Interaction between traditional Chinese medicine and western medicine in rats — In-Chen-How and acetaminophen

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Abstract: The purpose of this study is to evaluate the interaction effects of In-Chen-How (*Artemisia capillaries* Thunb.) on the pharmacokinetics of acetaminophen and on liver microsomal cytochrome P450 enzyme activity in rats. The rats were divided into control group ($n = 8$) without In-Chen-How and the pretreated group ($n = 8$) administered with In-Chen-How (approximately 1.0 mL·kg^{-1}, according to weight) for 5 consecutive days. Rats in the control group received water simultaneously. Each rat was then given acetaminophen. The pharmacokinetic parameters of acetaminophen of the two groups were significantly different. In the In-Chen-How pretreated group, the maximum concentration of acetaminophen and the area under the plasma concentration-time curve were reduced about 58.4%, 56.7% and 55.4%. To further explain the results, liver microsomal suspensions were obtained from rats that were randomly divided into control and In-Chen-How pretreated group. The levels of CYP1A2 and CYP2E1 in hepatic microsomal protein from pretreated group were increased as compared to that from the control group. It indicated that In-Chen-How can stimulate the activity of CYP isozymes. The changes in the pharmacokinetics of acetaminophen resulting from the administration of In-Chen-How are related to an increase in metabolic activity of CYP1A2 and CYP2E1.

Key words: In-Chen-How; acetaminophen; interaction

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中西药相互作用研究:茵陈蒿与对乙酰氨基酚

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关键词: 茵陈蒿; 对乙酰氨基酚; 相互作用

The international communities have placed increasing emphasis upon the use of traditional Chinese medicine (TCM) in recent years. Traditional Chinese medicines are believed by many to be safe and used for self-medication without supervision. Although the report of occurrence of adverse effects is rare, traditional Chinese medicine contains many compounds that influence the activity of cytochrome P450 isoforms. Modulation of CYP activity may cause pharmacokinetic changes in other drugs, resulting in a decrease in efficacy and increase in adverse effects[1]. Preliminary *in vitro* studies demonstrate that some TCM can inhibit CYP450 2C9, 2C19, 2D6 and 3A4 mediated-metabolism. Individuals taking a number of conventional and TCM products would be expected to be at a higher risk of experiencing a clinically

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significant adverse event due to interaction, particularly the increase of duration of use[2]. Some herbal products may potentially benefit people with liver disease; however, these "benefits" may result in a diagnostic delay and perpetuation or exacerbation of liver injury, especially when concomitant agents that induce cytochrome P450 enzymes may also increase individual susceptibility[3].

In-Chen-How is a commonly used Chinese medicine and is considered as a new choleretic[4]. It is used as an antipyretic and cholelitholytic to alleviate jaundice and acute hepatitis[5]. Many investigators of Japan found that In-Chen-How may have antihyperlipidemia activity, induce apoptosis in cultured rat hepatic stellate cells and suppress NF-kappaB activation[6-8]. In-Chen-How used in our study was purchased from Ko Da pharmaceuticals manufacturer, Taiwan. There is no report regarding the interactions on the concurrent use of In-Chen-How and western medicine. The main active components of In-Chen-How are scoparone and capillarisin which have been analyzed by high performance layer chromatography with fluorescence detector. The content of scoparone is about 0.005 6 mg• g^{-1}.

Acetaminophen (APAP) is mainly eliminated at a therapeutic dose through glucuronidation and sulfatation and a small fraction is oxidized by cytochromes P450 (CYP) 2E1, 3A4, and 1A2 to *N*-acetyl-p-benzoquinone-imine (NAPQI), which further conjugated with glutathione and then metabolized to APAP-cystein. If APAP overdose, the glucuronidation and sulfatation pathways are saturated and the production of NAPQI increases, causing hepatic injury[9].

Previous studies conducted by our laboratory showed that the effect of concurrent use of In-Chen-How and acetaminophen did not only provide no beneficial effect over acetaminophen-induced hepatic toxicity, but even exacerbated the adverse effects, such as the increase of mortality rate and serum alanine aminotransferase (ALT) /aspartate aminotransferase (AST) levels, and aggravations in the extent of hepatic tissue necrosis and depletion of glutathione[10]. In clinical practice, acetaminophen is commonly prescribed as an antipyretic and In-Chen-How is also commonly prescribed by traditional Chinese practitioner as an antipyretic and cholelitholytic. Chinese people also like to buy TCM from herb stores. It is very important to provide information about possible pharmacokinetic interactions between In-Chen-How and acetaminophen and its effects on CYP activity.

In this study, the effects of In-Chen-How on the pharmacokinetics of acetaminophen were studied in rats and the effects of In-Chen-How on CYP isozyme activity were also studied for further elucidation.

Materials and methods

Chemicals and reagents Acetaminophen (panadol 500 mg) tablets were obtained from Glaxo Smith Kline Pharmaceutical Company. In-Chen-How concentrated pharmaceutical powder (*Artemisia capillaries* Thunb.) was purchased from Ko Da pharmaceuticals manufacturer, Taiwan. All other chemicals used were of the highest purity available.

Animals Sixteen male Wistar rats, 2 months old, 200 - 250 g, were obtained from the National Laboratory Animal Breeding and Research Center, National Science Council, Taiwan. The rats were randomly divided into the control group, In-Chen-How free and In-Chen-How pretreated group. Rats were maintained in a clean room (Animal Center for Pharmaceutical Research, Institute of Medical Materia, Chia Nan University of Pharmacy and Science) at a temperature of 20 ℃, with light-dark cycle and 50% relative humidity. Rats were individually house in metabolic cages with a supply of filtered pathogen-free air. The rats of two groups were fed with water and standard rat chow (PMI Feeds Inc) every day. The rats in the control group were given plain water (1.0 mL• kg^{-1}) and rats in the pretreatment group were given In-Chen-How suspension (approximately 1.0 mL• kg^{-1}, according to weight) by artificial feeder at 8 AM, 1 PM, and 6 PM everyday for 5 consecutive days (food was removed 2 h before artificial feeding and at night so that food was limited during this period; there was no restrictions on water supply). The Animal Care and Use Committee of Institute of Medical Materia, Chia Nan University of Pharmacy and Science, approved the animal study protocol.

Preparation of In-Chen-How concentrated powder In-Chen-How concentrated powder 1 mg was accurately weighted and dissolved with enough water in 50 mL container and 50% methanol 40 mL was added and shakes with ultrasound shaker for 30 min, which was kept at 25 ℃. The solution was diluted with 50% methanol and centrifuged for 10 min and then filtered. After removing the methanol under 0.45 μm filter, the remaining aqueous solution was used to analyze the scoparone, main active component of In-Chen-How.

Scoparone was quantified with high performance liquid chromatography and fluorescence detector.

Preparation of acetaminophen suspension Acetaminophen tablets 500 mg were chopped into fine powder using pestles, then sodium carboxymethyl cellulose (CMC-Na) and Arabic gel were added, plus enough water to yield 10 mg· mL^{-1} suspension; the mixture was shaken well before used.

Pharmacokinetic experiments On the morning of day 6, after feeding In-Chen-How to the pretreated group for 5 consecutive days, each rat was anesthetized with ketamine (60 - 90 mg· kg^{-1}). Femoral arteries were annulated using polyethylene catheter (PE-50) and diazepam (5 mg· kg^{-1}) was administered to maintain stable anesthesia. Acetaminophen suspension 500 mg· kg^{-1} was administered by gastric gavages to the control rats (n = 8) and to the rats pretreated with In-Chen-How (1.0 mL· kg^{-1} daily for 5 days, n = 8).

A blood sample was withdrawn into heparinized tubes before acetaminophen administered and other blood samples were with drawn at 15 min, 30 min, 60 min, 90 min, 2 h, 3 h, 4 h, and 6 h separately after acetaminophen administered. The plasma was immediately centrifuged at 10 000 rpm for 10 min to obtain serum. Serum samples were stored under - 20 ℃ before TDx immunoassay.

The plasma acetaminophen concentrations were determined by Abbot TDx Analyzer (IL, USA). TDx/TDxFLx acetaminophen reagents (IL, USA) were added to 100 μL of serum sample. Abbott TDx Analyzer was set at 485 nm for excitation wavelength and 525 - 550 nm for radiation wavelength. The strength of radiation was used to calculate the concentration of testing substance in the sample. Prior to measurements, the calibration curve was drawn using 0.0, 10.0, 20.0, 50.0, 100.0 and 250.0 μg· mL^{-1} standard samples. After every 10 samples were tested, the analyzer was standardized with 150, 35, and 15 μg· mL^{-1} of control serum to ensure accuracy of reading.

Preparation of rat liver microsomes Rats of the control group (n = 8) and the pretreated group (n = 8) after 5 days of In-Chen-How feeding were sacrificed and livers were immediately removed using method developed by Mitchell *et al*[11], rinsed with ice-cold sodium chloride solution (0.9%) and homogenized in triple volumes of 1.15% KCl (Teflon pestle-glass homogenizer) and run 6 times. KCl was added to a solution 4 times the original tissue volume. The homogenate was centrifuged at 9 000 × g under 4 ℃ for 20 min using Beckman J2-21 (Beckman Instruments, Inc. Fullerton, CA; USA), in order to obtain supernatant containing microsomes; KCl solution was used again to make up to 4 times the original tissue volume, then centrifuged at 100 000 × g under 4 ℃ for 1 h to obtain microsomes in the deposited pellet; the microsome pellet was resuspended using KCl solution 4 times the original tissue volume and centrifuged at 100 000 × g under 4 ℃ for 1 h with Beckman LE-80 to obtain washed microsomes, which were stored in 20% glycerol / 0.1 mol· L^{-1} phosphate buffer under - 75 ℃[12].

Assays of CYP activity The induction of CYP1A2 and CYP2E1 enzymes by In-Chen-How has been studied by Western blotting. Discontinuous electrophoresis method developed by Laemmli[13] in this study. Acrylamide separation gel (7.5%) and acrylamide stacking gel (3%) were prepared by mixing different ratios of Tris-HCl buffer, acrylamide-bis and sodium dodecyl sulfate (SDS) solutions together and removed air, and then added ammonium perfulfate and TEMED to catalyze polymerization of acrylamide. Processing of samples involved boiling microsomes with 0.2 mol· L^{-1} Tris-HCl (pH 6.8), 0.3% glycerol, 6.25% SDS, 2.22 mmol· L^{-1} β-mercaptoethanol and 0.062 5% bromophenol blue for 3 min prior to electrophoresis. During electrophoresis, 20 mA and 40 mA were used to enhance separation. After electrophoresis, blotting of proteins was carried out according to methods developed by Towbin *et al*[14]. The nitrocellulose (NC) membrane was soaked with transfer buffer [including 25 mmol· L^{-1} Tris base with pH 8.3, 192 mmol· L^{-1} glycine and 20% (v/v) methanol]. The gel was placed flatly on the NC membrane and then covered by a sheet of filter paper that was also soaked with transfer buffer. The glass was gently pressed to remove any air bubble between the gel and the NC membrane. Then the sample was placed in a wet blotting trough at 4 ℃ and run at 0.1 A current for 16 h.

When blotting was completed, the NC membrane was placed into phosphate buffer saline (PBS; including 0.14 mol· L^{-1} NaCl, 8 mmol· L^{-1} Na_2HPO_4, 2.7 mmol· L^{-1} KCl and 1.5 mmol· L^{-1} KH_2PO_4) which contained 5% nonfat milk powder in 37 ℃ water bath and shaken for 1 h. The diluted 100 times of first-degree antibodies such as MAb 1-7-1

(anti-rat CYP1A2) and 1-98-1 (anti-rat CYP2E1) were added and allowed to sit at 4 ℃ for 16 h. After reactions occurred, the solution was washed with PBS which contained 0.5% Tween-20 for 15 min and repeated another 3 times to remove any non complementary bonds. Rabbit anti-mouse IgG (Pierce Chemical Co, Rockford, Ill) was added, diluting the solution 1 000 times and allowing reaction to take place under room temperature for 1 h, then washed with PBS containing 0.5% Tween-20 for 15 min with a total of 4 times. Lastly, enhanced chemiluminescence's (ECL) reagent (Amersham, Buckinghamshire, UK) was added and reaction was allowed for 1 min, then excess reagents were removed. The NC membrane was wrapped with cellophane, allowing 5 min for reactions, and then exposed on X-ray film. An automated film developer (X-OMAT M43, Kodak) processed the films and a digital image processing system ImageMaster (Pharmacia Biotech Ltd, Uppsala, Sweden) compared relative concentrations.

Statistical analysis One-way analysis of variance was used to estimate the differences of pharmacokinetic parameters between the control group and the pretreatment group. A difference with $P < 0.05$ was considered statistically significant. Plasma concentration-time data for individual rats were analyzed using PC NONLIN compartmental programs by nonlinear least squares regression analysis (SCI Software, Statistical Consultants Inc, Lexington, KY, USA).

Results

1 Pharmacokinetics of acetaminophen

Under the TDx immunoassay, the calibration curve for acetaminophen was linear over the concentration of 0.0, 10.0, 20.0, 50.0, 100.0, and 250.0 μg• mL^{-1}. The recovery rates for control serum samples of 150, 35, and 15 μg• mL^{-1} were 102.4%, 102.0%, and 101.2%, respectively.

The plasma concentration-time curves after a single oral administration of acetaminophen to rats are presented in Figure 1. The time to reach the maximum concentration was 0.371 and 0.603 h in the pretreated group and the control group respectively. The maximum concentration of acetaminophen decreased significantly from 199.875 to 116.803 μg• mL^{-1} by pretreatment with In-Chen-How.

The plasma concentration-time curves for both groups were adequately described by a first-order absorption two-compartment open model. The related

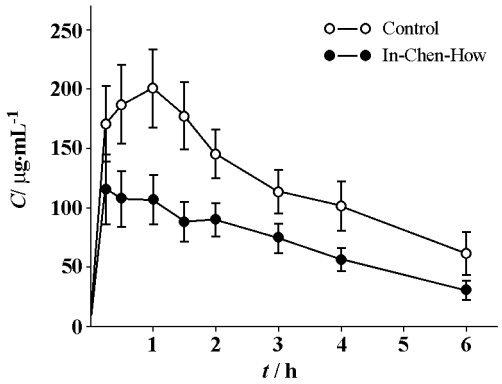

Figure 1 TDx analysis of plasma concentrations of acetaminophen after oral administration. Rats had been pretreated with In-Chen-How for 5 days before administration (•). Data represent mean ± SE of eight 2-month-old rats

data are summarized in Table 1. The elimination half-life ($T_{1/2}$) and area under the curve (AUC_{0-6} and $AUC_{0-\infty}$) were significantly different between the control group and the group pretreated with In-Chen-How. The $T_{1/2}$, C_{max}, AUC_{0-6}, and $AUC_{0-\infty}$ was reduced to approximately 99%, 58.4%, 56.7% and 55.4%, respectively, by pretreatment with In-Chen-How.

Table 1 Pharmacokinetic parameters of acetaminophen after oral administration to control rats and to rats pretreated with In-Chen-How

Parameter	Control group	In-Chen-How pretreated group
$T_{1/2}$ /h	3.047 ±0	3.018 ±0*
AUC_{0-6} /μg• mL^{-1}• h^{-1}	747.451 ±10.4	423.790 ±16.7*
$AUC_{0-\infty}$ /μg• mL^{-1}• h^{-1}	1 018.200 ±10.1	564.362 ±16.3*
T_{max} /h	0.603 ±0	0.371 ±0
C_{max} /μg• mL^{-1}	199.875 ±22.9	116.803 ±20.3
K_{01} /h^{-1}	5.510	10.732
K_{10} /h^{-1}	0.227 4	0.217 8

$n = 8$, mean ± SE. * $P < 0.001$ *vs* control group. AUC: Area under the curve; K_{01}, K_{10}: Intercompartmental rate constant; $T_{1/2}$: Drug half-life; T_{max}: Time point of C_{max}; C_{max}: Maximum concentration

2 CYP enzyme activity

Figure 2 shows the effects of In-Chen-How on the activity of CYP enzymes in rats. The immunoassay using MAb 1-7-1 to measure expression of CYP1A2 and MAb 1-98-1 to measure expression of CYP2E1 showed that after ingesting In-Chen-How, both CYP1A2 and CYP2E1 expressions increased.

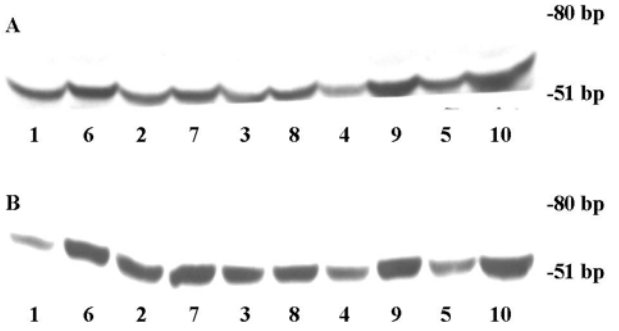

Figure 2　Immunoblot analysis of microsomal CYP enzymes in rat liver. Lanes 1, 2, 3, 4 and 5 represent hepatic microsomal protein from control rats. Lanes 6, 7, 8, 9 and 10 represent hepatic microsomal protein from In-Chen-How treated rats. Microsomal protein 25 μg was loaded for immunoreaction with mouse anti-rat CYP 1A2 (MAb 1-7-1, A). Microsomal protein 5 μg was loaded for immunoreaction with mouse anti-rat CYP 2E1 (MAb 1-98-1, B). Electrophoresis and immunodetection were carried out

Discussion

Traditional Chinese medicine is increasingly used as folk or supplementary medicine. It is usually co-administered with over-the-counter drugs by general public without any knowledge of the potential interactions that may occur. It is therefore important to obtain information about potential drug-drug interactions in order to assist people used TCM and over-the-counter drugs appropriately.

In this study, we found that the pharmacokinetics of acetaminophen changed when rats were pretreated with In-Chen-How. We assume that it is probably a result of an increase in CYP isozyme activity. The further experiments analyze the rat microsomal CYP isozymes in rat liver which showed that the levels of CYP1A2 and CYP2E1 in hepatic microsomal protein from pretreated group were increased as compared to that of the control group. It indicated that In-Chen-How can stimulate the activity of CYP isozymes. In addition, the pharmacokinetic parameters show that In-Chen-How can accelerate the metabolism of acetaminophen. The T_{max} decreased from 0.603 to 0.371 h; and the C_{max} reduced from 199.875 to 116.803 g·mL^{-1}. Based on the above results and the results of Western blotting analysis, we suppose that In-Chen-How may accelerated metabolism of acetaminophen by increasing the expression of CYP1A2 and CYP2E1. It is necessary to aware patients with this information when these two drugs used concurrently. More studies and further systematic studies are needed to investigate the mechanism of stimulation.

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