Heme oxygenase-1 prevents liver fibrosis in rats by regulating the expression of PPARγ and NF-κB

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Abstract

AIM: To investigate the effects of heme oxygenase (HO)-1 on liver fibrosis and the expression of peroxisome proliferator-activated receptor gamma (PPARγ) and nuclear factor-kappa B (NF-κB) in rats.

METHODS: Sixty Wistar rats were used to construct liver fibrosis models and were randomly divided into 5 groups: group A (normal, untreated), group B (model for 4 wk, untreated), group C (model for 6 wk, untreated), group D (model for 6 wk, treated with zinc protoporphyrin IX (ZnPP-IX) from week 4 to week 6), group E (model for 6 wk, treated with hemin from week 4 to week 6). Next, liver injury was assessed by measuring serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and albumin levels. The degree of hepatic fibrosis was evaluated by measuring serum hyaluronate acid (HA), type IV collagen (IV-C) and by histological examination. Hydroxyproline (Hyp) content in the liver homogenate was determined. The expression levels of alpha-smooth muscle actin (α-SMA) in liver tissue were measured by real-time quantitative polymerase chain reaction (RT-PCR). The expression levels of PPARγ and NF-κB were determined by RT-PCR and Western blotting.

RESULTS: The expression of HO-1 increased with the development of fibrosis. Induction of HO-1 by hemin significantly attenuated the severity of liver injury and the levels of liver fibrosis as compared with inhibition of HO-1 by ZnPP-IX. The concentrations of serum ALT, AST, HA and IV-C in group E decreased compared with group C and group D (P < 0.01). Amount of Hyp and α-SMA in the liver tissues in group E decreased compared with group C (0.62 ± 0.14 vs 0.84 ± 0.07, 1.42 ± 0.17 vs 1.84 ± 0.17, respectively, P < 0.01) and group D (0.62 ± 0.14 vs 1.11 ± 0.16, 1.42 ± 0.17 vs 2.56 ± 0.37, respectively, P < 0.01). The expression of PPARγ at levels of transcription and translation decreased with the development of fibrosis especially in group D; and it increased in group E compared with groups C and D (0.88 ± 0.15 vs 0.56 ± 0.19, 0.88 ± 0.15 vs 0.41 ± 0.11, respectively, P < 0.01). The expression of NF-κB increased with the development of fibrosis especially in group D; and it decreased in group E compared with groups C and D (1.43 ± 0.31 vs 1.89 ± 0.29, 1.43 ± 0.31 vs 2.53 ± 0.54, respectively, P < 0.01).

CONCLUSION: Our data demonstrate a potential mechanism that HO-1 can prevent liver fibrosis by enhancing the expression of PPARγ and decreasing the expression of NF-κB in liver tissues.
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INTRODUCTION

Liver fibrosis is the mechanism of compensation and reparation after chronic hepatic injury, which is a necessary pathological stage for the development of chronic hepatitis. Heme oxygenase-1 (HO-1), also known as heat shock protein 32, is a microsomal enzyme and rate-limiting enzyme that catalyzes the degradation of heme into biliverdin, iron atoms and carbon monoxide (CO)\(^\text{[1]}\). HO-1 and its breakdown products play vital physiological roles in anti-inflammation, anti-oxidation and regulation of apoptosis according to reports\(^\text{[2,3]}\). Many researchers have recently confirmed that HO-1 has protective effects on liver cells under such conditions as acute liver injury, alcoholic liver disease, liver transplantation and ischemia/reperfusion injury\(^\text{[4,5]}\). In chronic liver disease, induction of HO-1 is important to prevent the development of liver fibrosis\(^\text{[6]}\). However, the underlying molecular mechanisms are still unknown.

Peroxisome proliferator-activated receptor (PPAR) is a ligand-activated transcription factor which is widely distributed in tissues\(^\text{[10]}\). Three PPAR subtypes have been identified, namely α, β and γ. PPARγ is mainly expressed in hepatic stellate cells (HSC). Studies have shown that the expression of PPARγ benefits the maintenance of HSC static phenotype\(^\text{[11]}\). Up-regulation of PPARγ resulted in a significant reduction of HSC activation, and reversed the development of liver fibrosis\(^\text{[1]}\). However, the underlying molecular mechanisms are still unknown.

Nuclear factor-kappa B (NF-κB) is an important nuclear transcription factor, which plays an important role in the regulation of gene transcription such as cytokines, chemokines, adhesion molecules and other inflammatory mediators\(^\text{[12]}\). Up-regulating the activation of NF-κB promotes HSC proliferation and decreases HSC apoptosis. Therefore, inhibiting the activation of NF-κB resulted in a reduction of HSC activation, promoting HSC apoptosis and decreasing extracellular matrix production\(^\text{[13,14]}\).

In the present study, we have evaluated the role of HO-1 in liver fibrosis caused by carbon tetrachloride (CCL\(_4\)) in rat models, then observed the expression of PPARγ and NF-κB in liver after up-regulation of HO-1 by ferritroporphyrin IX chloride (hemin) or inhibition of HO-1 by zinc protoporphyrin IX (ZnPP-IX) pretreatment, and we finally hypothesize about a potential mechanism for the cytoprotection by HO-1.

MATERIALS AND METHODS

Reagents
ZnPP-IX is a selective HO inhibitor which can suppress the activity of HO-1 by blocking CO production and restricting the transformation of heme to biliverdin\(^\text{[23]}\). Hemin is a well-known physiological substrate and potent inducer of HO activity\(^\text{[14]}\). Both ZnPP-IX and hemin were purchased from Sigma Chemical Co. (St. Louis, MO, United States). Polyclonal antibodies HO-1 and PPARγ were purchased from Cell Signaling (Danver, MA, United States). Anti-phospho-NF-κB p65 monoclonal antibody was purchased from Santa Cruz (Santa Cruz, CA, United States). Anti-betactin antibody was purchased from Biogenesis (Bournemouth, United Kingdom). All other chemicals were of analytical grade and commercially available.

Animals and experimental design
Male Wistar rats (Medical University Laboratory Animal Center, Shanxi, China) weighing 220 g-250 g were used to establish fibrogenesis models\(^\text{[17-19]}\). All procedures used in this study were approved by the Ethics Committee for the use of experimental animals at Shanxi Medical University. All rats were kept at 21 °C-25 °C under a 12 h dark/light cycle, drank normal water and were fed with 79.5% corn meal, 20% lard and 0.5% cholesterol for the first two weeks, then 99.5% corn meal and 0.5% cholesterol thereafter. Sixty rats were randomly divided into five groups (twelve rats/group); groups B, C, D and E received subcutaneous injections of 40% CCL\(_4\) (a mixture of pure CCl\(_4\) and olive oil, 0.3 mL/100 g) every four days for six weeks. The rats in group A were fed with normal diet and received a 0.9% NaCl subcutaneous injection. In the fourth week, Group B and some of group A were killed. While group D and group E began to be peritoneally injected with ZnPP-IX (20 μmol/kg) or hemin (30 μmol/kg) every other day until the sixth week when they were killed with group C and the remnants of group A. The dose and preparation of ZnPP and hemin solution were based on our preliminary studies and references\(^\text{[20,21]}\). The numbers of rats were reduced to 11, 9, 9 and 10 in groups B, C, D, and E, respectively, due to deaths during the process. A small portion of the liver was removed for histological analysis by fixation with 10% formalin and subsequent embedding in paraffin. The remaining liver was cut into pieces and frozen in liquid nitrogen and kept at -80 °C until it was used for extraction of total RNA and proteins.

Serum biochemical and liver fibrosis indicator measurements
Markers of hepatic damage such as serum alanine aminotransferase (ALT), aspartate transaminase (AST) and albumin (ALB) levels were measured by using an automated biochemistry clinical analyzer (Hitachi, Japan) according to an automated procedure. The levels of serum hyaluronic acid (HA) and type IV collagen (IV-C) were determined using Chemiluminescence Quantitative Immunoassay Kit (Beijing Yuande Bio-Medical Engineering Co., Ltd.).

Quantification of hydroxyproline assay
Hydroxyproline (Hyp) content in the liver specimens represented the total amount of collagen in livers, which...
was quantified to evaluate the degree of liver fibrosis by using a colorimetric method[23]. Kits for the measurement were purchased from Nanjing Jiancheng Biotechnology. In brief, 100 mg of freeze-dried liver specimens were weighed and tested according to the manufacturer’s directions. At the end of the experiment, absorbance of each sample was read at 550 nm using a spectrophotometer. The content was obtained according to a formula and expressed as micrograms Hyp/milligram liver. Each sample was analyzed in triplicate.

**Histologic evaluation**

Liver tissues were fixed in 10% neutral formalin solution overnight, embedded in paraffin blocks, and then were sectioned at 4 μm thickness for staining with hematoxylin and cosin (HE) or Masson by using standard techniques. The results were analyzed by light microscopy. Representative views of liver sections are shown.

**RNA isolation and real-time polymerase chain reaction**

Total RNA was extracted from liver tissue using the RNA Trizol isolation reagent kit (Invitrogen, United States). cDNA was obtained by reverse transcription of RNA by using random primer and Moloney murine leukemia virus reverse transcriptase (Gibco BRL, Merelbeke, Belgium). The conditions were 25 ℃ for 5 min, 42 ℃ for 60 min and 70 ℃ for 5 min, finally cooling at 5 ℃ for use. Amplification reactions were performed with a SYBR green polymerase chain reaction (PCR) master mix (Applied Biosystems). Three microliters of diluted cDNA samples were used for quantitative analysis. The cycle conditions were 95 ℃ for 10 min for denaturation, followed by 50 cycles of 15 s at 95 ℃, 60 s at 55 ℃ and 70 ℃ for 15 s, finally cooling at 4 ℃ for 1 min. Amplification analysis were performed with the SYBR green polymerase chain reaction (PCR) master mix (Applied Biosystems). Three microliters of diluted cDNA samples were used for quantitative analysis. The cycle conditions were 95 ℃ for 10 min for denaturation, followed by 50 cycles of 15 s at 95 ℃, 60 s at 55 ℃ and 70 ℃ for 15 s, finally cooling at 4 ℃ for 1 min. Amplification analysis were performed with the SYBR green polymerase chain reaction (PCR) master mix (Applied Biosystems). Three microliters of diluted cDNA samples were used for quantitative analysis. The cycle conditions were 95 ℃ for 10 min for denaturation, followed by 50 cycles of 15 s at 95 ℃, 60 s at 55 ℃ and 70 ℃ for 15 s, finally cooling at 4 ℃ for 1 min. Amplification analysis were performed with the SYBR green polymerase chain reaction (PCR) master mix (Applied Biosystems). Three microliters of diluted cDNA samples were used for quantitative analysis. The cycle conditions were 95 ℃ for 10 min for denaturation, followed by 50 cycles of 15 s at 95 ℃, 60 s at 55 ℃ and 70 ℃ for 15 s, finally cooling at 4 ℃ for 1 min. Amplification analysis were performed with the SYBR green polymerase chain reaction (PCR) master mix (Applied Biosystems). Three microliters of diluted cDNA samples were used for quantitative analysis. The cycle conditions were 95 ℃ for 10 min for denaturation, followed by 50 cycles of 15 s at 95 ℃, 60 s at 55 ℃ and 70 ℃ for 15 s, finally cooling at 4 ℃ for 1 min. Amplification analysis were performed with the SYBR green polymerase chain reaction (PCR) master mix (Applied Biosystems). Three microliters of diluted cDNA samples were used for quantitative analysis. The cycle conditions were 95 ℃ for 10 min for denaturation, followed by 50 cycles of 15 s at 95 ℃, 60 s at 55 ℃ and 70 ℃ for 15 s, finally cooling at 4 ℃ for 1 min. Amplification analysis were performed with the SYBR green polymerase chain reaction (PCR) master mix (Applied Biosystems). Three microliters of diluted cDNA samples were used for quantitative analysis. The cycle conditions were 95 ℃ for 10 min for denaturation, followed by 50 cycles of 15 s at 95 ℃, 60 s at 55 ℃ and 70 ℃ for 15 s, finally cooling at 4 ℃ for 1 min. Amplification analysis were performed with the SYBR green polymerase chain reaction (PCR) master mix (Applied Biosystems). Three microliters of diluted cDNA samples were used for quantitative analysis. The cycle conditions were 95 ℃ for 10 min for denaturation, followed by 50 cycles of 15 s at 95 ℃, 60 s at 55 ℃ and 70 ℃ for 15 s, finally cooling at 4 ℃ for 1 min. Amplification analysis were performed with the SYBR green polymerase chain reaction (PCR) master mix (Applied Biosystems). Three microliters of diluted cDNA samples were used for quantitative analysis. The cycle conditions were 95 ℃ for 10 min for denaturation, followed by 50 cycles of 15 s at 95 ℃, 60 s at 55 ℃ and 70 ℃ for 15 s, finally cooling at 4 ℘ 0.05.

**RESULTS**

**Expression of HO-1 in the liver of rats in different groups**

Long-term application of CCl₄ can induce hepatic fibrogenesis not only in humans but also in rats. We established CCl₄ rat models to evaluate the effect of HO-1 expression on liver fibrogenesis (Figure 1). The process lasted for 6 wk. In this study, we used two opposite regents, i.e., hemin (induction of HO-1) and ZnPP-IX (inhibition of HO-1) from week 4 to week 6 to observe the regulation and mechanism of HO-1 in rat liver fibrosis. The mRNA and protein expressions of HO-1 in groups B and C were significantly higher than group A and increased with the severity of fibrosis, but all values were lower than in group E (P < 0.01); while those in group D were lower than in group C (P < 0.01), but still higher than in group A (P < 0.01).

**Effects of HO-1 expression on rat model of hepatic fibrogenesis**

The rat model for group B exhibited inflammatory infiltration, hepatic steatosis and slight fibrosis (Figure 2B and G), while group C showed obvious fibrosis (Figure 2C and H). Treatment with hemin from week 4 to week 6 markedly reduced the severity of hepatic inflammatory infiltration and fibrosis (Figure 2E and J), whereas hepatic steatosis, inflammatory infiltration, especially fibrosis in hepatic portal areas, varying degrees of fibrosis around the central vein and extension to the hepatic lobule were aggravated in groups treated with ZnPP-IX (Figure 2D and I). These results indicate that HO-1 induction could protect rats from CCl₄-induced liver injury and fibrosis.

**Effects of HO-1 on the levels of serum ALT, AST and ALB**

The levels of serum ALT and AST increased with the development of fibrosis, and were higher in group C than in group B (Table 1). The increase in serum ALT and AST was markedly augmented by ZnPP-IX in group D (P < 0.01), but attenuated by hemin in group E com-
pared to group C (P < 0.01). The levels of ALB in group E increased significantly compared to group C (P < 0.01). Meanwhile, ALB levels decreased in group D but did not differ significantly from group C.

Effects of HO-1 on the expression of fibrosis-related indicators
To evaluate the effect of HO-1 induction on fibrosis, we assessed the expression levels of hepatic fibrosis-related indicators, i.e., HA, IV-C, Hyp and α-SMA. Rats in group D injected with ZnPP-IX showed enhanced expression of hepatic α-SMA and Hyp, which correlated with the levels of serum HA and IV-C. Meanwhile mice in group E injected with hemin exhibited depressed expression of these compared with group C (P < 0.01), which still did not recover to the levels of group B (P > 0.05) (Figure 3).

Role of HO-1 in the expression of PPARγ and NF-κB at mRNA and protein levels
Studies have shown that up-regulating the activation of PPARγ resulted in a significant reduction of type I collagen and α-SMA expression, inhibited HSC proliferation and even reversed the development of liver fibrosis[24-26]. Studies also have found that up-regulating the activation of NF-κB inhibited HSC apoptosis and promoted the release of inflammatory response factors[18,19]. To evaluate the mechanism of the effect of HO-1 on fibrosis, we explored the expression of PPARγ and NF-κB at levels of transcription and translation. Unlike the trends of HO-1, real-time PCR and Western blotting showed that the expression of PPARγ decreased with the development of liver fibrosis (Figure 4A). The expression of PPARγ decreased more obviously after application of HO-1 inhibitor in group D as compared with group C (P < 0.05). On the contrary, PPARγ increased significantly after pretreatment with hemin in group E, and was higher than group C (P < 0.01). However, the expression of NF-κB gradually increased with the development of liver fibrosis, which was consistent with the change of HO-1 (Figure 4B). After using the inhibitor of HO-1 in group D, the expression of NF-κB increased as compared with group C, whereas expression decreased more significantly than group D and even group C when HO-1 was induced in group E (P < 0.01).

**DISCUSSION**
Liver fibrosis is the mechanism of compensation and reparation after chronic hepatic injury, which is a necessary pathologic stage from chronic hepatitis to cirrhosis. Previous studies have found that 25%-40% of liver fibrosis will eventually develop to cirrhosis and even liver cancer[27,28]. Therefore, it is essential to further clarify the mechanism of liver fibrosis in order to block and reverse the process of liver disease. We constructed liver fibrosis models in rats by using composite factors which had been confirmed successfully in the Department of Pathophysiology, Shanxi Medical University[18,19]. At the fourth week we observed inflammatory infiltration, hepatic steatosis and slight fibrosis in livers, and obvious liver cirrhosis could be seen at the sixth week.

HO-1 is the rate-limiting enzyme for heme degradation in a wide range of human and mammalian tissues. Prior clinical and animal research has confirmed that an external irritant could up-regulate the expression of HO-1 with increasing levels of oxygen-derived free radicals in cells[29]. It has previously been reported that induction of HO-1 is an important defense mechanism against many kinds of liver injuries. In chronic liver disease, especially liver fibrosis, induction of HO-1 can reduce the secretion of type I collagen, thus effectively preventing the development of liver fibrosis[24-26]. In this study, we also examined the effects of HO-1 inhibitor or inducer, and found that with the development of liver fibrosis, the expression of HO-1 was significantly
enhanced in liver of rats, whereas hemin pretreatment made this induction more prominent. We analyzed the biochemical parameters reflecting liver damage related to function and structure, such as serum ALT and AST levels, which indicated a remarkable decrease after HO-1 induction. Liver histopathology also clearly showed that HO-1 induction markedly reduced the severity of hepatic inflammatory infiltration and fibrosis. To further validate the protection by HO-1, we pretreated rats with concomitant ZnPP-IX (a competitive HO-1 inhibitor) and observed that the liver damage was more serious than in the control group and hemin-treated group. Then we assessed the levels of HA and IV-C in serum and detected the content of Hyp and α-SMA mRNA in rat liver tissues of the different groups, in order to determine the proliferation levels of fibrosis. Results showed that the induction of HO-1 could reduce all the biochemical indicators of fibrosis and attenuate the degree of fibrosis detected pathologically, while the inhibitor of HO-1 caused an opposite result. Taken together, we concluded that induction of HO-1 in hepatic tissues could produce anti-inflammatory effects and slow the process of liver fibrosis effectively; however the inhibition of HO-1 could enhance the liver fibrosis.

PPAR, including α, β and γ subtypes, are new steroid hormone receptors and ligand-activated transcription
NF-κB plays an important role in many biological processes, including adipogenesis, inflammatory reaction, cell growth regulation and cell differentiation. The expression of PPARγ is beneficial in maintaining the quiescent phenotype of HSC; however the inhibition of PPARγ may be an early event in HSC transformation from quiescent to activated states. Studies found that the PPARγ agonist rosiglitazone could be used to increase the expression of PPARγ in activated HSC, which could reduce oxidative stress, decrease the expression of α-SMA and the synthesis of type I collagen, inhibit cell proliferation and promote cell apoptosis. Recent studies also found that PPARγ activation reduced TGF-β1-induced CTGF expression at both transcriptional and posttranscriptional levels in HSCs. Enhancement of PPARγ activity might interrupt the signaling pathways for platelet-derived growth factor and epidermal growth factor, and then suppress hepatic fibrogenesis.

NF-κB is a nuclear transcriptional activator that plays a central role in stress response and inflammation. Activation of NF-κB can promote HSC proliferation, reduce HSC apoptosis and increase the production of collagen and inflammatory chemokines in the process of liver fibrosis. But inhibiting the activation of NF-κB can induce apoptosis of HSC. Studies have found that a decrease of PPARγ was accompanied with an increase of NF-κB in lung tissues, which played an important role in the development of lung fibrosis. PPARγ can inhibit the transcription and DNA synthesis of NF-κB by binding p50/p65 subunits to form transcriptional repressor complexes directly or by binding p300 and CBP co-activating factors to inhibit the transcription and expression of NF-κB competitively. Other research showed that a specific inducer of PPARγ such as troglitazone could interfere with NF-κB signaling pathway by activating PPARγ.

Studies in other areas have shown that co-regulation exists between HO-1 and PPARγ. Research regarding the interaction of HO-1 and PPARγ in human vascular endothelial cells demonstrated that HO-1 enzymatic activity mediated anti-inflammatory and anti-proliferative effects exerted by PPAR ligands, and that a clinically relevant (GT)n dinucleotide length polymorphism within the human HO-1 promoter significantly influenced the transcriptional regulation of HO-1 by both PPARγ and PPARα. Li et al. reported that induction of HO-1 could mediate the effect of PPARγ in suppressing the proliferation of rat pulmonary artery smooth muscle cells, but that this effect was blocked by knockdown of HO-1 through siRNA transfection. Recent studies demonstrated that HO-1, as an identifier of novel trophoblast invasion-related genes, controlled motility via up-regulation of PPARγ. Researchers found that up-regulation of PPARγ protein and activity by HO-1 was required to down-regulate cell motility, but blocking of PPARγ largely abolished the effect of HO-1. Studies also found that there was an NF-κB binding site in the HO-1 promoter region. The activity of HO-1 was directly related to NF-κB. HO-1 played an important role in suppressing HSC apoptosis and increase the production of collagen, inhibit cell proliferation, and promote cell apoptosis. Studies have found that a decrease of PPARγ was accompanied with an increase of NF-κB in lung tissues, which played an important role in the development of lung fibrosis.
role in the down-regulation of NF-κB activation. Yeh et al. showed that HO-1 activation could attenuate the surge of inflammation-related cytokines and decrease the occurrence of cardiomyocytic apoptosis via inhibition of NF-κB and AP-1 translocation. Liu et al. indicated that up-regulation of HO-1 could alleviate severe acute pancreatitis-associated lung injury in rats by decreasing NF-κB activity drastically and inhibiting the serum levels of tumour necrosis factor alpha (TNF-α) and interleukin-6 significantly. Overexpression of HO-1 could protect against TNF-α-mediated airway inflammation by diminishing NF-κB activation in both cultured human tracheal smooth muscle cells and the airways of mice.

In this study, we found that the expression of PPARγ was decreased in group D, which was treated with ZnPP-Ⅸ to inhibit the expression of HO-1, whereas the expression of NF-κB was increased. But PPARγ was overexpressed in group E by treating with hemin to induce the expression of HO-1, whereas the expression of NF-κB was reduced. By examining the HE stained liver histology, we found that the degree of liver fibrosis was significantly higher in group D than in groups C and E. Masson staining for collagen showed the same results: that the collagen content in group D was significantly increased compared to group C, but was markedly reduced in group E compared with groups D and C. Thus we hypothesize that induction of HO-1 could alleviate the liver injury and reverse the process of liver fibrosis by up-regulating the expression of PPARγ and down-regulating the expression of NF-κB, and then affect the releasing of inflammatory cytokines such as TNF-α in NF-κB-related signal pathways or induce HSC apoptosis. Among these, the inhibition of NF-κB may be regulated directly by HO-1 on the one hand, or on the other hand be regulated by the expression levels of PPARγ which are associated with the expression of HO-1 (Figure 5). In conclusion, our data...
demonstrate that the ability of HO-1 to alleviate liver fibrosis is correlated with the regulation of PPARγ and NF-κB, which construct a complex network system. Further study with regard to this mechanism will help us to form new strategies for the effective treatment of liver fibrosis.

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COMMENTS

Background
Heme oxygenase-1 (HO-1) is a microsomal enzyme and rate-limiting enzyme. HO-1 and degradation products are important defense mechanisms against many kinds of liver injuries. In chronic liver disease, induction of HO-1 is important to prevent the development of liver fibrosis effectively. However, the underlying molecular mechanisms are still unknown.

Research frontiers
Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-activated transcription factor which benefits the maintenance of the hepatic stellate cell (HSC) static phenotype. Nuclear factor-kappa B (NF-κB) plays an important role in the regulation of gene transcription which can promote HSC proliferation and decrease HSC apoptosis, then aggravate liver fibrosis. Studies have shown that co-regulation exists between HO-1 and PPARγ. HO-1 can mediate the effect of PPARγ. Studies have also found that there is an NF-κB binding site in the HO-1 promoter region. HO-1 plays an important role in the down-regulation of NF-κB activation.

Innovations and breakthroughs
In this study, by establishing a model of liver fibrosis in rats, the authors attempted to investigate the effects of HO-1 on liver fibrosis, and to evaluate whether the role of HO-1 in liver protection was achieved by regulating the expression of PPARγ and NF-κB, which are both important in the process of liver fibrosis.

Applications
This study further clarified one of the mechanisms of HO-1 in liver protection, which could help provide a new strategy for treating liver fibrosis.

Terminology
HO-1, also known as heat shock protein 32, is a microsomal enzyme and rate-limiting enzyme that catalyzes heme degradation into biliverdin, iron atoms and carbon monoxide. HO-1 and its breakdown products play vital physiological roles in anti-inflammation, anti-oxidation and regulation of apoptosis according to reports. PPAR is a ligand-activated transcription factor which is widely distributed in the tissues. Three PPAR subtypes have been identified, namely α, β and γ. NF-κB is an important nuclear transcription factor, which plays an important role in the regulation of gene transcription such as cytokines, chemokines, adhesion molecules and other inflammatory mediators. Zinc protoporphyrin IX is a selective HO inhibitor which can suppress the activity of HO-1 by blocking carbon monoxide production and restricting the transformation of heme to biliverdin. Hemin is a well-known physiological substrate and potent inducer of HO activity.

Peer review
The authors explored the protective effect of HO-1 in the CCl4 rat model of liver fibrosis. In order to determine the mechanism of such protection, the authors evaluated the expression of two important transcription factors, PPARγ and NF-κB. These transcription factors are involved in regulation of hepatic stellate cell activation, the primary cell responsible for liver fibrosis. This study proposes a pathway for the protective action of HO-1 in liver fibrosis. If reproduced by other investigators, this pathway could provide information that helps in designing new therapies for prevention and treatment of liver fibrosis.

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