>0.02** ± 0.0005</td>
<td>0.015** ± 0.0003</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.E.M ($n = 6$). * Significantly different from sham at $p < 0.05$. ** Significantly different from BDL at $p < 0.05$.

Table II. Effect of enalapril and aqueous garlic extract (AGE) on myeloperoxidase (MPO), angiotensin converting enzyme (ACE), reduced glutathione (GSH), and malondialdehyde (MDA) in bile-duct-ligated rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Sham</th>
<th>BDL</th>
<th>Enalapril</th>
<th>AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO [ng/(min g tissue)]</td>
<td>1.75 ± 0.13</td>
<td>2.93 ± 0.24</td>
<td>4.62* ± 0.19</td>
<td>3.94 ± 0.13</td>
<td>3.31** ± 0.22</td>
</tr>
<tr>
<td>ACE [nmol/(mL min)]</td>
<td>3.97 ± 0.18</td>
<td>5.23 ± 0.16</td>
<td>9.27* ± 0.21</td>
<td>5.1** ± 0.3</td>
<td>6.87** ± 0.19</td>
</tr>
<tr>
<td>GSH [µmol/g tissue]</td>
<td>2.7 ± 0.19</td>
<td>1.58 ± 0.07</td>
<td>0.38* ± 0.05</td>
<td>1** ± 0.07</td>
<td>1.04** ± 0.05</td>
</tr>
<tr>
<td>MDA [nmol/g tissue]</td>
<td>24.63 ± 0.1</td>
<td>41.07 ± 1.29</td>
<td>62.73* ± 2.27</td>
<td>49.57** ± 1.72</td>
<td>47.75** ± 1.88</td>
</tr>
</tbody>
</table>

All parameters were measured in liver tissue homogenates, except ACE activity which was measured in serum.

Data are presented as mean ± S.E.M. ($n = 6$). * Significantly different from sham at $p < 0.05$. ** Significantly different from BDL at $p < 0.05$. 
Fig. 1. Effect of oral administration of enalapril (5 mg/kg BW) and aqueous garlic extract (AGE) (250 mg/kg BW) for six weeks on the expression of the genes coding for (A) liver tumour necrosis factor alpha (TNF-α), (B) transforming growth factor beta-1 (TGF-β1), and (C) matrix metalloproteinase-13 (MMP-13) in bile-duct-ligated rats. * Significantly different from sham at $p < 0.05$. ** Significantly different from BDL at $p < 0.05$. The respective upper panels show the results of an agarose gel electrophoresis of the PCR products of TNF-α, TGF-β1, MMP-13 (upper lanes), and β-actin (lower lanes). Left lane, 100-bp DNA ladder.

**Oxidative stress**

BDL significantly increased the liver content of MDA, a lipid peroxidation product, as compared to sham rats ($p < 0.05$), and significantly decreased that of reduced GSH ($p < 0.05$). Both AGE and enalapril reduced lipid peroxidation and increased the GSH content in the liver ($p < 0.05$) with equal potency, as
shown in Table II. There was a strong positive correlation between serum TB and liver MDA content \((r = +0.8 \text{ at } p < 0.05)\), and a strong negative correlation between serum TB and liver GSH content \((r = -0.8 \text{ at } p < 0.05)\).

**Profibrogenic cytokines and collagen formation**

BDL induced a significant increase in the liver transcript levels of both TGF-\(\beta\)1 and MMP-13 (collagenase-3), as compared to sham rats. There was a strong positive correlation between both serum TB and transcript levels \((r = +0.9 \text{ at } p < 0.05)\). A significant reduction of the TGF-\(\beta\)1 and MMP-13 transcript levels \((p < 0.05)\) occurred in both AGE- and enalapril-treated groups compared with the BDL group (Figs. 1B, C).

**Discussion**

AGE was found to have protective effects on cholestatic liver injury and the associated hepatorenal syndrome in rats subjected to BDL. These effects were comparable to that of enalapril, a potent ACE inhibitor with hepatoprotective effects. RAS has been shown previously to play an important role in the development of cholestatic liver injury in BDL rats (Karimian et al., 2008). Although AGE reduced the ACE activity and prevented some vascular complications of diabetes mellitus in diabetic rats (Hosseini et al., 2007), to our knowledge, the effect of AGE on RAS in cholestatic liver injury has not been evaluated yet. The present study showed that AGE reduces the serum ACE activity similar to enalapril. It improved the antioxidant status and reduced oxidative stress. Additionally, the inflammatory process elicited by BDL was alleviated by AGE treatment. Liver and renal functions were also improved. The most important consequence was the transcriptional suppression of TGF-\(\beta\)1 and MMP-13 which delay or even prevent the progression of cholestasis to fibrosis and cirrhosis. These findings are in accordance with the previous study of Gedik et al. (2005) who injected a similar dose of AGE intraperitoneally into rats for 28 days and measured its effect on oxidative stress and inflammation. However, the intraperitoneal injection is not similar to the traditional consumption of garlic by the oral route. Furthermore, a period of 28 days is not sufficient to allow the development of the hepatorenal syndrome observed in the present study, and these authors did not determine the effect of AGE on ACE, TGF-\(\beta\)1, and MMP-13.

The present study showed that BDL increases the ACE activity in serum, and it had previously been demonstrated that this enzyme is up-regulated in the liver of BDL animals (Herath et al., 2007, 2009). This enzyme catalyzes the conversion of angiotensin I to angiotensin II. Angiotensin II plays an important role in ROS formation by activating NADPH oxidase in hepatic stellate cells (HSCs) (De Minicis and Brenner, 2007). Furthermore, angiotensin II is profibrogenic as it activates HSCs (Tharaux et al., 2000) and increases the expression of TGF-\(\beta\)1 as shown in the present study. Furthermore, TGF-\(\beta\)1 increases the collagen fiber formation in liver tissue of BDL rats. Inhibition of the ACE activity by AGE and enalapril may lead to the availability of angiotensin (1–7). It is known that angiotensin (1–7) has opposing actions to angiotensin II. Angiotensin (1–7) is a vasodilator that suppresses proliferation, hypertrophy, and fibrosis in contrast to angiotensin II which is a vasoconstrictor and participates in proliferation, hypertrophy, and fibrosis. RAS could be described as a dual function system in which the vasoconstrictor/proliferative or vasodilator/antiproliferative actions are primarily driven by the ACE/angiotensin-converting enzyme 2 (ACE2) balance. ACE2 is a newly discovered homologue of ACE, which plays a key role as the central negative regulator of RAS. It counter-regulates the actions of angiotensin II by facilitating its breakdown to angiotensin (1–7) (Zhuo et al., 2013). If the ACE/ACE2 activity ratio is increased, angiotensin II generation and catabolism of angiotensin (1–7) are also increased, favouring vasoconstriction, while an opposite ratio will decrease angiotensin II and increase angiotensin (1–7) levels facilitating vasodilatation. Paizis et al. (2005) showed an increased activity and widespread gene expression of ACE2 in the liver of BDL animals and in human cirrhotic liver. Lubel et al. (2009) also reported that angiotensin (1–7) has a protective role against liver fibrosis.

In the present study, MPO was used as an indicator of the accumulation of activated neutrophils in the liver, because infiltration of neutrophils into tissues is commonly assessed by changes in the activity of MPO, which is an enzyme found only in neutrophils (Ohta et al., 2003). There are several reports on increased levels of MPO following BDL in rats (Ohta et al., 2003). Recruitment of neutrophils to the liver increases the production of TNF-\(\alpha\) which in turn increases neutrophil infiltration and increases hepatocytes damage, as shown in the present study by elevation of the ALT, AST, and LDH activities.
in serum. Large amounts of TNF-α are released in response to lipopolysaccharide, other bacterial products, and interleukin-1 (Walsh et al., 1991). Inflammation is an important feature of cholestatic liver disease and leads to fibrogenic changes in this organ, such as HSCs activation. Cytokines released from activated Kupffer cells stimulate proliferation and activation of HSCs (Friedman, 1999). We have shown here that AGE exerts a potent anti-inflammatory effect as it reduced both the TNF-α gene expression and enalapril reduced only the TNF-α gene expression without affecting the MPO activity. Previous studies showed a similar effect of AGE in the 3-galactosamine-induced liver damage model (El-Beshbishy, 2008).

Previous studies suggested that oxidative stress occurs during cholestasis and likely plays a role in cholestasis-induced liver injury (Sokol et al., 1998). It has been demonstrated that oxygen-derived free radicals and lipid peroxidation play a critical role in the pathogenesis of various liver diseases including hepatic fibrosis (Das et al., 2005). GSH and MDA levels are indicators of the oxidative stress state of the liver. GSH is a key intracellular antioxidant that is capable of interacting with free radicals to repair membrane lipid peroxides. MDA is the end product of the peroxidation of polyunsaturated fatty acids. Therefore, MDA is used as an indicator of lipid peroxidation (Huang et al., 2009). ROS derived from neutrophils stimulate collagen synthesis in HSCs (Casini et al., 1997). AGE produced a significant decrease in the liver content of MDA and a significant increase in the GSH level compared with the BDL group. The antioxidant properties of AGE in the present study may be attributed to water-soluble organosulfur compounds (Sener et al., 2007) and the inhibition of ACE activity. In accordance with Karimian et al. (2008), enalapril was found to attenuate liver damage in BDL rats due to its indirect antioxidant effect. In this study, it produced a significant decrease in the liver content of MDA and a significant increase in the GSH level compared with bile-duct-ligated control rats. It was reported previously that cholestasis-induced fibrosis is associated with increased liver TGF-β1 expression (Kim et al., 2013). The present study also showed an increase in TGF-β1 transcripts in BDL rats. TGF-β1, derived from both paracrine and autocrine sources, is the most potent fibrogenic cytokine in the liver (Friedman, 1999).

Once activated, TGF-β1 activates quiescent HSCs that differentiate into myofibroblasts and produce collagen (Lee and Friedman, 2011). We found that AGE and enalapril suppressed the TGF-β1 gene expression, thus reducing the fibrotic process associated with cholestasis. The reduction of TGF-β1 gene expression may be due to the inhibition of ACE and the reduction of angiotensin II levels. The ACE constituent S-allylmercaptocysteine may be an active component in this process, as this compound was recently found not only to attenuate the gene expression level of TGF-β1, but also to reduce collagen accumulation around the centrilobular veins (Xiao et al., 2013).

Matrix metalloproteinases are enzymes produced by activated HSCs that are capable of degrading collagen. They play an important role in resolving liver fibrosis (Siller-Lopez et al., 2004). One of them is MMP-13, which is the interstitial collagenase of rodents. It is a highly specific protease capable of degrading collagen, especially type I, thus MMP-13 could play an important role in liver fibrogenesis. We found an increased expression of the MMP-13 gene in bile-duct-ligated rats which was suppressed by both AGE and enalapril. Previous studies showed that MMP-13 levels are up-regulated in BDL rats which confirms the current finding. MMP-13 may activate MMP-2, which in turn is able to activate chemokines (Parks et al., 2004) and increase leukocyte infiltration and inflammation. This is confirmed in the present study by the increased MPO enzyme activity along with an increased expression of MMP-13. It has been observed by others that acute liver injury and inflammation after BDL are reduced in mice deficient in MMP-13 suggesting that interstitial collagenase is required to induce fibrosis and inflammation during the initial injury phase in BDL (Uchinami et al., 2006). Thus the reduction of MMP-13 expression by garlic may be responsible in part for the anti-inflammatory and antifibrotic effects.

Conclusion

Our findings suggest that AGE possesses reno- and hepatoprotective effects, similar to enalapril, probably by modulating the levels of proteins such as TNF-α, TGF-β1, and MMP-13, in a mechanism dependent on the reduction of ACE activity and oxidative stress. This suggests that AGE may be used as adjuvant therapy in cholestasis patients.

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