• BASIC RESEARCH •

Effects of estradiol on liver estrogen receptor-α and its mRNA expression in hepatic fibrosis in rats

Jun-Wang Xu, Jun Gong, Xin-Ming Chang, Jin-Yan Luo, Lei Dong, Ai Jia, Gui-Ping Xu

Jun-Wang Xu, Xin-Ming Chang, Ai Jia, Gui-Ping Xu, Department of Gastroenterology, First Hospital of Xi' an Jiaotong University, Xi' an 710061, Shaanxi Province, China
Jun Gong, Jin-Yan Luo, Lei Dong, Department of Gastroenterology, Second Hospital of Xi' an Jiaotong University, Xi' an 710031, Shaanxi Province, China
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Correspondence to: Dr. Jun-Wang Xu, Department of Gastroenterology, First Hospital of Xi' an Jiaotong University, Xi' an 710061, Shaanxi Province, China. xujw@pub.xaonline.com
Telephone: +86-29-85323507   **Fax:** +86-29-85263190
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Abstract

AIM: Estradiol treatment regulates estrogen receptor (ER) level in normal rat liver. However, little information is available concerning the role of estrogen in regulating liver ER in hepatic fibrosis in rats. The present study was conducted to determine whether estradiol treatment in CCl_4-induced liver fibrosis of female and ovariectomized rats altered liver ERα and its mRNA expression, and to investigate the possible mechanisms.

METHODS: Seventy female rats were divided into seven groups with ten rats in each. The ovariectomy groups were initiated with ovariectomies and the sham operation groups were initiated with just sham operations. The CCl_4 toxic fibrosis groups received 400 mL/L CCl_4 subcutaneously at a dose of 2 mL/kg twice weekly. Estrogen groups were treated subcutaneously with estradiol 1 mg/kg, the normal control group and an ovariectomy group received injection of peanut oil vehicle twice weekly. At the end of 8 weeks, all the rats were killed to detect their serum and hepatic indicators, their hepatic collagen content, and liver ER and ER mRNA expression.

RESULTS: Estradiol treatment in both ovariectomy and sham ovariectomy groups reduced liver levels of ALT (from 658±220 nkat/L to 311±146 nkat/L and 540±252 nkat/L to 314±163 nkat/L, $P<0.05$) and AST (from 697±240 nkat/L to 321±121 nkat/L and 631±268 nkat/L to 302±153 nkat/L, $P<0.05$), increased serum nitric oxide (NO) level (from 53.7±17.1 μmol/L to 93.3±24.2 μmol/L and 55.3±23.1 μmol/L to 87.5±23.6 μmol/L, $P<0.05$) and hepatic nitric oxide synthase (NOS) activity (from 1.73±0.71 KU/g to 2.49±1.20 KU/g and 1.65±0.46 KU/g to 2.68±1.17 KU/g, $P<0.05$), diminished the accumulation of hepatic collagen, decreased centrolobular necrotic areas as well as the inflammatory reaction in rats subjected to CCl_4. The positive signal of ER and ER mRNA distributed in parenchymal and non-parenchymal hepatic cells, especially near the hepatic centrolobular and periportal areas. Ovariectomy decreased ER level (from 10.2±3.2 to 4.3±1.3) and ER mRNA expression (from 12.8±2.1 to 10.9±1.3) significantly ($P<0.05$). Hepatic ER and ER mRNA concentrations were elevated after treatment with estradiol in both ovariectomy (15.8±2.4, 20.8±3.1) and sham ovariectomy (18.7±3.8, 23.1±3.7) fibrotic groups ($P<0.05$).

CONCLUSION: The increase in hepatic ER and mRNA expression may be part of the molecular mechanisms underlying the suppressive effect of estradiol on liver fibrosis induced by CCl_4 administration.

INTRODUCTION

The progression of various forms of chronic liver diseases is more rapid in men than in women. This has been specifically noted in hepatic cirrhosis and hepatocellular carcinoma[1-3]. The specific mechanisms underlying these clinical findings are unclear. Hepatic fibrosis is a consequence of severe liver damage, which occurs in many chronic liver diseases as a forerunner to cirrhosis. We recently found[4] that estradiol treatment inhibited the proliferation of hepatic stellate cells, suppressed hepatic collagen content and reduced hepatic type I collagen in fibrotic rats induced by CCl_4 administration, and thus improved liver fibrosis. The mechanisms of the antifibrogenic effect of estradiol have been hypothesized by an indirect way-a hepatocellular membrane protection and a radical scavenging action.

Chronic fibrotic diseases can differ from each other in etiology. But, in terms of pathogenesis, they share some basic common features[5]. For instance, three serious chronic diseases-atherosclerosis, glomerulosclerosis, and liver fibrosis-have many properties in common. Therefore, factors that affect the development of atherosclerosis or glomerulosclerosis may affect liver fibrosis by similar mechanisms. Studies showed[6] that estradiol could suppress atherosclerosis and glomerulosclerosis in rats by directly affecting the estrogen receptor (ER) on smooth muscle cells and mesangial cells. The liver is not considered as a kind of classic estrogen-dependent tissue, as are the breast and uterus, but livers in both male and female rats have shown to contain high affinity, low capacity of ER and respond to estrogen by regulating liver function[7]. Previous data have shown[4] that tamoxifen, an antiestrogen agent, acts by occupying the estrogen-binding site of the receptor protein, increases fibrogenesis in CCl_4-induced fibrosis of the liver. It was proposed that estrogen might suppress hepatic fibrosis also by a receptor mechanism. In the liver, ERα is the dominant form of ER, while the other subtype, ERβ, has not yet been demonstrated[8]. Hence, only ERα would be further considered in this paper.

Previously published data indicated that 17 β-estradiol treatment could regulate the levels of ER in normal rat liver[9]. However, little information is available concerning the role of estrogen in the regulation of liver ER in fibrotic rats. The present study was conducted to determine whether estradiol treatment in CCl_4-induced liver fibrosis of female and

ovariectomy rats altered the liver ER and its mRNA expression, and to investigate the possible mechanisms.

MATERIALS AND METHODS

Animals

Seventy female Sprague-Dawley rats (obtained from the Experimental Animal Holding Unit of Shaanxi Province, China), weighing 209±19 g, with an average age of approximately 10 weeks, were housed in a temperature-humidity-controlled environment with 12 h light-dark cycles (lights on from 07:00 AM to 19:00 PM) and had free access to food and water. They were randomly divided into seven groups with ten rats in each. Ovariectomy groups were initiated with a bilateral ovariectomy and sham operation groups were initiated with just a sham operation. For two CCl_4 toxic fibrosis groups, with bilateral ovariectomy (CCl_4+Ovx) and sham operation (CCl_4), 400 mL/L CCl_4 in peanut oil was injected subcutaneously at a dose of 2 mL/kg twice weekly, and the first dosage was doubled. The two estrogen groups, with bilateral ovariectomy (CCl_4+Ovx+E) and sham operation (CCl_4+E), were treated subcutaneously with estradiol (benzoic estradiol) 1 mg/kg twice weekly (The Ninth Pharmaceutical Factory of Shanghai, China). All of the above four groups were fed with a modified high fat diet containing 5 g/kg cholesterol and 200 g/kg pig oil. CCl_4 and estradiol were used 2 weeks after operation. A control ovariectomy treated group (Ovx+E) was just given estradiol 1 mg/kg twice weekly. The normal control group (Control) and an ovariectomy group (Ovx) were given normal food and water, and received injection of peanut oil vehicle twice weekly. At the end of a 8-week experimental period, all the rats were fasted overnight and sacrificed by cervical dislocation after anaesthetised by intramuscular injection of sodium pentobarbital (40 mg/kg). Blood was collected from the animals and serum was analysed. The livers were removed immediately.

Estimation of serum indicators and hepatic nitric oxide synthase

Activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by a 917-Hitachi automatic analyzer. Serum nitric oxide (NO) and hepatic nitric oxide synthase (NOS) were measured following the instructions of the reagent kit (purchased from Jiancheng Medical Institute, Nanjing, China).

Histopathological study

Tiver tissues excised from each rat were fixed in 100 mL/L neutral formalin, embedded in paraffin, and stained with hematoxylin-eosin (HE) and Masson's trichrome. Evaluation of hepatic fibrosis was determined by a semi-quantitative method to assess the degree of histologic injury, applying the following scores[10,11]: 0, absence of fibrosis; 1, perivenular and/or pericellular fibrosis; 2, septal fibrosis; 3, bridging fibrosis (incomplete cirrhosis); 4, complete cirrhosis.

Immunohistochemical examination for estrogen receptor-*a*

Liver tissue sections were mounted on slides, deparaffinized in xylene, and rehydrated in alcohol. Estrogen receptor-α(ERα) was assessed and semi-quantitated by immunohistochemistry using a commercial antibody against ERα (Boster, Wuhan, China) followed by DAB detection. For each sample, ten random fields were evaluated for positively stained cells. The results were expressed as the percentage of positive cells to the total number of cells counted.

In situ hybridization for estrogen receptor mRNA of liver tissue

In situ hybridization kit was purchased from Boster Biological

Technology Limited Company (Wuhan, China, No. MK1069α). *In situ* hybridization was performed according to the manufacturer's instructions. Briefly, the paraffin embedded serial sections (thickness of 4 μm) were dried at 80 ℃, and their paraffin was removed by xylene and rehydrated with graded ethanol. The sections were acidified in HCl for 30 min, and blocked in 3 mL of 300 mL/L H_2O_2 for 10 min before digestion in proteinase K for 30 min, and then dehydrated with graded ethanol. After prehybridization at 37-40 ℃ for 2 h, the labeled cDNA probes of ERα were denatured in hybridization buffer at 95 ℃ for 10 min, then at -20 ℃ for 10 min, then added into tissues prehybridized at 37 ℃ overnight. Sections were washed in turn with 2×SSC, 1×SSC, 0.2×SSC, and Buffer I, blocking water was added at room temperature for 20 min, and then mouse anti-digoxin serum at 37 ℃ for 60 min, biotinylated goat anti-mouse serum at 37 ℃ for 30 min, SABC at 37 ℃ for 30 min, finally DAB was added to be stained. After several times of washing, the sections were counterstained with hematoxylin, dehydrated with ethanol, rinsed in xylene and mounted with gum for microscopic examination and photography. For each sample, ten random fields were evaluated for positively stained cells. The results were expressed as the percentage of positive cells to the total number of cells counted.

Statistical analysis

Data were presented as $\bar{x}{\pm}s$ unless otherwise indicated. Mann-Whitney *u* test for nonparametric and unpaired values, Student's *t*-test or Fisher's exact test was used as appropriate. Results were considered significant when *P*<0.05.

RESULTS

Estimation of serum indicators and hepatic nitric oxide synthase

At the end of a 8-week experimental period, 5 rats died because of infection at the site of injection and hepatic crack due to improper handling. It was evident that CCl_4 produced a marked increase in the activities of serum ALT and AST in both ovariectomy and sham ovariectomy rats. The extent of increase was lower in sham ovariectomy group than in ovariectomy group, but without statistical significance (*P*>0.05). CCl_4 plus estradiol in both ovariectomy and sham ovariectomy groups showed a significant decrease in enzyme levels. However, they were still higher than those of control groups. The enzyme levels in the ovariectomy and estradiol treatment groups were similar to those in control group. The levels of serum NO_2^-/NO_3^- and hepatic NOS activity increased significantly in both ovariectomy and sham ovariectomy rats when CCl_4 was injected, especially in estradiol treatment groups (Table 1). Ovariectomy itself had no influence on the above parameters.

Table 1 Serum ALT, AST, NO_2^-/NO_3^- and hepatic NOS activity ($\bar{x}{\pm}s$)

Group	n	ALT (nkat/L)	AST (nkat/L)	NO_2^-/NO_3^- (μmol/L)	NOS (KU/g)
Control	10	35.8±7.9	64.5±20.8	21.8±13.7	0.65±0.08
Ovx	10	31.6±6.5	66.4±18.3	19.3±11.2	0.54±0.33
Ovx+E	9	38.5±11.2	68.4±21.2	84.8±24.9ac	2.57±1.06ac
CCl_4	9	540±252a	631±268a	55.3±23.1a	1.65±0.46a
CCl_4+Ovx	10	658±220a	697±240a	53.7±17.1a	1.73±0.71a
CCl_4+E	8	314±163c	302±153c	87.5±23.6c	2.68±1.17c
CCl_4+Ovx+E	9	311±146c	321±121c	93.3±24.2c	2.49±1.20c

a*P*<0.05, *vs* control; c*P*<0.05, *vs* CCl_4.

Histopathological changes

The control livers showed a normal lobular architecture with central veins and radiating hepatic cords. Prolonged administration of CCl_4 caused severe pathological damages such as fat accumulation, inflammation, necrosis, and collagen deposition, especially in ovariectomy group. Administration of estradiol reduced the accumulation of hepatic collagen, decreased centrolobular necrotic areas as well as inflammatory reaction in rats subjected to CCl_4. Semi-quantitative hepatic collagen staging scores are shown in Table 2.

Table 2 Effects of ovariectomy and estradiol on histological scores of CCl_4 – induced hepatic fibrosis

Group	n	Hepatic fibrosis scores				
		-	+	++	+++	++++
Control	10	10	0	0	0	0
Ovx	10	10	0	0	0	0
Ovx+E	9	9	0	0	0	0
CCl_4	9	0	2	2	3	1
CCl_4+Ovx[a]	10	0	0	2	5	3
CCl_4+E[a]	8	0	4	2	1	0
CCl_4+Ovx+E[a]	9	0	5	2	2	0

[a] $P<0.05$, vs control.

Immunohistochemical staining and in situ hybridization for estrogen receptor-a

By immunohistochemical detection, the positive signal of ERα as brown particles was scattered or diffused only in cytoplasm other than in nuclei of parenchymal (hepatocytes) and non-parenchymal hepatic cells, especially near the hepatic centrolobular and periportal areas. The positive signal of in situ hybridization for ERα mRNA also showed brown particles, and distributed in cytoplasm and partly in nuclei (Figure 1).
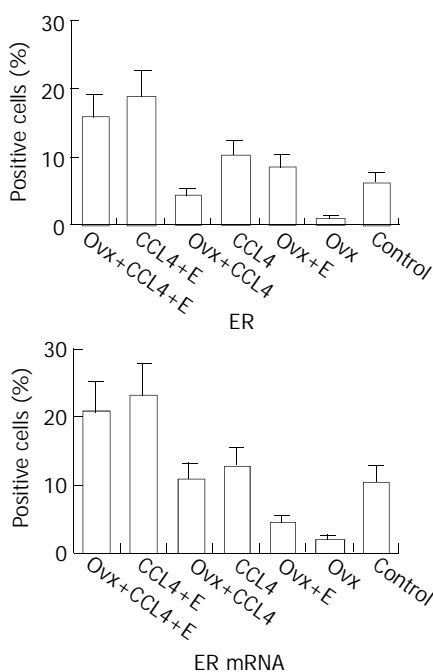

Figure 1 ERα and ERα mRNA in female rat liver.

It showed that livers of female rats contained ER, and ovariectomy decreased ER level and ER mRNA expression significantly ($P<0.05$). Treatment with estradiol at a high dose restored the liver ER and ER mRNA levels. CCl_4 administration

in control and ovariectomized groups was associated with an increase in ER level and ER mRNA expression, but the difference was not significant in control group ($P>0.05$). Hepatic ER and ER mRNA concentrations were elevated significantly after treatment with estradiol in CCl_4 induced fibrotic rats ($P<0.05$, Figures 2 and 3).
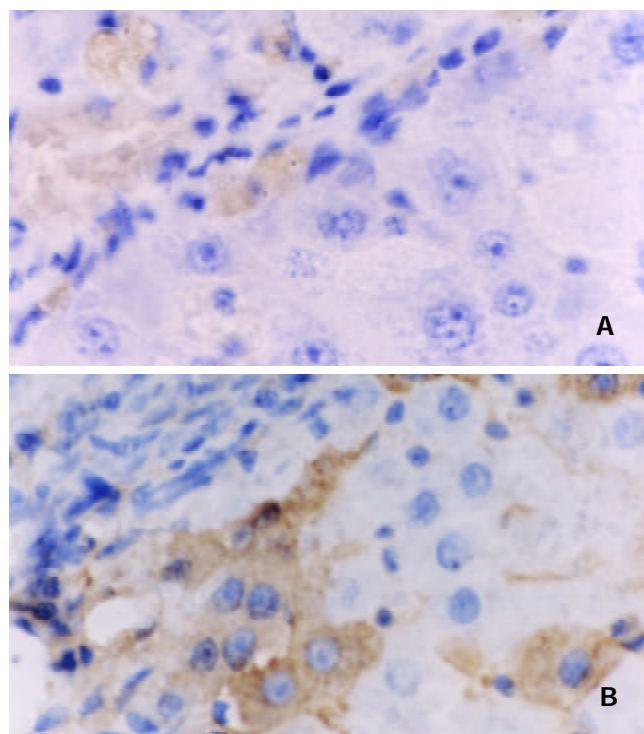

Figure 2 Positive signal of ER in female rat liver (Immunohisto-chemistry staining PAP method, magnification ×400). 2A: CCl_4 group, 2B: Estradiol treatment group shows increased ER expression compared with 2A.
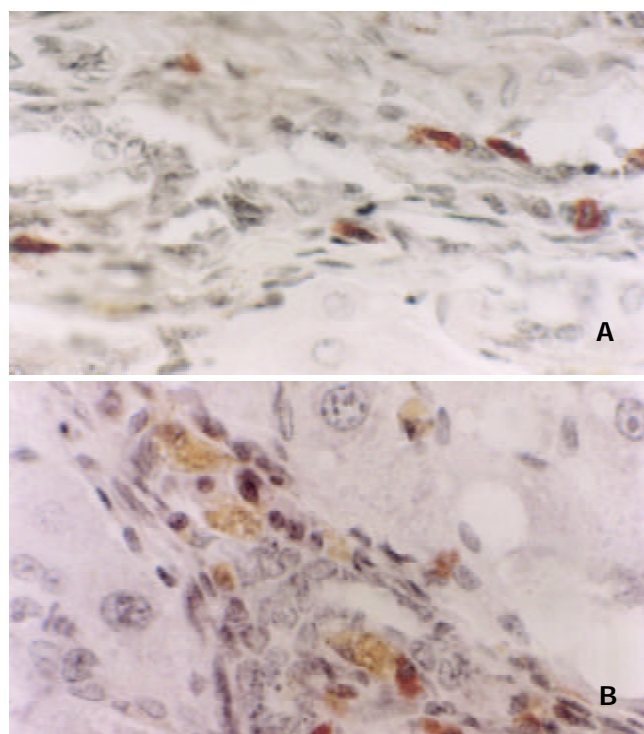

Figure 3 Positive signal of ER mRNA in female rat liver (in situ hybridization staining, SP method, magnification ×400). 3A: CCl_4 group, 3B: Estradiol treatment group shows increased ER mRNA expression compared with 3A.

DISCUSSION

Hormone receptors are generally down regulated by high concentrations of their own ligands. This also seems to be the case with ER in reproductive tissues such as the uterus, ovaries, and mammary glands where estrogens are involved in cell growth and differentiation[12]. In the liver, however, ER has an entirely different function and the hepatic responses to estrogens appear to require high concentrations of estrogens. Some data indicated[9] that treatment with a low (physiological) dose of estradiol could increase ER and ER mRNA in livers of male rats. But the regulation of hepatic ER and ER mRNA in female rats by estrogen has been reported differently[13,14]. The majority of studies concerning estrogenic effects have been performed in ovariectomized female rats. Some data showed[15] that hepatic sinusoidal endothelial cell and Kupffer cell in the livers of female rats contained ER, and they were decreased by ovariectomy. Long-term treatment of 17β-estradiol elevated the level of ER in hepatic sinusoidal endothelial cell and Kupffer cell in ovariectomized rats. On the other hand, ovariectomy has also been reported to increase ER level and decrease ER mRNA level in hepatocytes from female rats[12]. This is somewhat divergent to what was seen in this study, where as ovariectomy decreased ER level and ER mRNA expression in parenchymal and non-parenchymal hepatic cells of female rats, and treatment with estradiol at a high dose restored their levels. These observations confirmed a reported increase in hepatic ER mRNA after estrogen treatment in ovariectomized female rats[16]. An explanation for the mechanism whereby hepatic ER was increased is difficult based on our limited observations. It is possible that the effect of estrogen-treatment on ER levels in ovariectomized female rats was affected indirectly through the pituitary, or by increased hepatic growth hormone receptors. Treatment with 17β-estradiol in combination with growth hormone and dexamethasone was reported to increase ER levels to eightfold, while estradiol treatment alone had a minimal influence on them in cultured hepatocytes from female rats[12]. This may suggest that the regulation of hepatic ER is under a more complex control involving other factors.

It has been shown[17] that prolonged alcohol abuse induced a marked increase in ER levels in livers of both male and female patients, especially in patients who had histological evidence of acute liver damage (alcoholic hepatitis). As we know, CCl_4-induced hepatitis and fibrosis shared several characteristics with human hepatitis and fibrosis of different etiologies[18-24]. In this study, prolonged administration of CCl_4 induced increases in hepatic ER and ER mRNA expression in female rats, especially in ovariectomized group. The increase extent of ER and ER mRNA was correlated histologically with inflammation, fat accumulation, necrosis, activities of serum ALT and AST, and hepatic collagen diposition. As it is impossible to provide a detailed explanation of the regulation of hepatic ER in normal female rats, we can not establish whether the increase in ER is due CCl_4 administration in our study. Further investigations are thus required to clarify this point. Within these limitations, our data indicate that prolonged CCl_4 administration can affect ER levels and ER mRNA expression in female rat livers.

In our study, hepatic ER and ER mRNA concentrations were significantly elevated after treatment with estradiol in CCl_4-induced fibrotic rats, and the elevation of ER was correlated with a marked decrease in hepatic damage and fibrosis. It suggested that estradiol might suppress hepatic fibrosis by a receptor mechanism. This finding has not been reported previously[25,26] but is in line with the experimental data obtained from cultured hepatic sinusoidal endothelial cells in rats. In the experiment[27], ER was demonstrated in hepatic sinusoidal endothelial cells, and estrogen increased NOS activity and upregulated NO production in the cells through an ER-mediated system, and then regulated the hepatic sinusoidal microcirculation. It has been found that in the liver, NO is produced by hepatic sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells and hepatocytes[28-32], and NO is synthesized by NOS from L-arginine as a substrate[33,34]. It was observed that endogenous NO could protect the liver from lipid peroxidation, damage, and fibrosis[35]. In the present study, estradiol treatment led to a paralleled increase in ER with serum NO and hepatic NOS activity in fibrotic rats. These findings suggest that in liver fibrosis, estrogen may promote NO synthesis through hepatic ER, resulting in improvements of liver damage and fibrosis in rats.

The liver has been found to be extremely sensitive to the action of sex hormones[4,26], estrogen-dependent regulation of hepatic function could occur through ER present in the liver[7]. We demonstrated that administration of estradiol elevated ER levels in hepatic fibrotic rats, and ER was distributed in parenchymal (hepatocytes) and non-parenchymal hepatic cells, especially near the hepatic centrolobular and periportal areas where non-parenchymal cells were mainly located. Although we could not identify the exact location of ER in hepatic cells in the present study, we know that the high affinity ER in Kupffer cells of rat liver could exhibit the same characteristics as that presented in hepatocytes[15]. Therefore, estrogen may modulate Kupffer cell function. Kupffer cells upon stimulation, could produce mediators such as TNF, IL-6 and IL-10, and therefore playing a central role in the regulation of CCl_4-induced liver injury and fibrotic progression[17,36-42].

As is generally believed that the expression of specific genes and cell responses to steroid hormones are related to the amount of receptors, the increase in hepatic ER and its mRNA as described in this paper may be part of the molecular mechanisms underlying the suppressive effect of estradiol on liver fibrosis induced by CCl_4 administration.

REFERENCES

1   **Becker U,** Deis A, Sorensen TI, Gronbaek M, Borch-Johnsen K, Muller CF, Schnohr P, Jensen G. Prediction of risk of liver disease by alcohol intake, sex, and age: a prospective population study. *Hepatology* 1996; **23**: 1025-1029
2   **Pinzani M,** Romanelli RG, Magli S. Progression of fibrosis in chronic liver diseases: time to tally the score. *J Hepatol* 2001; **34**: 764-767
3   **Yan JC,** Ma JY, Pan BR, Ma LS. Study of hepatitis B in China. *Shijie Huaren Xiaohua Zazhi* 2001; **9**: 611-616
4   **Xu JW,** Gong J, Chang XM, Luo JY, Dong L, Hao ZM, Jia A, Xu GP. Estrogen reduces CCL_4-induced liver fibrosis in rats. *World J Gastroenterol* 2002; **8**: 883-887
5   **Tan E,** Gurjar MV, Sharma RV, Bhalla RC. Estrogen receptor-α gene transfer into bovine aortic endothelial cells induces eNOS gene expression and inhibits cell migration. *Cardiovasc Res* 1999; **43**: 788-797
6   **Kwan G,** Neugarten J, Sherman M, Ding Q, Fotadar U, Lei J, Silbiger S. Effect of sex hormones on mesangial cell proliferation and collagen synthesis. *Kindey Int* 1996; **50**: 1173-1179
7   **Porter LE,** Elm MS, Van Thiel DH, Dugas MC, Eagon PK. Characterization and quantitation of human hepatic estrogen receptor. *Gastroenterology* 1983; **84**: 704-712
8   **Kuiper GG,** Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptorα and β. *Endocrinology* 1997; **138**: 863-870
9   **Koritnik DR,** Koshy A, Hoversland RC. 17β-estradiol treatment increases the levels of estrogen receptor and its mRNA in male rat liver. *Steroids* 1995; **60**: 519-529
10 **George J,** Rao KR, Stern R, Chandrakasan G. Dimethylnitrosamine-induced liver injury in rats: the early deposition of collagen. *Toxicology* 2001; **156**: 129-138
11 **Pilette C,** Rousselet MC, Bedossa P, Chappard D, Oberti F, Rifflet H, Maiga MY, Gallois Y, Cales P. Histopathological evaluation

of liver fibrosis: quantitative image analysis *vs* Semi-quantitative scores. Comparison with serum markers. *J Hepatol* 1998; **28:** 439-446

12   **Stavreus-Evers AC,** Feyschuss B, Eriksson HA. Hormonal regulation of the estrogen receptor in primary cultures of hepatocytes from female rats. *Steroids* 1997; **62:** 647-654

13   **Stavreus-Evers A,** Parini P, Freyschuss B, Elger W, Reddersen G, Sahlin L, Eriksson H. Estrogenic influence on the regulation of hepatic estrogen receptor-α and serum level of angiotensinogen in female rats. *J Steroid Biochem Mol Biol* 2001; **78:** 83-88

14   **Ignatenko LL,** Mataradze GD, Rozen VB. Endocrine mechanisms for the formation of sex-related differences in hepatic estrogen receptor content and their significance for the realization of an estrogen effect on angiotensinogen blood level in rats. *Hepatology* 1992; **15:** 1092-1098

15   **Vickers AE,** Lucier GW. Estrogen receptor levels and occupancy in hepatic sinusoidal endothelial and Kupffer cells are enhanced by initiation with diethylnitrosamine and promotion with 17alpha-ethinylestradiol in rats. *Carcinogenesis* 1996; **17:** 1235-1242

16   **Shupnik MA,** Gordon MS, Chin WW. Tissue-specific regulation of rat estrogen receptor mRNAs. *Mol Endocrinol* 1989; **3:** 660-665

17   **Colantoni A,** Emanuele MA, Kovacs EJ, Villa E, Van Thiel DH. Hepatic estrogen receptors and alconol intake. *Mol Cell Endocrinol* 2002; **193:** 101-104

18   **Du WD,** Zhang YE, Zhai WR, Zhou XM. Dynamic changes of type I, III and IV collagen synthesis and distribution of collagen-producing cells in carbon tetrachloride induced rat liver fibrosis. *World J Gastroenterol* 1999; **5:** 397-403

19   **Wu CH.** Fibrodynamics-elucidation of the mechanisms and sites of liver fibrogenesis. *World J Gastroenterol* 1999; **5:** 388-390

20   **Liu HL,** Li XH, Wang DY, Yang SP. Matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase –1 expression in fibrotic rat liver. *World J Gastroenterol* 2000; **6:** 881-884

21   **Wei HS,** Li DG, Lu HM, Zhan YT, Wang ZR, Huang X, Zhang J, Cheng JL, Xu QF. Effects of AT1 receptor antagonist, losartan, on rat hepatic fibrosis induced by CCl$_4$. *World J Gastroenterol* 2000; **6:** 540-545

22   **Wang JY,** Zhang QS, Guo JS, Hu MY. Effects of glycyrrhetinic acid on collagen metabolism of hepatic stellate cells at different stages of liver fibrosis in rats. *World J Gastroenterol* 2001; **7:** 115-119

23   **Zhang YT,** Chang XM, Li X, Li HL. Effects of spironolactone on expression of type I/III collagen proteins in rat hepatic fibrosis. *Shijie Huaren Xiaohua Zazhi* 2001; **9:** 1120-1124

24   **Wang LT,** Zhang B, Chen JJ. Effect of anti-fibrosis compound on collagen expression of hepatic cells in experimental liver fibrosis of rats. *World J Gastroenterol* 2000; **6:** 877-880

25   **Shimizu I,** Mizobuchi Y, Yasuda M, Shiba M, Ma YR, Horie T, Liu F, Ito S. Inhibitory effect of oestradiol on activation of rat hepatic stellate cells *in vivo* and *in vitro*. *Gut* 1999; **44:** 127-136

26   **Liu Y,** Shimizu I, Omoya T, Ito S, Gu XS, Zuo J. Protective effect of estradiol on hepatocytic oxidation damage. *World J Gastroenterol* 2002; **8:** 363-366

27   **Sakamoto M,** Uen T, Nakamura T, Hashimoto O, Sakata R, Kin M, Ogata R, Kawaguch T, Torimura T, Sata M. Estrogen upregulates nitric oxide synthase expression in cultured rat hepatic sinusoidal endothelial cells. *J Hepatol* 2001; **34:** 858-864

28   **Huang YQ,** Zhang DZ, Mo JZ, Li RR, Xiao SD. Nitric oxide concentration of esophageal tissues and hemodynamics in cirrhotic rats. *Xin Xiaohuabingxue Zazhi* 1997; **5:** 558-559

29   **Yang CJ,** Zhen CE, Yao XX. Study on relationship between plasma NO and sex hormones in patients with hepatic cirrhosis. *Huaren Xiaohua Zazhi* 1998; **6:** 976-978

30   **Huang YQ,** Xiao SD, Zhang DZ, Mo J. Nitric oxide synthase distribution in esophageal mucosa and hemodynamic changes in rats with cirrhosis. *World J Gastroenterol* 1999; **5:** 213-216

31   **Qin JM,** Zhang YD. Intestinal expressions of eNOSmRNA and iNOSmRNA in rats with acute liver failure. *World J Gastroenterol* 2001; **7:** 652-656

32   **Wang X,** Zhong YX, Zhang ZY, Lu J, Lan M, Miao JY, Guo XG, Shi YQ, Ding J, Wu KC, Pan BR, Fan DM. Effect of L-NAME on nitric oxide and gastrointestinal motility alterations in cirrhotic rats. *World J Gastroenterol* 2002; **8:** 328-332

33   **Zhou JF,** Cai D, Zhu YG, Yang JL, Peng CH, Yu YH. A study on relationship of nitric oxide, oxidation, peroxidation, lipoperoxidation with chronic chole-cystitis. *World J Gastroenterol* 2000; **6:** 501-507

34   **Pei WF,** Xu GS, Sun Y, Zhu SL, Zhang DQ. Protective effect of electroacupuncture and moxibustion on gastric mucosal damage and its relation with nitric oxide in rats. *World J Gastroenterol* 2000; **6:** 424-427

35   **Muriel P.** Nitric oxide protection of rat liver from lipid peroxidation, collagen accumulation, and liver damage induced by carbon tetrachloride. *Biochem Pharmacol* 1998; **56:** 773-779

36   **Li D,** Zhang LJ, Chen ZX, Huang YH, Whang XZ. Effects of TNFα IL-6 and IL-10 on the development of experimental rat liver fibrosis. *Shijie Huaren Xiaohua Zazhi* 2001; **9:** 1242-1245

37   **Jian HQ,** Zhang XL. The mechanisms of hepatic fibrosis. *Shijie Huaren Xiaohua Zazhi* 2000; **8:** 687-689

38   **Weng HL,** Cai WM, Liu RH. Animal experiment and clinical study of effect of gamma-interferon on hepatic fibrosis. *World J Gastroenterol* 2001; **7:** 42-48

39   **Dai WJ,** Jiang HC. Advances in gene therapy of liver cirrhosis: a review. *World J Gastroenterol* 2001; **7:** 1-8

40   **Tang NH,** Chen YL, Wang XQ, Li XJ, Yin FZ, Wang XZ. Construction of IL-2 gene-modified human hepatocyte and its cultivation with microcarrier. *World J Gastroenterol* 2003; **9:** 79-83

41   **Li D,** Wang XZ. Relationship of liver fibrosis and TNFα, IL-6 and IL-10. *Shijie Huaren Xiaohua Zazhi* 2001; **9:** 808-810

42   **Parola M,** Robino G. Oxidative stress-related molecules and liver fibrosis. *J Hepatol* 2001; **35:** 297-306

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