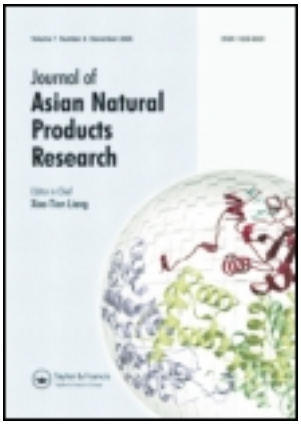

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Effects of bicyclol on the activity and expression of cytochrome P450 after hepatic warm ischemia/reperfusion in rats

Xiao-Min Yao [a b], Bao-Lian Wang [a], Hong-Yan Yu [a] & Yan Li [a]

[a] Department of New Drug Development, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100050, China

[b] Department of Pharmacy, Zhejiang Pharmaceutical College, Ningbo, 315100, China

Available online: 22 Jun 2011

To cite this article: Xiao-Min Yao, Bao-Lian Wang, Hong-Yan Yu & Yan Li (2011): Effects of bicyclol on the activity and expression of cytochrome P450 after hepatic warm ischemia/reperfusion in rats, Journal of Asian Natural Products Research, 13:7, 634-644

To link to this article: http://dx.doi.org/10.1080/10286020.2011.583643

Journal of Asian Natural Products Research
Vol. 13, No. 7, July 2011, 634–644

Effects of bicyclol on the activity and expression of cytochrome P450 after hepatic warm ischemia/reperfusion in rats

Xiao-Min Yao[ab], Bao-Lian Wang[a], Hong-Yan Yu[a] and Yan Li[a]*

[a]*Department of New Drug Development, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China;* [b]*Department of Pharmacy, Zhejiang Pharmaceutical College, Ningbo 315100, China*

(*Received 1 March 2011; final version received 20 April 2011*)

Bicyclol is a synthetic antihepatitis drug with antioxidative property. This study was designed to investigate the effects of bicyclol on the activity, gene and protein expressions of hepatic microsomal cytochrome P450s (CYPs) during hepatic ischemia and reperfusion (I/R) in rats. The rats were subjected to 90 min of hepatic ischemia followed by reperfusion for 3 and 24 h. Bicyclol (300 mg/kg) was orally administered three times before hepatic ischemia in rats. Liver injury was evaluated by biochemical examinations. Hepatic microsomal malondialdehyde (MDA) was measured spectrophotometrically. Total hepatic CYP content and activities of four CYP isozymes were evaluated with a differential spectrophotometer and liquid chromatography–mass spectrometry analysis. The gene and protein expressions of four CYP isozymes were determined by reverse transcriptional-polymerase chain reaction and Western blotting assay. As a result, bicyclol significantly inhibited the elevation of serum alanine aminotransferase and hepatic microsomal MDA and prevented the decrease of total hepatic CYP content in I/R rats. In addition, bicyclol markedly attenuated the decrease of 2C6, 2C11, 3A1/2 activity and reduction of mRNA or protein expression of 2C6 and 3A in I/R rats. As for CYP2E1, bicyclol further aggravated the decrease of protein expression in I/R rats. These results suggested that bicyclol may ameliorate the abnormalities in the activity and expression of certain CYP isoforms during I/R, and this protective effect was likely due to its antioxidative and hepatoprotective properties.

Keywords: ischemia–reperfusion; bicyclol; liver; antioxidant; cytochrome P450

1. Introduction

Hepatic ischemia–reperfusion (I/R) injury is implicated in the pathogenesis of a variety of clinical conditions, such as liver resection, liver transplantation, hypovolemic shock, and trauma [1]. Reoxygenation of ischemic liver leads to the generation of reactive oxygen species (ROS), which are thought to be one of the earliest and most important factors responsible for hepatic I/R injury [2].

ROS were known to cause direct cellular damage via protein oxidation and degradation, lipid peroxidation, and DNA damage. It has been reported that cytochrome P450 (CYP) is susceptible to peroxidative damage [3]. For example, several chemical agents may stimulate lipid peroxidation, which results in CYP degradation, and purified CYP is destroyed in the presence of various lipid peroxides [4]. A number of previous studies have shown that hepatic microsomal CYP plays an important role in the biotransformation of various substances, such as the synthesis of steroid hormones and prostaglandins,

*Corresponding author. Email: yanli@imm.ac.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2011.583643
http://www.informaworld.com

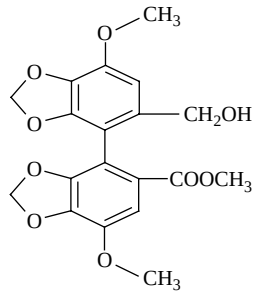

Figure 1. Chemical structure of bicyclol.

activation of vitamin D_3 and bile acids, and metabolism and detoxication of drugs [5]. Therefore, the generation of ROS after hepatic I/R may damage CYP protein (heme protein) and then influence the liver function.

Bicyclol (Figure 1) is a synthetic antihepatitis drug for the treatment of chronic hepatitis B patients in clinic [6]. It has been demonstrated that bicyclol has significant pharmacological properties including antioxidation, anti-inflammation, and antiapoptosis [7–8]. In addition, the effect of bicyclol on metabolic enzymes, especially hepatic CYP activity, has been reported. After multiple administration of bicyclol, CYP2E1 activity in rats was mildly induced, whereas the activities of CYP2C and 2D were inhibited in certain extents [9]. However, there is limited information about the effect of bicyclol on individual CYP isoforms during liver injury.

The purpose of this study was to investigate the effect of bicyclol on hepatic I/R injury, especially the profile of major CYP isozyme activity, and gene/protein expressions.

2. Results and discussion

2.1 Effect of bicyclol on serum alanine aminotransferase and lipid peroxidation in hepatic I/R rats

To assess the effect of bicyclol on hepatic injury induced by I/R, serum alanine aminotransferase (ALT) was detected at 3 and 24 h after reperfusion. The results showed that serum ALT was raised by 29.8-fold at 3 h and 2.5-fold at 24 h after reperfusion in I/R rats compared with those of sham-operated (SM) rats. Bicyclol pretreatment significantly inhibited the elevation of serum ALT at different time points after reperfusion.

Our data also indicated that lipid peroxidation occurred in liver microsomes of I/R rats, because a significant increase (24 and 38%) of malondialdehyde (MDA) content was found at both 3 and 24 h after reperfusion. Bicyclol showed a remarkable inhibition on the elevation of liver microsomal MDA in I/R rats (Table 1).

2.2 Effect of bicyclol on liver microsomal CYP content in hepatic I/R rats

Hepatic microsomal CYP content was found to be significantly decreased by 32

Table 1. Effect of bicyclol on serum ALT and lipid peroxidation of liver microsomes in I/R rats.

	ALT (IU/l)		MDA (nmol/mg protein)	
Groups	3 h	24 h	3 h	24 h
SM	37.1 ± 3.6	76.9 ± 13.1	4.27 ± 0.41	4.43 ± 0.32
I/R	1107.2 ± 118.9$^{\#\#}$	192.6 ± 43.0$^{\#\#}$	5.31 ± 0.38$^{\#}$	6.10 ± 1.02$^{\#\#}$
Bicyclol	131.9 ± 17.4**	98.2 ± 38.7**	4.08 ± 0.76**	4.87 ± 0.38*

Notes: Bicyclol (300 mg/kg) was administered three times before operation. Samples were collected at 3 and 24 h after reperfusion.

$^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. SM; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. I/R.

and 45% at 3 and 24 h after reperfusion in I/R rats, compared with that in SM rats. Bicyclol administration markedly prevented such decrease (Figure 2).

2.3 *Effect of bicyclol on the activities of CYP isozyme in hepatic I/R rats*

As shown in Table 2, the activities of hepatic microsomal CYP2C6, 2C11, and 3A1/2 were significantly reduced by 50, 21, and 20% at 24 h after reperfusion, although no changes were observed at 3 h in I/R rats. These decreases (CYP2C6, 2C11, and 3A1/2) were markedly ameliorated by bicyclol pretreatment in certain extents. Moreover, a 16% decrease in CYP2E1 activity was found at 3 h after reperfusion, which was further decreased to 60% at 24 h, whereas bicyclol had no effect on the decrease of CYP2E1 in I/R rats.

2.4 *Effect of bicyclol on mRNA expressions of CYP isozyme in hepatic I/R rats*

The mRNA expression of CYP2C6 in I/R rats was dramatically decreased by 13 and 32% at 3 and 24 h after reperfusion, which was attenuated by bicyclol pretreatment, respectively. A similar reduction (18%) of CYP3A1 was also found at late stage of reperfusion, and bicyclol showed a strong inhibition on the reduction of CYP3A1 in I/R rats. In addition, no changes on the mRNA expressions of CYP2C11, 2E1, and 3A2 were observed in I/R rats after reperfusion (Figure 3).

2.5 *Effect of bicyclol on the protein expressions of CYP2E1 and 3A in hepatic I/R rats*

The protein expression of CYP2E1 in liver microsomes was downregulated at 3 and 24 h after reperfusion, whereas bicyclol can further reduce CYP2E1 protein expression in I/R rats. In addition, no significant change of CYP3A expression was observed at 3 h after reperfusion, but CYP3A expression was markedly reduced at the late phase (24 h) in I/R rats. Bicyclol pretreatment was found to suppress the reduction in CYP3A expression significantly (Figure 4).

2.6 *Discussion*

Liver injury after hepatic I/R comprises two distinct phases [10], i.e. the acute phase of injury, which is mainly characterized by increased production of oxygen radical species at 3–6 h after reperfusion, and a subacute inflammatory phase, which ensues at 18–24 h after reperfusion and leads to the cellular damage mediated primarily by neutrophil infiltration.

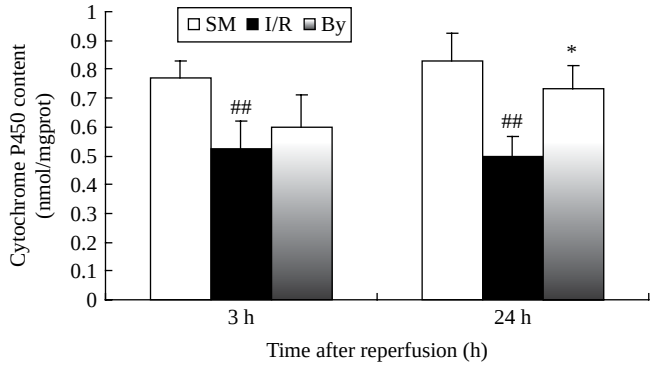

Figure 2. Effect of bicyclol on total CYP content in hepatic I/R rats ($n = 8$). Bicyclol (300 mg/kg) was given orally to rats three times before operation. Liver microsomes were collected at 3 and 24 h after reperfusion. $^{\#\#}P < 0.01$ vs. SM; $^{*}P < 0.01$ vs. I/R.

Table 2. Effect of bicyclol on hepatic microsomal CYP isozyme activities in I/R rats.

Groups	CYP2C6 (pmol/mg protein/min)		CYP2C11 (pmol/mg protein/min)		CYP2E1 (pmol/mg protein/min)		CYP3A1/2 (pmol/mg protein/min)	
	3 h	24 h	3 h	24 h	3 h	24 h	3 h	24 h
SM	1070.2 ± 34.3	1103.4 ± 109.8	20.1 ± 6.0	20.6 ± 4.0	626.8 ± 44.0	615.7 ± 85.1	127.0 ± 10.0	126.5 ± 10.8
I/R	1033.9 ± 233.0	546.8 ± 74.6$^{\#\#}$	20.2 ± 5.9	16.2 ± 1.9$^{\#\#}$	528.9 ± 57.4$^{\#}$	369.2 ± 50.0$^{\#\#}$	144.5 ± 18.2	101.2 ± 11.7$^{\#\#}$
Bicyclol	767.7 ± 85.7	766.0 ± 182.0*	16.2 ± 3.6	17.2 ± 1.6*	518.3 ± 46.5	420.4 ± 104.9	123.3 ± 6.23	165.9 ± 25.9**

Notes: Bicyclol (300 mg/kg) was administered three times before operation. Liver microsomes were collected at 3 and 24 h after reperfusion. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. SM; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. I/R.

The results of this study demonstrated that bicyclol had an overall protective effect against hepatic I/R injury in both acute and subacute phases by the inhibition of ALT elevation, attenuation of lipid peroxidation, and improvement of the activities and expressions of CYP isozymes.

A previous study has shown that ROS is generated and released from hepatocytes, Kupffer cells, and the adherent leukocyte-sinusoidal endothelium system after reperfusion [11]. Antioxidative systems, such as superoxide dismutase (SOD) and glutathion (GSH), can offer protection from cell damage by scavenging superoxide anion radical in the upper stream of reactive oxygen metabolism cascade. Our previous study has shown that bicyclol can offer an antioxidative protection by restoring SOD activity in I/R rats [12]. In this study, lipid peroxidation in liver microsomes was significantly increased after reperfusion, which was consistent with previous study [12]. Bicyclol showed a remarkable protective effect, as indicated by the alleviation of the formation of hepatic microsomal MDA, a marker of ROS-mediated lipid peroxidation.

It is well known that serum ALT level is a common biomarker for hepatocytes damage. In addition, total hepatic CYP content may partially reflect the ability of detoxification and metabolism mediated by drug-metabolizing enzymes. Our results indicated that serum ALT level in I/R rats was strikingly increased at acute phase after reperfusion. With the increase in reperfusion time, the elevation in serum ALT level declined, but it still remained higher than that in SM rats. In contrast, total hepatic microsomal CYP content decreased at 3 h and further aggravated at 24 h after reperfusion, which was consistent with previous studies [13–14]. Bicyclol not only attenuated the hepatocellular damage by the inhibition of elevated serum alanine aminotransferase (sALT) but also improved hepatic drug-metabolizing

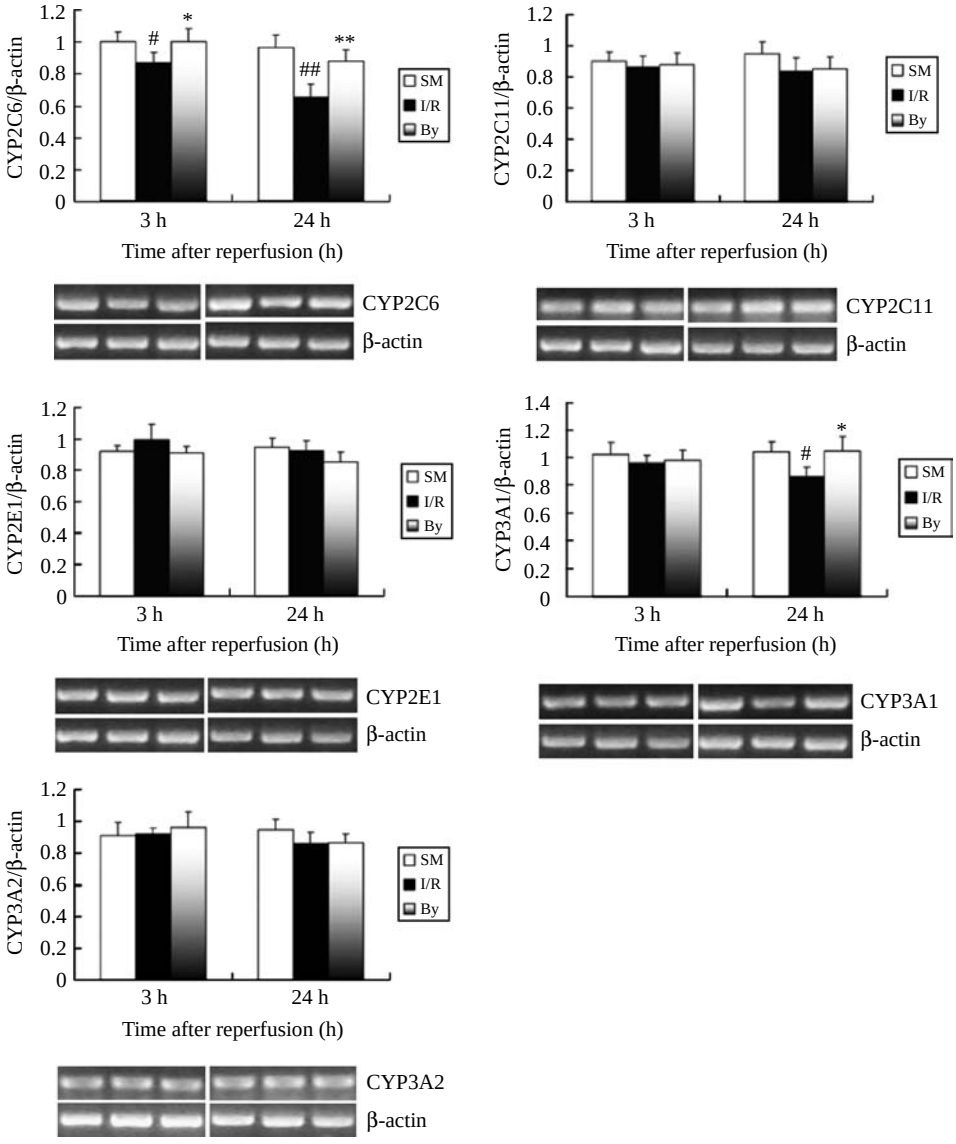

Figure 3. Effect of bicyclol on the mRNA expression of CYP isozymes in hepatic I/R rats. Bicyclol (300 mg/kg) was administered orally three times before operation. Lane 1, SM-3 h; Lane 2, I/R-3 h; Lane 3, By-3 h; Lane 4, SM-24 h; Lane 5, I/R-24 h; Lane 6, By-24 h. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. SM; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. I/R.

dysfunction by increasing the total CYP content.

Hepatic CYP enzymes, a key factor in drug elimination, are involved in clinically significant inhibitory and inductive drug interactions. Isozymes belonging to CYP2C subfamily were known to participate in the process of drug metabolism, such as warfarin, mephenytoin, tolbutamide and so on. Previous studies have demonstrated that the generation of ROS during hepatic I/R may damage CYP protein. In addition to ROS, cytokines, such as tumor necrosis factor-α (TNF-α), are also believed to be

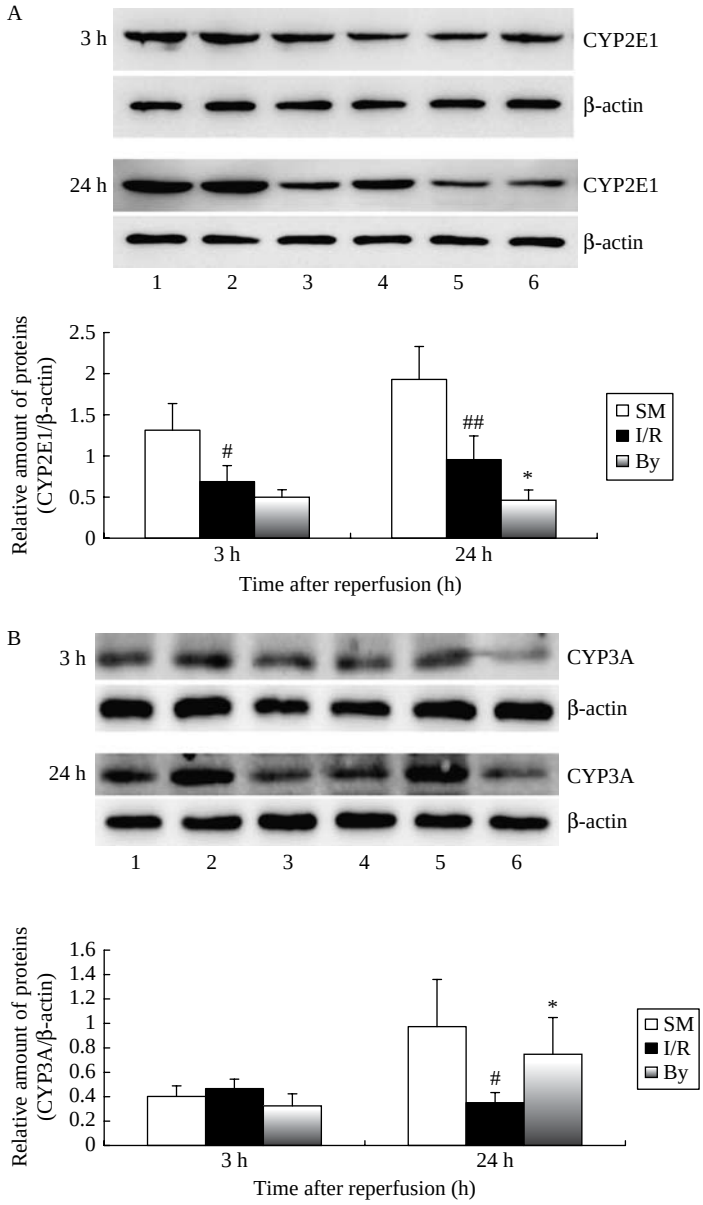

Figure 4. Effect of bicyclol on the expression of CYP2E1 and 3A in hepatic I/R rats ($n = 8$). Bicyclol (300 mg/kg) was administered orally three times before operation. Lane 1–2, SM; Lane 3–4, I/R; Lane 5–6, bicyclol. A, CYP2E1; B, CYP3A.

involved in the regulation of CYP isozymes [15]. TNF-α downregulates CYP2C11 and 3A2 in rat liver, whereas two other constitutive CYPs, 2A1 and 2C6, seem to be refractory to TNF-α [16]. The activities of both CYP2C6 and 2C11 were remarkably reduced after reperfusion in this study, whereas the extent of CYP2C6 reduction at activity and mRNA levels was much more than that of CYP2C11 in I/R rats. Our previous study has demonstrated that bicyclol can alleviate the secretion of

inflammatory factors, such as TNF-α, and inhibit oxidative stress to protect against hepatic I/R injury in rats [12]. Therefore, bicyclol pretreatment might attenuate the decrease in their expressions and activities through its antioxidative and anti-inflammatory properties.

CYP2E1, an ethanol-inducible isoform for the oxidation of small molecular compounds, is considered to contribute the hepatotoxicity induced by those chemical toxins [17]. Induction of CYP2E1 leads to several folds of increase in ROS formation and lipid peroxidation. CYP2E1 inhibitors, such as cyclosporine A and diallyl sulfide, have been shown to reduce ethanol-induced liver injury [18–19]. In this study, the activity of CYP2E1 was significantly suppressed at both early and late phases after reperfusion, with a coincidental reduction in its protein level, whereas the mRNA expression of CYP2E1 was unchanged in I/R rats. The above results were consistent with the previous studies [20–21]. Bicyclol was found to further reduce the protein expression of CYP2E1, whereas it had no influence on the activity of CYP2E1. The above results, in combination with oxidative injury induced by I/R, suggested that ROS may involve in the downregulation of CYP2E1 activity at the translational level. Interestingly, bicyclol could only downregulate the expression of CYP2E1, but it had no influence on the decrease in CYP2E1 activity. The exact underlying mechanisms, such as protein inactivation, degradation, and synthesis, need further investigation.

CYP3A subfamily plays an important role in the metabolism of xenobiotics and the first pass elimination of many drugs. The activity of CYP3A1/2 was strikingly diminished after reperfusion in I/R rats, which was consistent with the previous studies [13]. Moreover, the mRNA expression of CYP3A1 was also markedly decreased at the same time, whereas that of CYP3A2 was unchanged. The change in CYP3A protein expression had a similar trend to the activity and mRNA expression. Bicyclol showed the protection on the decrease of CYP3A1 at all three levels. The above results suggested that the generation of ROS in I/R can modulate the transcription of CYP3A1 rather than CYP3A2, whereas bicyclol attenuated the decrease in CYP3A1/2 activity and expression due to its antioxidative and anti-inflammatory properties.

In conclusion, bicyclol ameliorated the abnormalities of certain CYP isoforms at activity and expression levels during I/R, and this protective effect was likely due to its antioxidative and hepatoprotective properties. Although the production of ROS during I/R was believed to damage CYP isozymes, the individual CYP isozyme was differentially changed at activity, mRNA and protein levels. The complicated regulatory mechanism on CYP isozymes by bicyclol needs to be further investigated.

3. Materials and methods

3.1 Chemicals

Bicyclol was kindly provided by Beijing Union Pharmaceutical Plant (Beijing, China), and its purity is more than 99%. ALT assay kit was purchased from Beijing Chemical Plant (Beijing, China). Coomassie blue assay kit was obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Trizol was obtained from BioDev Tech Co., Ltd. (Beijing, China). Reverse transcriptional–polymerase chain reaction (RT–PCR) kit was obtained from Takara Biotechnology Co. (Osaka, Japan). CYP2E1 and 3A antibodies were the products from Abcam plc.322 Cambridge Science Park (London, UK) and Santa Cruz Biotechnology (Santa Cruz, CA, USA). The compounds mentioned below were all purchased from Sigma (St Louis, MO, USA): diclofenac, mephenytoin, chlorzoxazone, 4-hydroxydiclofenac, 4-hydroxymephenytoin, 6-hydroxychlorzoxazone, 1-hydroxymidazolam, and

NADPH. Midazolam was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Other chemicals were of analytical grade and were obtained from the local market.

3.2 Animals

Male Sprague-Dawley rats weighing 220–240 g were obtained from Beijing Vital River Experimental Animal Co., Ltd (Beijing, China). The animal study protocol was in compliance with the guidelines of China for animal care, which was conformed to internationally accepted principles in the care and use of experimental animals.

3.3 Hepatic I/R model

The segmental (70%) hepatic ischemia model was used in this study [22]. Briefly, a midline laparotomy was performed under anesthesia with 10% chloral hydrate (0.3 g/kg, intraperitoneally) in rats. All structures in the portal triad (hepatic artery, portal vein, and bile duct) of the left and median liver lobes were occluded with a microvascular clamp for 90 min, and then, the clamp was removed for reperfusion. The abdomen was immediately closed with continuous 4–0 silk suture. The animals were sacrificed at 3 and 24 h after reperfusion, and then, blood and liver tissue samples were collected for further analysis.

3.4 Animal treatment

Three groups of male rats were included in the study: SM group, in which rats underwent anesthesia, laparotomy, and exposure of the portal triad without hepatic ischemia ($n = 6$); I/R group, in which rats were subjected to I/R as described above ($n = 8$); bicyclol-pretreated (By) groups, in which rats were given bicyclol (300 mg/kg, suspended in 0.5% sodium carboxymethyl cellulose) orally for three times in two consecutive days, and ischemia was performed at 1 h after the last administration ($n = 8$). At the same time, the rats in SM and I/R groups received an equivalent volume of vehicle as control.

3.5 Preparation of liver microsomes

Liver tissues were homogenized in 50 mM Tris–HCl buffer (pH 7.4) containing 200 mM sucrose. The homogenates were centrifuged at 10,000 × g for 20 min. The resulted supernatant was further centrifuged at 105,000 × g for 60 min. Then, the pellet was resuspended with 50 mM Tris–HCl buffer (pH 7.4) containing 250 mM sucrose. All procedures were carried out at 4°C. Protein concentrations of liver microsomes were determined using a Coomassie blue assay kit. Microsomal fractions were stored at − 80°C for further analysis.

3.6 Detection of serum ALT and hepatic microsomal lipid peroxidation

Serum ALT levels and hepatic microsomal MDA contents were determined by using commercial assay kits according to the standard procedure.

3.7 Assay of CYP enzyme activity

Total CYP contents were quantified by the method of Omura and Sato [23]. The activities of CYP isozymes were determined by the formation of probe metabolites and liquid chromatography–mass spectrometry (LC–MS) methods after microsomal incubations as reported previously [24].

Microsomal incubations were performed according to the following method. In brief, the system of microsomal incubation contained 1 mg/ml microsomal proteins, 1.2 mM NADPH, 50 mM Tris–HCl (pH 7.4), 100 μM mephenytoin or 20 μM diclofenac, or 100/20 μM chlorzoxazone/midazolam in a final volume of 500 μl. The reaction was initiated by addition of NADPH after 5 min preincubation at 37°C. After a given incubation time (10 or 20 min),

the reaction was terminated by adding 500 μl of cold acetonitrile. Then, the samples were centrifuged at 14000 × *g* for 5 min, the supernatant was transferred to a disposable vial in an autosampler and 5 μl was injected for LC–MS analysis.

The LC separation was carried out with a Zorbax SB-C_{18} column (3.5 μm, 2.1 × 100 mm, Agilent, Santa Clara, CA, USA). The optimum ESI conditions included the nitrogen nebulizer pressure of 40–60 psi, the nitrogen drying gas temperature of 350°C at 7–9 l/min, and spray voltage of 4000–4500 V. Mobile phase, ion source, and multiple reaction monitoring for metabolites of the probe substrates are shown in Table 3.

3.8 RT–PCR analysis

Total RNA was extracted from liver tissue using Trizol reagent. cDNA was reverse transcribed from 0.5 μg of total RNA using an RT–PCR kit. Corresponding primer sets for PCR are shown in Table 4, and the final volume of the reaction was 40 μl. The samples were loaded into a thermal cycler after determining the optimal number of cycles. For each gene, the final cycle was followed by extension at 72°C for 10 min. RT–PCR products were subjected to electrophoresis using 1.2% agarose gel, and then, the bands were visualized with ethidium bromide and analyzed by Pro31 software. Results were expressed as ratios relative to β-actin (density of product/density of β-actin).

3.9 Western blot assay

Microsomal proteins (30 μg) were separated by 12% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membrane (Amersham, Inc., London, UK). Bands were then immunologically detected by specific primary antibodies to CYP2E1 (ab28146), CYP3A (sc-25845), or β-actin (sc-1616) and followed by incubation in horseradish peroxidase-conjugated

Table 3. CYP enzymes, substrate concentration, mobile phases, ion sources, and multiple reactions monitoring for probe substrates metabolites.

Enzyme	Substrate	Concentration (μmol/l)	Metabolite	Mobile phases	Ion source	Precursor (m/z)	Product (m/z)
CYP2C6	Diclofenac	20	4-Hydroxydiclofenac	Acetonitrile:water (50:50, 0.1% formic acid)	ESI(+)	312	268
CYP2C11	Mephenytoin	100	4-Hydroxymephenytoin	Methanol:water (45:55, 0.1% formic acid)	APCI(+)	235	150
CYP2E1	Chlorzoxazone	100	6-Hydroxychlorzoxazone	Acetonitrile:water (50:50, 0.005% ammonia water)	ESI(−)	184	148
CYP3A1/2	Midazolam	20	1-Hydroxymidazolam		ESI(+)	342	203

Table 4. PCR primer sets for CYP isozyme.

Gene	Primer sequences	Size (bp)	Annealing temperature (°C)	PCR cycle no.
CYP2C6	Forward CGGGAAGTCATACGACATTAGC Reverse GCAGAGAGGCAAATCCATTG	759	61	20
CYP2C11	Forward CTGCTGCTGCTGAAACACGTG Reverse GGATGACAGCGTACTATCAC	248	58	25
CYP2E1	Forward CTCCTCGTCATATCCATCTG Reverse GCAGCCAATCAGAAATGTGG	473	58	22
CYP3A1	Forward ATCCGATATGGAGATCAC Reverse GAAGAAGTCCTTGTCTGC	579	58	25
CYP3A2	Forward AGTAGTGACGATTCCAACATAT Reverse TCAGAGGATTCTGTGTTTCCT	252	58	27
β-actin	Forward TGGAATCCTGTGGCATCCATGAAAC Reverse TAAAACGCAGCTCAGTA-ACAGTCCG	200	62	25

anti-rabbit IgG antibody. Finally, the immunoreactive bands were visualized by enhanced chemiluminescence (ECL) Western blot detection system (Kodak, Inc., San Leandro, CA, USA).

3.10 Statistical analysis

All results expressed as mean ± SD were analyzed by one-way analysis of variance with SPSS 11.0 statistical software package. The differences between means were analyzed by Student–Newman–Keuls test for multiple comparisons. *P* value of less than 0.05 was considered statistically significant.

References

[1] H. Jaeschke, *J. Invest. Surg.* **16**, 127 (2003).

[2] C. Fondevila, R.W. Busuttil, and J.W. Kupiec-Weglinski, *Exp. Mol. Pathol.* **74**, 86 (2003).

[3] E. Jeffery, A. Kotake, and R.E. Azhary, *Mol. Pharmacol.* **13**, 415 (1977).

[4] R.S. Britton, B.R. Bacon, and R.O. Recknagel, *Chem. Phys. Lipids.* **45**, 207 (1987).

[5] A. Hiwatashi and Y. Ichikawa, *Biochim. Biophys. Acta.* **664**, 33 (1981).

[6] G.T. Liu, *Chin. J. New Drugs* **10**, 325 (2001).

[7] G.T. Liu, Y. Li, H.L. Wei, H. Zhang, J.Y. Xu, and L.H. Yu, *Liver Int.* **25**, 872 (2005).

[8] H.P. Wang and Y. Li, *Eur. J. Pharmacol.* **534**, 194 (2006).

[9] J.P. Hu, H. Chen, and Y. Li, *Chin. J. New Drugs* **18**, 340 (2009).

[10] S. Arii, K. Teramoto and T. Kawamura, *J. Hepatobiliary Pancreat. Surg.* **10**, 189 (2003).

[11] U. Rauen, R. Viebahn, W. Lauchart, and H. de Groot, *Hepatogastroenterology* **41**, 333 (1994).

[12] X.M. Yao, H. Chen, and Y. Li, *Hepatol. Res.* **39**, 833 (2009).

[13] K. Izuishi, H. Wakabayashi, T. Maeba, M. Ryu, and H. Maeta, *World J. Surg.* **24**, 49 (2000).

[14] H.A. Eum and S.M. Lee, *Br. J. Pharmacol.* **142**, 35 (2004).

[15] P.C. Kuo, R.A. Schroeder, and J. Loscalzo, *J. Pharmacol. Exp. Ther.* **282**, 1072 (1997).
[16] L. Nadin, A.M. Butler, G.C. Farrell, and M. Murray, *Gastroenterology* **109**, 198 (1995).
[17] Y. Lu and A.I. Cederbaum, *Fre. Rad. Biol. Med.* **44**, 723 (2008).
[18] M. Morimoto, A.L. Hagbjörk, Y.J. Wan, P.C. Fu, P. Clot, E. Albano, M. Ingelman-Sundberg, and S.W. French, *Hepatology* **21**, 1610 (1995).
[19] A. Dey and A.I. Cederbaum, *Hepatology* **45**, 1355 (2007).
[20] I.H. Shaik, J.M. George, T.J. Thekkumkara, and R. Mehvar, *Pharm. Res.* **25**, 2231 (2008).
[21] K. Pahan, B.T. Smith, A.K. Singh, and I. Singh, *Fre. Rad. Biol. Med.* **23**, 963 (1997).
[22] Y. Nakashima, H. Kohno, O.N. EL-Assal, D.K. Dhar, T. Ono, A. Yamanoi, and N. Nagasue, *Hepatol. Res.* **27**, 67 (2003).
[23] T. Omura and R. Sato, *J. Biol. Chem.* **239**, 2370 (1964).
[24] D. Smith, N. Sadagopan, M. Zientek, A. Reddy, and L. Cohen, *J. Chromatogr. B Analyt Technol Biomed Life Sci.* **850**, 455 (2007).