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• BRIEF REPORT •

# Overexpression of hepatic plasminogen activator inhibitor type 1 mRNA in rabbits with fatty liver

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## INTRODUCTION

Plasminogen activator inhibitor type 1 (PAI-1), an approximately $M_r$50000 glycoprotein, is the major physiological inhibitor of plasminogen activators. It is not only the priming factor for atherosclerosis and coronary thrombosis[1-3], but also participates in the genesis of chronic hepatitis and liver fibrosis[4-11]. However, there has been no available report yet about the research of hepatic PAI-1 gene expression in hyperlipidemia and fatty liver. The present study AIMed to explore the change of hepatic PAI-1 mRNA and its plasma activity by means of animal model.

## MATERIALS AND METHODS

### Animal model

Seventeen male New Zealand white rabbits weighing 2 200 g-2 500 g were randomly divided into two groups. The seven rabbits of control group were fed with normal rabbit diet. The ten rabbits of model group were fed with a fat-rich diet, which was prepared by addition of 10 g·kg$^{-1}$ cholesterol and 100 g·kg$^{1}$ lard oil to the normal diet. Vein blood samples of rabbits were collected before the beginning of study and at the 6th week and 12th week of the study respectively. Then the plasma samples were obtained and maintained at -70℃ until assayed. When the rabbits were sacrificed at the 12th week, their body mass and wet liver mass were measured. And two small samples of liver tissue were obtained from the right hepatic lobe. One sample was fixed in formalin, embedded in paraffin and stained with H&E. The other sample was stored in liquid nitrogen for detection of PAI-1 mRNA.

### Measurement of plasma biochemistry

Plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase ( AST ), triglycerides ( TG ) and total cholesterol (TCH) were determined by automatic biochemical analysis instrument ( Olympus AU 1000, made in Japan ). Plasma PAI-1 activity was determined by a chromogenic activity kit purchased from Biotechnology Co., Ltd. of the former Shanghai Medical University.

### Detection of hepatic PAI-1 Mrna

The primer for rabbit PAI-1 was designed based on its complement DNA (cDNA) sequence published previously[6,12,13] and synthesized by DNA synthesis instrument. The sequence is 5' ATG GAA TTC CCG TGG AAC AAG AAT GAG ATC AG 3'and 5' TGA GCC ATC ATG GGC ACA GAG. The primer for β-actin was given by Shanghai Institute of Cell Biology of the Chinese Academy of Sciences, and the sequence is 5' ATC TGG CAC CAC ACC TTC TAC AAT GAG CTGC 3' and 5' CGT CAT ACT CCT GCT TGC TGA TCC ACA TCT GC 3'. The samples of liver tissues were cut into pieces quickly and the total RNAs were extracted with Trizol Reagent (Gibco BRL Co., Ltd.). Reverse transcription-polymerase chain reaction (RT-PCR) was carried out to amplificate the mRNA of PAI-1 and β-actin, and followed by electrophoresis of the products, photography and computer image scan. And the results were validated by the OD value of β-actin because of its equal expression in various tissues.

### Statistical analysis

All results were expressed as $\bar{x}\pm s$. Statistical differences between means were determined by Student *t* test. $P<0.05$ was selected to reflect significance.

## RESULTS

### General condition

During the experiment, no animal death occurred in both groups. The body mass of rabbits in the model group increased more rapidly than in the control group. The model group had significantly greater body mass and liver index (wet liver mass per body mass) than the control group ($P<0.05$ respectively, Table 1). Compared with the control group, the plasma TCh and TG levels in the model group already increased significantly at the 6th week ($P<0.05$ *vs* controls) and increased more significantly at the 12th week ($P<0.01$ *vs* controls). However, the plasma levels of ALT and AST in the model group only had a tendency to increase as compared with those of control group (Table 2).

**Table 1** The changes of body mass and liver index

| Groups | *n* | m (body)/(kg) | | Liver index/(g·kg$^{-1}$) |
|---|---|---|---|---|
| | | Start | End | End |
| Control | 7 | 2.4±0.3 | 3.1±0.2 | 26±3 |
| Model | 10 | 2.3±0.6 | 3.4±0.1[a] | 31±2[a] |

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

**Table 2** Plasma biochemical test at the 12th week

| Groups | *n* | c(TG)/(mmol·L$^{-1}$) | c(TCh)/(mmol·L$^{-1}$) | b(ALT)/(nkat·L$^{-1}$) | b(AST)/(nkat·L$^{-1}$) |
|---|---|---|---|---|---|
| Control | 7 | 0.34±0.11 | 0.76±0.17 | 372±130 | 215±45 |
| Model | 10 | 0.54±0.17[b] | 26.40±3.89[b] | 505±215 | 263±127 |

[b]$P<0.01$ *vs* control.

### *Changes of plasma PAI-1 activity*

In the model group, the plasma PAI-1 activity of rabbits already increased significantly at the 6th week ($P<0.05$) and increased more significantly at the 12th week ($P<0.01$, Table 3) compared with those at the beginning of the experiment. Moreover, the difference in plasma PAI-1 activity between the two groups at the same period also had highly statistical significance ($P<0.05$ and $P<0.01$, respectively).

**Table 3** The changes of plasma PAI-1 activity ($\times 10^3 AU \cdot L^{-1}$)

| Groups | *n* | 0 wk | 6 wk | 12 wk |
|---|---|---|---|---|
| Control | 7 | 5.6±2.6 | 6.8±1.4 | 4.8±2.2 |
| Model | 10 | 4.9±3.1 | 7.7±1.1[a] | 14.0±2.5[b] |

[a]$P<0.05$, [b]$P<0.01$ *vs* control.

### *Changes of liver pathology*

In the gross, the livers of the model group enlarged markedly as compared with those of control group. Under the light microscope, all the liver tissue sections stained with H&E of the model group showed diffuse and severe foamy steatosis of hepatocyte with mild necrosis and inflammatory cell infiltration, among which monocytes were dominant. And these lesions were located mainly in portal areas.

### *Expression of hepatic PAI-1 mRNA*

The mRNA fragments of PAI-1 and β-actin were 360 bp and 828 bp respectively (Figures 1, 2). The lanes (No.3,4,5) of hepatic PAI-1 mRNA of the model group were markedly more intense than those of control group (No.1,2). The ratio of hepatic PAI-1 mRNA to β-actin mRNA of model group was significantly greater than that of the control group (3.474±0.051 *vs* 1.210±0.031, $P<0.01$).

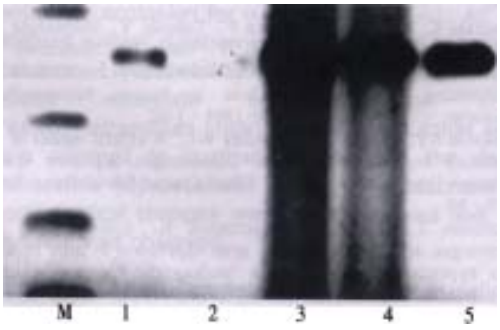


**Figure 1** The expression of hepatic PAI-1 mRNA. 1,2: control group; 3,4,5: model group; M: marker

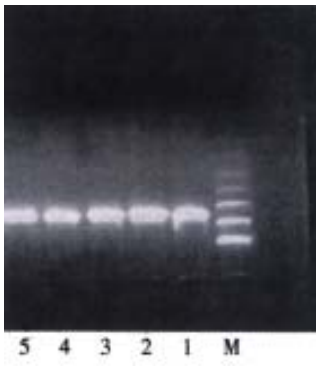


**Figure 2** The expression of hepatic β-action mRNA. 1,2: control group; 3,4,5: model group; M: marker

## DISCUSSION

Fibrinolysis system has an inactive zymogen that is called plasminogen. It can be activated by plasminogen activators and be converted to plasmin[11]. Plasmin can not only degrade the fibrin and remove it from the blood circulation, but also degrade extracellular matrix (ECM) directly and protect the tissues from fibrosis[1-11]. Among all fibrinolysis components, PAI-1 plays a central role in the pathophysiology of cardioangiological diseases. It is the major physiological inhibitor of urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA). The plasma PAI-1 regulates the plasmin cascade by its interaction with the tPA[1,2]. The increased activity of plasma PAI-1 could inactive the tPA in circulation, and the PAI-1 depositing in ECM could accelerate the clearance of tPA in tissues. PAI-1 exists in all of human tissues. Analysis of human tissues for PAI-1 antigen and activity has shown that the greatest quantity and highest specific activity were found in the liver. In plasma and ECM, the free PAI-1 produced by hepatocytes, endothelial cells and smooth muscle cells binds vitronectin (VN) to maintain the stable activity. The binding PAI-1 which increases the anti-fibrinolysis activity of blood vessels and accelerates the deposition of ECM can induce the genesis of atherosclerosis, coronary heart disease, pulmonary fibrosis and kidney fibrosis[1-3]. Recent studies have shown that the activity of PAI-1 is also associated with the occurrence and progression of chronic viral hepatitis and liver fibrosis. With the progression of chronic liver disease, the plasma PAI-1 antigen level and expression of PAI-1 in the liver increased gradually and culminated in the stage of liver cirrhosis, then decreased with the deterioration of liver function (Child-Pugh classification A to C)[4-8]. In vitro, hepatic stellate cells (HSC), also known as hepatic lipocytes, fat-storing cells, or Ito cells, could express PAI-1 when activated[12-15]. And previous studies have already demonstrated that HSC played a critical role in the genesis and development of liver fibrosis[16,17]. However, there has been no report yet about the effect of PAI-1 on the pathogenesis of fatty liver.

The present study established a rabbit model with overweight, hyperlipidemia and fatty liver via a fat-and-cholesterol-rich diet. The rabbits of this model had significantly greater body weight and higher plasma lipid levels than the normal control rabbits and their serum levels of ALT and AST had a tendency to increase. In hepatic histopathology, this model showed severe hepatocyte steatosis with mild hepatocyte necrosis and inflammatory cell infiltration. These characteristics resemble the presentation of human fatty liver associated with obesity and hyperlipidemia[18]. So this model can be used to explore the pathogenesis of nonalcoholic fatty liver. In the present study, while hyperlipidemia occurred in the model rabbits after 6 weeks of the fatty-rich diets, the plasma PAI-1 activity also increased. Then the plasma lipid and the plasma PAI-1 activity increased more significantly at the 12th week. Moreover, it was shown in the RT-PCR that PAI-1 mRNA was markedly intense in the liver with steatosis.

It is known that various factors could influence the expression and translation of hepatic PAI-1 gene. In the non-alcoholic fatty liver with obesity and hyperlipidemia, many accompanying factors such as lipoprotein (VLDL, LDL), hyperinsulinemia or gut-derived endotoxemia can stimulate hepatic PAI-1 production[19-22]. When the cytokines such as IL-1, TNF, PDGF, TGF-β or IGF-1 exist simultaneously, hepatic PAI-1 production will be further increased[23-28]. Besides, other factors such as dexamethasone, prothrombin, or angiotensin II (Ang II) can also accelerate the secretion of

PAI-1[29-32]. Subsequently, the increase in PAI-1 caused the imbalance of tPA and PAI-1, which leads to the decrease in fibrinolysis activity of hepatic microcirculation and degrading of hepatic ECM. Then the dysfunction of hepatic microcirculation, even the microthrombosis could be induced and the occurrence and progression of liver fibrosis promoted[5-11]. However, some studies have indicated that PAI-1 can inhibit the conversion of inactive TGF-β (transforming growth factor-β) into active TGF-β, so that PAI-1 might inhibit liver fibrogenesis since TGF-β can promote the production of ECM and inhibit its degrading[4]. It is therefore, necessary to reinforce the study of the relationship between PAI-1 and fibrosis in fatty liver in order to find an effective method for the prevention and treatment of liver fibrosis.

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