Int J Clin Exp Med 2017;10(5):7957-7964
www.ijcem.com /ISSN:1940-5901/IJCEM0042994

Original Article

Dynamic monitoring of blood biochemistry and CYP450 metabolic changes in cirrhotic rats

Lichuan Hu[1], Jiong Yu[2], Zhong Lin[1], Xiufei Yang[1], Congcong Wen[3], Lufeng Hu[4], Xi Xu[5]

[1]Department of Pharmacy, Wenzhou Central Hospital, Wenzhou 325000, China; [2]The State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, China; [3]Analytical and Testing Center & Department of Pharmacology, Wenzhou Medical University, Wenzhou 325000, China; Departments of [4]Pharmacy, [5]Hematology, The First Affliated Hospital of Wenzhou Medical University, Wenzhou 325000, China

Received April 9, 2016; Accepted January 3, 2017; Epub May 15, 2017; Published May 30, 2017

Abstract: Liver cirrhosis, a progressive disease, presents no obvious symptoms in the early stage. So far, no comprehensive study has focused on the dynamic changes of liver function and the metabolic ability of CYP450 enzymes. In this study, thirty-two Sprague-Dawley Rats were randomly divided into two groups: Control-group (n=10) and cirrhosis-group (n=22). The cirrhotic rat model was developed by subcutaneous injection of CCl4 for 8 weeks. The blood biochemistry was monitored at 0, 2, 4, 6, 8, and 10 weeks. The metabolic ability of CYP450 was evaluated by pharmacokinetics of six CYP450 probe drugs at 10 weeks. The results showed that the level of alkaline phosphatase (AKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bile acid (BA) were all increased significantly at 2 weeks, and were subsequently maintained at a high level. Albumin gradually decreased and globulin (GLO) gradually increased, showing a statistically significant difference at 4 weeks ($P<0.05$). The pharmacokinetic study showed that except for testosterone, the $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, $MRT_{(0-t)}$, $MRT_{(0-\infty)}$, $t_{1/2}$, and T_{max} of the five probe drugs phenacetin, bupropoin, omeprazole, tolbutamide, and metoprolol were all increased in cirrhotic rats compared with the control group ($P<0.05$). In conclusion, ALT, AST, AKP, BA, and GLO can be used as sensitive indices for the early stage development of cirrhosis, and the metabolic ability of CYP450 enzymes was significantly inhibited.

Keywords: Cirrhosis, blood biochemistry, CYP450 enzymes, rat

Introduction

Liver cirrhosis is commonly caused by long-term alcohol abuse, hepatitis B and C infection, and fatty liver disease, and is a major cause of morbidity and mortality [1]. According to a systematic analysis of liver cirrhosis mortality in 187 countries, global liver cirrhosis deaths increased from around 676,000 in 1980 to over 1 million in 2010 [2]. Liver cirrhosis is a progressive disease. There are no obvious symptoms in the early stage of the disease, but at the advanced stage, various symptoms appear, such as visible blood capillaries on the skin of the upper abdomen, fatigue, itchy skin, loss of appetite, nausea, and other symptoms. During the past ten years, numerous studies have performed on the progression of advanced cirrhosis [3-5]. However, few studies focus on the dynamic changes of blood biochemistry that occur during the progression of cirrhosis. Additionally, the change of metabolic ability of the intrahepatic cytochrome P450 (CYP450) enzyme has not been studied in depth. CYP450 enzymes play an important role in the metabolism of many molecules including steroid hormones and certain fats and drugs [6].

A study of gene expression during liver cirrhosis showed there were 19 genes of CYP450 enzymes associated with inflammatory response, immune response, and oxidative stress [7]. CYP450 was confirmed to be involved in the progression of cirrhosis. CYP1A2, CYP2B6, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 are widely presented in liver, and are considered important isozymes in drug metabolism [8, 9]. The changed metabolic ability of CYP450 enzyme will lead to different plasma levels of substrate drugs [10]. Without a comprehensive study of liver function and the metabolic ability of CYP450 enzymes in the liver, it is difficult for doctors to make accurate drug dosage adjustments for patients with cirrhosis. This study aims to dynamically monitor the blood biochemical changes and metabolic ability of the CYP450 enzyme in cirrhotic rats.

Material and methods

Drugs and reagents

The probe drugs omeprazole, phenacetin, tolbutamide, bupropion, metoprolol, and testosterone (all >98%) and the internal standard diazepam (IS) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Tetrachloromethane (CCl4) was analytical grade and purchased from Siyou Company (Tianjin, China). Ultra-pure water was prepared by a Millipore Milli-Q purification system (Bedford, USA, resistance >18 mΩ). The methanol and acetonitrile were both HPLC grade and purchased from Merck Company (Darmstadt, Germany). All other chemicals were of analytical grade.

Instruments

A fully automatic biochemical analysis system (AU5800 series, USA Beckman) was used to monitor blood biochemistry, a UPLC-MS/MS system (Waters Corp., Milford, MA, USA) composed of an ACQUITY I-Class UPLC and a XEVO TQD triple quadrupole mass spectrometer was used to analyze concentration of probe drugs, and the Masslynx 4.1 software (Waters Corp., Milford, MA, USA) was used for data acquisition and instrument control.

Animals

A total of 32 Sprague-Dawley (SD) rats aged 8 weeks and weighing 200-230 g were purchased from the Laboratory Animal Center of Wenzhou Medical University (Wenzhou, China). The rats were housed in standard plastic cages and maintained on normal diets and fresh water at 23-25°C with relative humidity 55±10% and a natural light-dark cycle. All experimental procedures were conducted according to the Guide of Animal Care and Use Committee of Wenzhou Medical University.

Experimental design

The rats were randomly divided into 2 groups: the cirrhosis group (n=22) and the control group (n=10). CCl4 was dissolved in corn oil at 40% concentration, and 0.3 ml/100 g was administrated to the backs of rats by subcutaneous injection in the cirrhosis group. The chemical was administrated twice every week for 8 weeks. Rats in the control group were given normal saline with the same administration method.

The blood biochemistry test was monitored at 0, 2, 4, 6, 8, and 10 weeks and the metabolic change of CYP450 was studied at 10 weeks. At 10 weeks, the rats were sacrificed and the cirrhotic rat models were validated by pathological examination.

Dynamic monitoring of blood biochemistry

Starting on a Saturday (0) and then again on the Saturday of the 2nd, 4th, 6th, 8th, and 10th week of treatment, 0.5 mL blood sample was drawn from the caudal vein into a 1.5 mL centrifuge tube that contained 80 IU heparin. The plasma samples were separated immediately by centrifugation at 8000 rpm for 10 min, and analyzed using the fully automatic biochemistry analyzer. The blood biochemistry indexes including liver and kidney indexes were analyzed with the independent-samples T test using the statistic package for social science (SPSS) 16.0.

Metabolic change of CYP450 enzymes

The metabolic ability of CYP450 was evaluated by a cocktail probe method. A mixture of six CYP450 probe drugs, bupropion, metoprolol, omeprazole, tolbutamide, phenacetin and testosterone (at concentrations of 10, 10, 10, 1, 10, and 10 mg/kg, respectively) were administered to rats by intragastric administration at the 10th week.

Sample collection and preparation

After administration of the cocktail of six CYP450 probe drugs, 0.3 mL blood samples were collected into heparinized 1.5 mL centrifuge tubes from the caudal vein after 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h and immediately centrifuged at 8000 rpm for 10 min.

Table 1. Dynamic monitoring of blood biochemistry in cirrhosis group and control group (mean ± SD)

Index		0 Week (n=22)	2 Week (n=22)	4 Week (n=19)	6 Week (n=17)	8 Week (n=17)	10 Week (n=14)
TP	Cirrhosis	61.2±6.6	58.9±6.8	61.2±9.5	62.3±7.3	61.6±3.8	63.8±7.4
	Control	61.2±6.9	60.4±6.6	62.2±5.6	60.1±5.8	58.2±4.1	58.4±4.4
ALB	Cirrhosis	37.0±7.3	34.0±2.3	33.5±6.8	33.7±5.9	33.3±2.2	32.0±4.2
	Control	37.1±7.6	36.1±6.8	37.1±7.0	35.2±6.2	33.7±5.4	33.1±5.6
GLO	Cirrhosis	23.4±4.5	24.8±5.2	27.6±5.7	28.6±4.0	28.2±4.3	31.7±4.0
	Control	23.2±4.6	22.6±4.3	23.0±4.6*	21.9±2.9**	20.8±2.0**	21.0±2.12**
ALT	Cirrhosis	81.1±19.2	301.3±90.5	422.1±99.6	398.5±182.3	512.2±348.1	380.2±180.0
	Control	77.7±10.8	69.3±19.8**	74.2±15.5**	71.0±19.8**	70.3±21.3**	58.1±17.9**
AST	Cirrhosis	181.7±51.1	438.2±247.7	735.6±203.7	743.3±481.8	647.6±494.1	627.0±483.8
	Control	182.4±38.4	207.8±22.5**	190.0±29.1**	223.8±35.7**	234.2±34.7**	230.6±33.7**
AKP	Cirrhosis	110.5±17.6	242.7±88.2	277.4±157.3	358.9±132.4	347.1±160.9	327.31±135.9
	Control	242.7±90.7	252.7±92.4**	214.8±57.5*	239.2±62.1*	258.1±51.7**	252.5±56.8
BA	Cirrhosis	6.9±2.6	19.6±7.2	29.4±18.3	30.3±19.8	38.0±32.3	33.8±37.3
	Control	8.4±3.6	11.7±5.8**	12.1±6.0*	12.4±6.2*	13.9±6.0**	13.8±6.4
TBIL	Cirrhosis	1.5±0.2	1.9±0.9	2.8±2.6	3.5±2.3	3.2±2.3	2.7±2.2
	Control	1.8±0.6	1.7±0.7	1.8±0.6	1.9±0.6	2.0±0.5	1.9±0.4
CR	Cirrhosis	23.0±3.6	27.9±6.2	25.6±5.4	24.5±3.0	24.8±3.3	21.8±2.0
	Control	21.9±2.9	20.1±1.9	20.0±2.00	19.9±2.0	23.8±2.3	20.0±2.5

*P<0.05, **P<0.01.

Subsequently, 0.1 mL plasma was drawn into a separate 1.5 mL centrifuge tubes. To this sample, 10 μL of the internal standard working solution (1.0 μg/mL) was added, and then 0.2 mL acetonitrile was added to precipitate the protein. The mixture was shaken for 30 s, centrifuged for 10 min at 15000 rpm, and 2 μL of the supernatant was injected into the UPLC-MS/MS system for analysis.

Analytical condition

The analytes were eluted within 3 min by gradient elution using 0.1% formic acid and acetonitrile at a flow rate of 0.4 mL/min using a BEH C18 column (2.1 mm×100 mm, 1.7 μm) at 40°C. Nitrogen was used as the cone gas (50 L/h) and the desolvation gas (1000 L/h). The mass conditions were set as follows: source temperature 150°C; capillary voltage 2.5 kV; and desolvation temperature 500°C.

Omeprazole, tolbutamide, bupropion, phenacetin, metoprolol, and testosterone and the IS were detected. The multiple reaction monitoring (MRM) model was set as follows: m/z 240.1→184.1 for bupropion, m/z 180.1→109.9 for phenacetin, m/z 268.1→115.8 for metroprolol, m/z 346.1→ 197.8 for omeprazole, m/z 304.2→124.0 for testosterone, m/z 271.2→155.1 for tolbutamide, and m/z 285.1→193.1 for IS.

Method validation

The dependent linear equations for bupropion, omeprazole, tolbutamide, phenacetin, metoprolol, and testosterone were constructed in the range of 10-10000 ng/mL (10, 50, 250, 500, 1000, 2500, 5000, and 10000 ng/mL) by the ratios of peak area to concentrations. Three different quality-control (QC) samples (80, 800, and 8000 ng/mL) were prepared to validate the accuracy, precision, and recovery according to published literature [11]. Each validation run consisted of one set of calibration standards and six replicates of QC plasma samples.

Pharmacokinetic analysis

The plasma concentrations of probe drugs were determined by UPLC-MS/MS and the pharmacokinetic parameters were calculated. The time and concentrations were analyzed with a two compartment model and 1/cc weight coefficients using the Drug and Statistics 2.0 software (DAS 2.0). The differences in the pharmacokinetic parameters and blood biochemistry indexes between the two groups were analyzed by SPSS16.0.

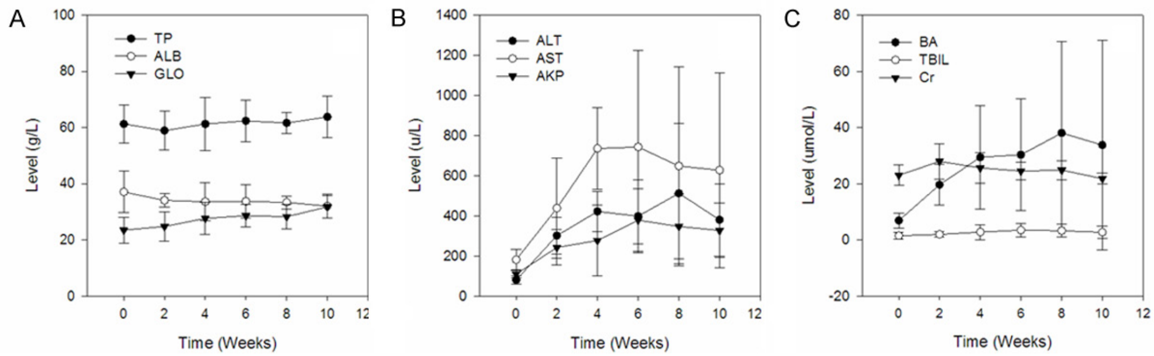

Figure 1. Dynamic change of blood biochemistry in Cirrhosis rats (A: TP, ALB, GLO; B: ALT, AST, AKP; C: BA, TBIL, Cr).

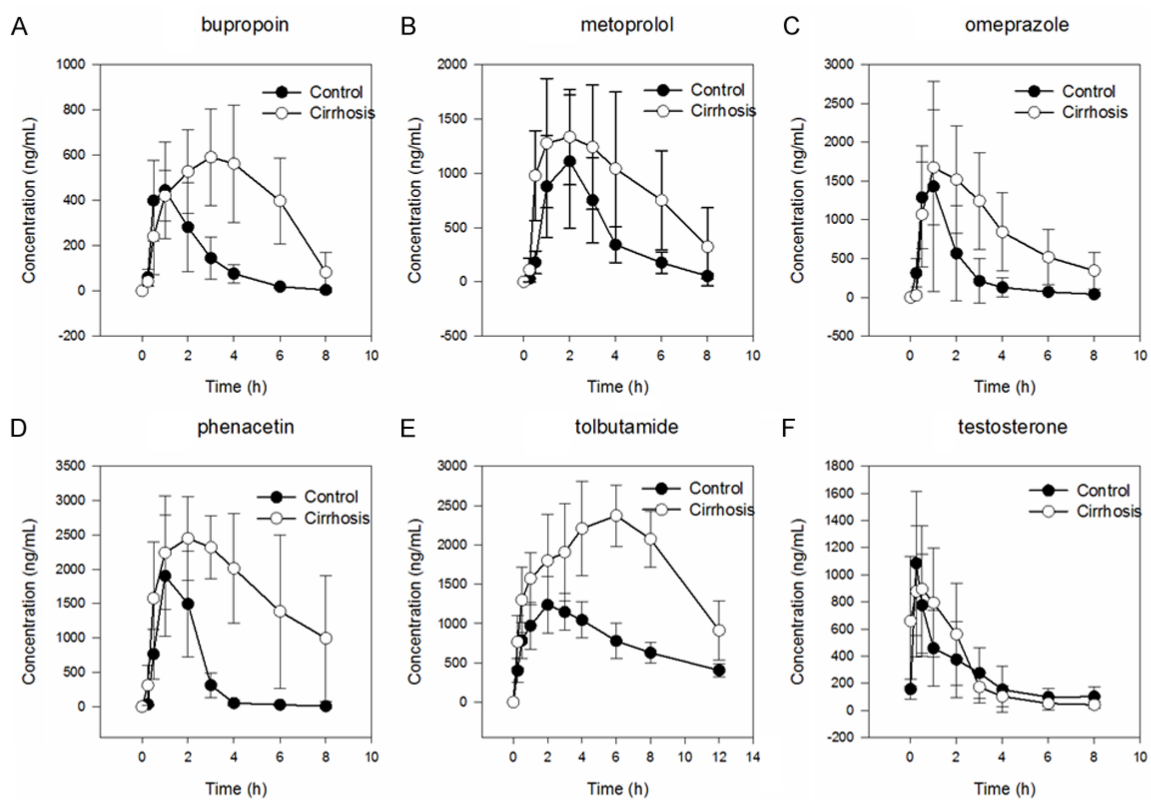

Figure 2. The concentration-time curves of six probe drugs in rats of Cirrhosis-group and Control-group, bupropoin (A), metoprolol (B), omeprazole (C), phenacetin (D), tolbutamide (E), testosterone (F), Data were presented as mean ± SD in each group.

Pathological observation

All rats of the two groups were examined by pathological observation to validate the success of the cirrhotic rat models. After pharmacokinetic study, the rats were anesthetized with 10% chloral hydrate (i.p., 20 mg/kg), and then liver tissues were rapidly isolated and immersed into 4% w/v formaldehyde. Subsequently, histologic sections of liver tissues were prepared by embedding in paraffin, and liver sections were stained with hematoxylin and eosin by routine hematein eosin (HE) method.

Results

Dynamic blood biochemistry changes

As shown in **Table 1** and **Figure 1**, there were 9 indexes of blood biochemistry analyzed in this

Table 2. Pharmacokinetic parameter of bupropoin, omeprazole, phenacetin in Control and Cirrhosis group (mean ± SD)

Parameters	Bupropoin		Omeprazole		Phenacetin	
	Control	Cirrhosis	Control	Cirrhosis	Control	Cirrhosis
$AUC_{(0-t)}$ ug/L*h	1073.0±521.8	3135.8±926.1**	2916.0±2281.0	7039.5±3418.1**	3690.3±1533.0	13308.9±3756.7**
$AUC_{(0-\infty)}$ ug/L*h	1155.2±546.6	5420.0±3076.3**	3005.5±2274.7	8712.8±4695.7**	3704.1±1535.1	24735.7±15800.5**
$MRT_{(0-t)}$ h	1.8±0.2	3.3±0.4**	1.6±0.3	3.0±0.5**	1.6±0.1	3.2±0.8**
$MRT_{(0-\infty)}$ h	2.2±0.5	5.2±3.4**	2.1±0.6	5.0±2.4**	1.7±0.1	7.6±5.2**
$T_{1/2z}$ h	1.2±0.5	3.5±2.5**	1.6±0.8	2.8±1.7*	1.0±0.4	4.3±3.6**
T_{max} h	0.8±0.4	2.7±0.9**	0.7±0.2	1.1±0.3**	1.0±0.2	2.54±1.55**
CL_z L/h/kg	10.6±4.9	2.5±1.5**	4.2±1.6	1.5±0.9**	3.1±1.2	0.6±0.3**
V_z L/kg	19.7±12.1	10.0±5.4*	9.9±6.0	5.3±2.9*	4.8±2.9	2.1±0.9*
C_{max} ug/L	500.6±184.1	648.4±174.7	1637.2±1266.9	1706.1±697.2	1955.9±14.7	2775.0±470.9**

*Compared with Control group P<0.05, **Compared with Control group P<0.01.

Table 3. Pharmacokinetic parameter of metoprolol, testosterone, tolbutamide in Control and Cirrhosis group (mean ± SD)

Parameters	Metoprolol		Testosterone		Tolbutamide	
	Control	Cirrhosis	Control	Cirrhosis	Control	Cirrhosis
$AUC_{(0-t)}$ ug/L*h	3557.7±1309.9	7271.5±3348.7**	2884.5±806.7	4094.9±1799.5*	10419.4±1542.7	21863.8±2858.2**
$AUC_{(0-\infty)}$ ug/L*h	3724.7±1294.6	12187.9±9595.4**	3167.3±945.1	4439.5±1824.4*	20072.1±7328.6	40316.5±17421.3**
$MRT_{(0-t)}$ h	2.7±0.3	3.0±0.6	2.4±0.7	2.6±0.4	5.7±0.4	5.5±0.7
$MRT_{(0-\infty)}$ h	3.0±0.4	3.7±1.8	3.5±1.9	3.3±1.3	16.1±7.4	14.1±11.0
$T_{1/2z}$ h	1.4±0.3	2.7±2.1*	2.0±1.4	1.7±1.2	10.2±5.3	9.6±7.8
T_{max} h	1.8±0.8	2.1±1.1	1.0±1.0	1.5±1.0	3.1±1.2	5.7±1.3**
CL_z L/h/kg	2.9±1.0	1.4±1.2*	3.3±0.8	2.6±1.3	0.5±0.1	0.2±0.1**
V_z L/kg	6.5±3.1	3.7±1.8*	9.7±7.2	6.4±4.6	7.1±1.8	3.1±1.4**
C_{max} ug/L	1171.9±582.8	1615.5±507.4*	1172.7±15.3	1193.8±461.7	1385.2±257.1	2529.6±440.4**

*Compared with Control group P<0.05, **Compared with Control group P<0.01.

study including total protein (TP), albumin (ALB), globulin (GLO), alkaline phosphatase (AKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), bile acid (BA), total bilrubin (TBIL), and creatinine (CR). The ALB level gradually decreased and GLO gradually increased, showing statistical differences starting at 4 weeks (P<0.05). Additionally, ALT, AST, AKP, and BA were obviously increased at 2 weeks and maintained at a high level, with significant statistical differences (P<0.01). The CR and TBIL were both unchanged indexes in this study, which indicated that there was no kidney function damaged during the modeling period.

Pharmacokinetics of probe drugs

Using the UPLC-MS/MS method, the plasma concentrations of bupropion, metoprolol, omeprazole, tolbutamide, phenacetin, and testosterone were determined. The concentration-time curves and pharmacokinetic parameters of the six CYP450 probe drugs are shown in **Figure 2** and **Tables 2, 3.** The concentrations of omeprazole, bupropion, metoprolol, phenacetin, and testosterone were all under the lowest detectable limit of the UPLC-MS/MS method after 8 hours in the control group, so the time profile of concentrations was presented from 0 to 8 hours.

According to the pharmacokinetic results calculated by DAS, there was no statistical difference in testosterone, and the $MRT_{(0-t)}$, $MRT_{(0-\infty)}$, $t_{1/2}$, and T_{max} were not obviously increased or decreased. However, the pharmacokinetic parameters of the other five probe drugs were dramatically changed in the cirrhosis-group. Their $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ were all increased and CL and V_z were decreased (P<0.05). The $MRT_{(0-t)}$, $MRT_{(0-\infty)}$, and T_{max} of bupropion, omeprazole, and phenacetin were all increased (P<0.01). For all except testosterone and tolbutamide, the $t_{1/2z}$ was increased.

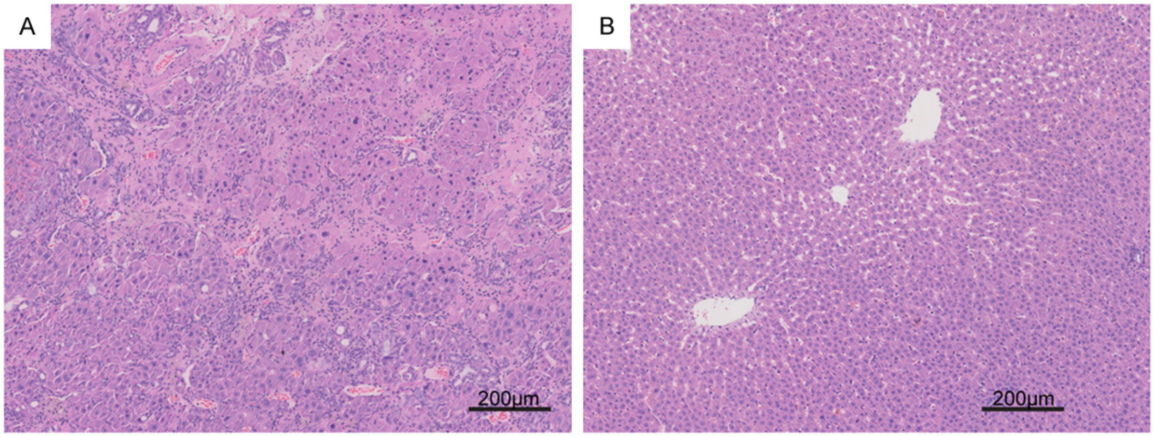

Figure 3. Morphological changes of liver in Cirrhosis-group (A) and Control-group (B).

Morphological changes of liver

The pathological examination of the liver samples revealed significant morphologic differences between the cirrhosis group and the control group. There was no morphologic damage observed in the control group specimens. The normal structure of hepatic lobules, portal area, and central vein could be observed clearly. The hepatic cells were tightly connected and radically arranged along central veins.

Compared with the control group, there were significant changes in the cirrhotic rats. The collagen fibrils and hepatic cells exhibited significant hyperplasia and there was liver cell cord derangement. The structures of hepatic lobules were disordered and difficult to recognize. There were congestion in hepatic sinusoid and central vein, and the normal structure of the portal phase disappeared, the regenerative nodules of hepatocytes were surrounded by the deposition of fibrous tissue. In summary, the pathology indicated that the liver cirrhotic model was successfully developed. The pathological examination is shown in **Figure 3**.

Discussion

According to the dynamic monitoring of blood biochemistry, the levels of ALT, AST, and AKP changed significantly compared with other blood indexes, which indicated there were a large number of liver cells damaged in the development of cirrhotic model. This effect was long lasting, as the level of ALT, AST, and AKP remained at a high concentration even at the end of experiment. Although there was sustained liver cell damage, the fundamental functions remained. For example, the synthetic function for proteins was not affected, as there was no significant reduction in protein synthesis for TP and ALB. Nevertheless, the level of GLO was gradually increased in a statistically significant manner by 4 weeks ($P<0.05$), and continued to increase with modeling time. This change is consistent with the pathological examination that revealed evidence of cirrhosis, as shown in **Figure 3**. Compared with ALB, GLO was a more sensitive index for the development of cirrhosis. Cr was previously confirmed as a sensitive index for kidney function. Since there was no change in Cr levels, we can confirm there was no kidney function damage in the cirrhotic rats.

The blood biochemistry and pathological examination confirmed the liver cirrhosis. As there was no obviously low level of ALB and ascites, these cirrhotic rats were in early cirrhotic phase. However, the metabolic abilities of CYP450 enzymes were influenced significantly, as indicated by the pharmacokinetic results of the cocktail of probes. CYP450 enzymes participate in the metabolism of most clinically administered drugs. Commonly, CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 are considered as six important metabolic enzymes in humans [12]. The metabolic ability of these enzymes can be evaluated using probe drugs, such as phenacetin (CYP1A2), metoprolol (CYP2D6), testosterone (CYP3A4), tolbutamide (CYP2C9), omeprazole (CYP2C19), and bupropion (CYP2B6) [13]. In this study, except for testosterone, there were significant changes in the pharmacokinetic parameters of the probe drugs in the cirrhotic rats. CYP3A4 is responsible for the metabolism of approximately 55% of marketed drugs, and it is one of the most abundant enzymes in the liver relative to the other five CYP450 enzymes [14]. Nevertheless, rats are different from humans, for example, CYP3A1 (the main CYP3A form in the rat liver) is not induced by rifampicin [15]. The species-specific differences may partially explain why no pharmacokinetic parameters were changed for testosterone.

Conclusions

In this study, a cirrhotic model of rat was successfully developed by subcutaneous injection of CCl4. The dynamic monitoring of blood biochemistry showed that the levels of ALT, AST, AKP, and BA were all increased significantly at 2 weeks, and subsequently maintained at a high level. GLO gradually increased and was statistically different at 4 weeks. These blood biochemistry indices can be used to monitor the development of cirrhosis. Moreover, the $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, $MRT_{(0-t)}$, $MRT_{(0-\infty)}$, $t_{1/2}$, and T_{max} of four probe drugs, bupropoin, omeprazole, tolbutamide, and metoprolol were all increased in cirrhotic rats, which indicated inhibition of the metabolic ability of CYP450 enzymes in the liver.

Acknowledgements

This work was supported by the Natural Science Foundation of Zhejiang poince (LY16H300005), the Health Department of Zhejiang province (2014KYB100, 2015KYA154, 2015KYB114); the Youth Talent Program Foundation of The Affiliated Hospital of Wenzhou Medical University (qncy010).

Disclosure of conflict of interest

None.

Address correspondence to: Lufeng Hu, Department of Pharmacy, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China. E-mail: hulufeng79@sina.com; Xi Xu, Department of Hematology, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China. E-mail: aximine@126.com

References

[1] Hsiang JC, Bai WW, Raos Z, Stableforth W, Upton A, Selvaratnam S, Gane EJ and Gerred SJ. Epidemiology, disease burden and outcomes of cirrhosis in a large secondary care hospital in South Auckland, New Zealand. Intern Med J 2015; 45: 160-169.

[2] Mokdad AA, Lopez AD, Shahraz S, Lozano R, Mokdad AH, Stanaway J, Murray CJL and Naghavi M. Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. Bmc Medicine 2014; 12: 145.

[3] Sharma S, Khalili K and Nguyen GC. Non-invasive diagnosis of advanced fibrosis and cirrhosis. World J Gastroenterol 2014; 20: 16820-16830.

[4] Telem DA, Schiano T, Goldstone R, Han DK, Buch KE, Chin EH, Nguyen SQ and Divino CM. Factors that predict outcome of abdominal operations in patients with advanced cirrhosis. Clin Gastroenterol Hepatol 2010; 8: 451-457.

[5] Farr M and Schulze PC. Recent advances in the diagnosis and management of cirrhosis-associated cardiomyopathy in liver transplant candidates: advanced echo imaging, cardiac biomarkers, and advanced heart failure therapies. Clin Med Insights Cardiol 2014; 8 Suppl 1: 67-74.

[6] Wang S, Wang Z, Chen D, Chen M, Lin Y, Liu Z, Zhang L, Wen C, Wang X and Ma J. Effect of acute paraquat poisoning on CYP450 isoforms activity in rats by cocktail method. Int J Clin Exp Med 2015; 8: 19100-19106.

[7] Liu YF, Zha BS, Zhang HL, Zhu XJ, Li YH, Zhu J, Guan XH, Feng ZQ and Zhang JP. Characteristic gene expression profiles in the progression from liver cirrhosis to carcinoma induced by diethylnitrosamine in a rat model. J Exp Clin Cancer Res 2009; 28: 107.

[8] Lewis DF. P450 structures and oxidative metabolism of xenobiotics. Pharmacogenomics 2003; 4: 387-395.

[9] Wang X, Chen M, Chen X, Ma J, Wen C, Pan J, Hu L and Lin G. The effects of acute hydrogen sulfide poisoning on cytochrome P450 isoforms activity in rats. Biomed Res Int 2014; 2014: 209393.

[10] Zhou ZX, Wang LP, Song ZY, Saari JT, McClain CJ and Kang YJ. Zinc supplementation prevents alcoholic liver injury in mice through attenuation of oxidative stress. Am J Pathol 2005; 166: 1681-1690.

[11] Hu LF, Xu RA, Wang Z, Chen XL, Ma JS, Pan JC and Wang XQ. Simultaneous determination of four cytochrome P450 probe drugs in rat plasma by a simple liquid chromatography-mass spectrometry method. Latin American Journal of Pharmacy 2012; 31: 889-895.

[12] Wu Q, Zhang Q, Wen C, Hu L, Wang X and Lin G. The effect of MS-275 on CYP450 isoforms activity in rats by cocktail method. Int J Clin Exp Pathol 2015; 8: 9360-9367.

[13] Zhuang ZS, Tang MM, Zheng YM, Hu LF and Lin FY. Effect of dimethoate on the activity of hepatic CYP450 based on pharmacokinetics of probe drugs. Pharmacology 2015; 95: 243-250.

[14] Foti RS, Rock DA, Wienkers LC and Wahlstrom JL. Selection of alternative CYP3A4 probe substrates for clinical drug interaction studies using in vitro data and in vivo simulation. Drug Metab Dispos 2010; 38: 981-987.

[15] Martignoni M, Groothuis GMM and de Kanter R. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Opin Drug Metab Toxicol 2006; 2: 875-894.