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The enhancement of cardiotoxicity that results from inhibiton of CYP 3A4 activity and hERG channel by berberine in combination with statins

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ABSTRACT

Metabolism of most endogenous and exogenous compounds is usually produced by the oxidation of cytochrome P450. Due to drug-drug interactions caused by the inhibition or induction of cytochrome P450 enzymes, changes in drug metabolism are the major causes of drug toxicity, CYP3A4 is one of the key isozymes, and involved in the metabolism of over 60% of clinical drugs. Human ether-a-go-go related genes (hERG) potassium channel is the most important target of many drugs and plays an important role in cardiac repolarization. Blockade of this channel may lead to long QT syndrome (LQTS), leading to sudden cardiac death. Therefore, it is necessary to evaluate the inhibitory properties of drugs on cytochrome P450 enzymes and hERG channel. We primarily evaluate the safety of berberine in combination with statins. Based on these findings, berberine in combination with statins has a greater inhibitory effect on CYP3A4 activity and CYP3A4 protein and mRNA expression than berberine alone. Simvastatin and atorvastatin reduce hERG current by accelerating channel inactivation. At the same time, the inhibitory effect of berberine and statin combination increased on hERG current by reducing the time constant of inactivation than the single drug alone. These results indicate that berberine in combination with statins can increase cardiotoxicity by inhibiting CYP3A4 and hERG channel.

1. Introduction

CYP enzymes are important oxidases that help metabolize about 75% of the commonly used drugs for clinical treatment [1]. Drug metabolism is divided into phase I metabolism and phase II metabolism. CYP enzymes are mainly involved in phase I metabolism of drugs [2]. Cytochrome has many subtypes. CYP3A4 is one of the key isozymes, widely distributed in the small intestine and liver, and involved in the metabolism of over 60% of clinical drugs [3]. Drug-induced CYP3A4 induction or inhibition may influence the pharmacokinetics of the drug with which they are co-administered, thereby altering their efficacy or toxicity [4].

Human ether-a-go-go related gene (hERG) encodes the potassium channel responsible for the rapid delayed rectifier potassium current (I_{Kr}), which plays an important role in the 3 phase repolarization of action potential duration [5]. hERG channel is an important target of many drugs [6]. Blockade of hERG channels can lead to long QT syndromes (LQTS), torsades de pointes, and then to sudden death [7]. The primary mechanism of drug-induced LQTS is the disruption of hERG potassium channel function [8–10].

Berberine is a kind of isoquinoline alkaloids extracted from the Chinese medicine berberine, with a variety of pharmacological effects. Berberine is widely used in medical treatment such as anti-inflammatory [11], anti-diabetic [12] and anti-hyperlipidemic [13]. Jiang et al. demonstrated that berberine reduces blood lipids by acting on stable low-density lipoprotein receptor in the 3' UTR regions, completely different from statins [13]. Berberine blocks hERG channels and prolongs action potential duration [14]. Berberine converts itself into dihydroerberine through the nitro reductase of the intestinal microflora. dihydroerberine is not stable in solution and recovers to BBR in intestine tissues via oxidization [15]. In addition, previous study also found that dihydroerberine also inhibits hERG channels [16]. Previous studies have shown that berberine inhibits CYP3A activity [17]. According to the results of our research group, berberine blocks open-channel hERG channels with an IC50 of 3.1 ± 0.5 μM [18]. Therefore, taking berberine in vivo can strongly inhibit hERG channel, which may lead to cardiotoxicity.

It is well known that statins such as simvastatin and atorvastatin are

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widely used in the treatment of hyperlipidemia [19]. Previous studies have shown that some statins can inhibit hERG channels and lead to LQTS [20]. However, the effects of atorvastatin and simvastatin on hERG currents remain unclear. At present, more attention is focused on the combination drug treatment due to the advantages of enhanced efficacy and reducing toxicity. Berberine can be used in combination with statins to treat hyperlipidemia [21,22]. Most statins are metabolized in the liver [23]. However, to our knowledge, there have been no previous studies investigating the effects of these two statins or in combination with berberine on CYP3A4 enzymes. The purpose of this study was to investigate the effects of combination of berberine and statins on CYP3A4 activity and hERG current, evaluate the possibility of heart toxicity, and provide guidance for the clinical application of co-administration of berberine and statins.

2. Materials and methods

2.1. Chemicals and reagents

Berberine (Sigma Chemical Co, St Louis, USA) was dissolved in 40 °C deionized water to obtain a 1 mM stock solution. Simvastatin (Sigma Chemical Co, St. Louis, USA) and atorvastatin (Sigma Chemical Co, St. Louis, USA) were dissolved in methanol to obtain a 1 mM stock solution. Nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) was purchased from Roche (Basel, Switzerland). 6β-hydroxytestosterone was obtained from Sigma-Aldrich (St. Louis, USA). Testosterone was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Cortisone acetate was purchased from China's food and drug inspection institute (Beijing, China). ReverTra Ace qPCR RT Kit and SYBR Green Realtime PCR Master Mix were purchased from Toyobo Co Ltd (Osaka, Japan). The CYP3A4 primers and GADPH pimers were purchased from Invitrogen Biotechnology Co Ltd (Calif, USA). CYP3A4 antibody was purchased from Santa Cruz Biotechnology (California, USA). Goat anti-mouse secondary antibodies were purchased from LI-COR (Lincoln, USA). Acetonitrile and methanol (Dikma, Beijing, China) used were of the HPLC grade.

2.2. hERG-HEK cell and $HepG_2$ cell culture

HEK293 cells stably expressing hERG gene and $HepG_2$ cells were cultured at 37 °C in a humidifited atmosphere of 5% CO_2 in DMEM medium with high glucose (DMEM, Hyclone, Logan, Utah, USA) containing 10% fetal bovine serum (FBS, Gibco, NY). The difference is that the medium contain 400 μg/ml gentamycin (G418, Invitrogen, USA) for the hERG-HEK cells and 1% pencilin-streptomycin solution for $HepG_2$ cells.

2.3. Rat liver microsomes (RLM) preparation

All rats used in this study were 200 ± 50 g male Sprague-Dawley rats from Harbin Medical University Animal Center (Harbin, China), housed and maintained in a temperature-controlled animal room with 12 h light/darkness cycle and free access to food. All animal experiments were approved by Harbin Medical University Ethics Committee (Harbin, China). RLM samples were prepared based on the method described in with only minor changes [24]. Rats were sacrificed by cervical dislocation and the liver removed immediately. Liver tissue was washed with ice-cold 0.9% NaCl solution, blotted with filter paper, weighed and homogenized in 4 vol of homogenization buffer (0.05 M Tris-HCl, pH = 7.4) in the homogenizer. The homogenate was centrifuged at 9000 g for 20 min and the supernatant was collected. It was further centrifuged at 100000 g for 60 min. All experiments were carried out at 4 °C. The precipitate is liver microsome and resuspended with 25% sucrose and stored at 80 °C until use. The RLM protein concentration was measured using the BCA method.

2.4. Liver microsomal incubation conditions

The effect of the drugs on CYP3A4 activity was tested by the formation of 6β-hydroxytestosterone by testosterone, microsomal incubation was performed in a total volume of 500 μL [25–27]. Briefly, the incubation mixture consisted of 1 mg/mL rat liver microsomes, 100 μM testosterone, 10 mM magnesium chloride, 100 mM potassium phosphate buffer, drug. The pre-incubation step was performed at 37 °C for 5 min. The reaction was started by adding NADPH enzyme. The concentration of berberine was 1–30 μM, simvastatin 1–5 μM, atorvastatin 1–4 μM. The reaction was incubated in a shaking incubator for 30 min. At the end of the incubation, the reaction was quenched by the addition of ice-cold acetonitrile and cortisone acetate. The mixture was then vortexed and then centrifuged at 13500 rpm for 15 min. Twenty microliters of supernatant was analyzed by HPLC. All experiments were in quintuplicate.

2.5. Assessment of CYP3A4 mRNA expression by RT-PCR

The effects of berberine and statins on CYP3A4 mRNA expression were determined by RT-PCR. Total RNA was extracted from HepG2 cells with trizol reagent (Invitrogen, USA) according to the instruction and was subjected to Dnase treatment by GeneAmp PCR System 9700 kit (Appliedbiosystems, USA) prior to RT-PCR. Primers were designed based on the CYP3A4 sequence from the NCBI website. The CYP3A4 primers used in the study were 5′-AAGTCGCCTCGAAGATACACA-3' (forward) and 5′-AAGGAGAGAACACTGCTCGTG-3' (reverse). Human GADPH primers were ATCATCCCT (forward) and CTGCTTCAC (reverse). The reaction consisted of 1 μL of 10 μM primer, 10 μL of SYBR Green Realtime PCR Master Mix (Toyobo, Japan), 100 ng of cDNA and deionized water to a volume of 20 μL. RT-PCR reaction program was 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s, 60 °C for 30 s, 72 °C for 30s. CYP3A4 mRNA expression was calculated as a fold change in GAPDH. All experiments were done in triplicate.

2.6. Western blot analysis

Forty-eight hours after drug treatment, 6-well plate cells were washed with pre-cooled physiologically buffered saline (PBS) and then added to each well with 100 μL of RIPA Lysis Buffer (Bi Yun Tian, China) supplemented with 1 μL of protease inhibitor. The cells were scraped from the plate with a cell scraper and transferred to a 1.5 mL EP tube. The cells were sonicated, then centrifuged at 13500 rpm for 15 min at 4 °C and the supernatant was collected. Protein concentration was measured by the BCA method. 100 μg CYP3A protein was seperated using SDS-PAGE with 10% (v/v) resolving gel. Briefly, fractionated proteins were transferred to nitrocellulose membrane (Pall Life Science, USA) and then blocked with 5% (w/v) skim milk for 2 h and incubated with primary antibody at 4 °C overnight, the membrane was washed three times for 10 min with PBST and then incubated with the corresponding secondary antibody for 1 h at room temperature. Membranes were washed three times again with PBST for 10 min and analyzed by Odyssey Infrared Fluorescence Scanning System (LI-COR, Lincoln, NE). The intensity of the protein band was quantified on the Scion Image software (Scion, Frederick, MD).

2.7. Cellular electrophysiology

Functional analysis of I_{hERG} was done using the whole-cell patch clamp technique as described previously [28]. The external solution contained: 136 mM NaCl, 5 mM HEPES, 1 mM $MgCl_2$ 6H2O, 1 mM $CaCl_2$ and 10 mM glucose (pH 7.4 with NaOH) and an internal pipette solution contained: 130 mM KCl, 1 mM $MgCl_2$.6H2O, 10 mM HEPES, 5 mM Mg-ATP, 5 mM EGTA and 0.1 mM GTP (pH 7.3 with KOH). Recordings were taken at room temperature using an Axopatch-200 B amplifier (Axon Instruments, Foster City, CA) controlled by PC/Amp