Abstract. The present study evaluated the ability of a Saccharomyces cerevisiae expression system to predict the pharmacokinetic (PK) activity of a calcium channel blocker in patients with distinct cytochrome P450 3A5 (CYP3A5) polymorphisms. The blood pressure lowering activity of amlodipine in 57 hypertensive patients with CYP3A5*1/*1, CYP3A5*1/*3, CYP3A5*4 and CYP3A5*6 polymorphisms was evaluated by the current study. Subsequently, a Saccharomyces cerevisiae expression system for CYP3A5 gene polymorphisms was constructed to examine the PK activity of amlodipine and was compared with the in vivo data from different gene polymorphism groups. The blood pressure lowering activity of amlodipine in hypertensive patients varied among CYP3A5 polymorphisms. The in vivo results demonstrated that CYP3A5*6 exhibited the highest metabolic rate, followed by CYP3A5*1/*1, CYP3A5*4 and CYP3A5*1/*3. The difference between CYP3A5*6 and CYP3A5*1/*1 was not statistically significant (P=0.5). In accordance with in vivo data, CYP3A5*1/*1 exhibited the highest in vitro metabolic rate, followed by CYP3A5*6 and CYP3A5*4. With the exception of the comparison between CYP3A5*6 and CYP3A5*1/*1, polymorphisms exhibited statistically significant differences compared with CYP3A5*1/*1 (P<0.05). The Saccharomyces cerevisiae expression system may be a cost effective and potentially useful tool for assessing the PK activity of drugs that are metabolized by CYP3A5.

Introduction

Cytochrome P450 (CYP) are a family of hemoproteins involved in multiple enzymatic reactions, such as drug metabolism. Previous studies have indicated that gene polymorphisms may be a major cause of the variability in drug metabolism that exists among individuals. The CYP3A subfamilies are the most abundant CYPs in the human liver and intestine. CYP3A catalyzes the metabolism of drugs, and contributes to variations in the bioavailability and clearance of CYP3A substrates (1-4). CYP3A4 is the most abundant hepatic and intestinal CYP3A isoform. Researchers previously thought that CYP3A4 was the primary contributor to metabolism within this family, however, this view was recently challenged by data that indicated that the relative contribution of CYP3A5 may represent >50% of total CYP3A in certain individuals and may exert a greater metabolic action than previously considered in individuals that possess at least one polymorphic CYP3A5 allele (3,5,6). The wild type allele of CYP3A5 is defined as CYP3A5*.1. Mutations include the following: CYP3A5*2; CYP3A5*3A; CYP3A5*3C; CYP3A5*4; CYP3A5*5; CYP3A5*6; and CYP3A5*7. The penetrance of polymorphic CYP3A5 genotypes varies among different ethnic groups and polymorphic CYP3A5 genotypes have varying pharmacokinetics (PK) and pharmacodynamic (PD) among CYP3A substrates, which include the calcium channel blocker, amlodipine (7,8). The effects of amlodipine vary depending on which CYP3A5 allele an individual has. For example, among Korean and Chinese patients that were treated with amlodipine, homozygous CYP3A5*3 carriers (2,9) exhibited a greater oral clearance of amlodipine and reduced mean area under the plasma concentration-time curve compared with CYP3A5*1 carriers (10-12). Additionally, Zhang et al (2) demonstrated that CYP3A5*3/*3 patients exhibited lower plasma levels of amlodipine compared with CYP3A5*1 patients, which indicated that patients with polymorphisms may have faster amlodipine metabolism and increased CYP3A enzyme activity (2). This heterogeneity in enzyme action indicates the requirement for a reliable method to assess the impact...
of polymorphisms in amlodipine metabolism. To investigate the impact of CYP3A5 genetic polymorphisms on amlodipine metabolism, the present study enrolled 57 Chinese hypertensive patients with four different polymorphisms: CYP3A5*1/*1 (n=7); CYP3A5*1/*3 (n=28); CYP3A5*4 (n=2); and CYP3A5*6 (n=2). Changes in the blood pressure of patients following administration of amlodipine were recorded. In addition, a well-investigated and cost-effective Saccharomyces cerevisiae expression system was constructed to validate the results obtained in the in vivo assessment.

Materials and methods

Patients and study design. The study protocol and consent for prospectively enrolled patients were approved by the Ethics Committee of the Third Xiangya Hospital (Changsha, China). Informed consent was obtained from all individual participants included in the study. The study included 57 hypertensive patients that were assigned to receive amlodipine from a single center in China between May 2012 and February 2013. Patients were included in the study if they were between the ages of 18 and 70, and diagnosed with a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg. Patients were excluded if they exhibited any of the following complications: Cerebrovascular accident history, ≥90 mmHg. Patients were excluded if they exhibited any of the following complications: Cerebrovascular accident history, heart failure, serious organic heart disease, large aneurysm and aortic dissection, second degree atrioventricular block, sick sinus syndrome, arrhythmia of clinical significance, ventricular tachycardia, atrial fibrillation or flutter, untreated thyroid disease, gastrointestinal lesions or prior gastrointestinal surgeries that may affect drug absorption, history of malignancy or any liver, kidney, heart and lung function congruent with a life-threatening illness, liver dysfunction, allergy to amlodipine, pregnancy or lactation, mental illness, active use of other antihypertensive drugs during the trial period or participating in other clinical trials within 3 months of enrolling in the current study. The suitability of all subjects was determined using medical history, routine physical examination, clinical laboratory testing, and measuring the heart rate and blood pressure. Following a 1-week washout period, baseline blood pressure values were recorded. All subjects received a single oral dose of 5 mg/day amlodipine (once 5 mg tablet; Pfizer Inc., New York, NY, USA) with water (200 ml) in the morning for 4 weeks. A series of 5 ml venous blood samples were collected into heparinized tubes from the antecubital vein of patients. Aliquots of plasma (2 ml) isolated from the resultant recombinant plasmids were transformed into E. coli JM109 using the CYP3A5 expression vector and cultured at 37˚C and 210 rpm. Subsequently, plasmids were extracted. Changes in the blood pressure of patients following administration of amlodipine were recorded. In addition, a well-investigated and cost-effective Saccharomyces cerevisiae expression system was constructed to validate the results obtained in the in vivo assessment.

Construction of CYP3A5 gene polymorphism expression vectors. The pYES2/CT vector was purchased from Invitrogen (Thermo Fisher Scientific, Inc.). All primers used in this study are presented in Table 1 (Shenzhen Huada Gene Science and Technology Co., Ltd., Shenzhen, China) (5). The total CYP3A5 RNA was extracted from human liver cells (collected from a 33 year old male patient who had undergone surgery and signed the informed consent) following the Qiagen RNeasy Mini Kit (Qiagen, GmbH, Hilden, Germany) protocol. The tissue sections were homogenized in RLT buffer supplemented with 2 M dithiothreitol using a 1 ml syringe and a needle. Homogenized cells were applied to an RNeasy spin column, washed, and then eluted in 50 ml of water and stored at -20°C until required. cDNA was obtained via RNA reverse transcription using a reverse transcription kit (Qiagen, GmbH) according to the manufacturer's protocol. cDNA fragments were amplified using PCR as presented in Tables II and III, according to the manufacturer's protocol.

The CYP3A5-CDS-F/CYP3A5-CDS-R primers are presented in Table I, and GAPDH served as a control. Gel electrophoresis products of PCR were analyzed using agarose gel electrophoresis. Agarose gels of 2% (w/v) concentration were made in 0.5 Tris/Borate/EDTA buffer. Loading dye was added to the PCR products prior to being run at 120 V for 90 min alongside Hyperladder II (Bioline UK, London England) for comparison. Ethidium bromide at a concentration of 0.5 mg/ml (CLP, US) was used to stain the DNA for visualization using a UV transilluminator. RT-PCR products were alternatively analyzed using a standard procedure on a Capillary Gel Electrophoresis CEQ 8000 Genetic Analyser (Beckman Coulter, Inc., Brea, CA, USA).

The human liver cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen, Thermo Fisher Scientific, Inc.) supplemented with 20% fetal bovine serum at a temperature of 37°C in an environment containing 5% CO₂. PCR products of the cDNA fragments (Table I) were purified and ligated into the pMD18-T simple vector (Invitrogen; Thermo Fisher Scientific, Inc.). The resulting pMD18-T-CYP3A5 plasmid was transformed into E. coli JM109 (Auragene Bioscience Corporation Inc, Changsha, China). The E. coli strains carrying the recombinant pMD18-T-CYP3A5 plasmid were cultured on LB agar supplemented with 50 µg/ml ampicillin at 37°C overnight. A single colony was transferred into 5 ml of LB medium with ampicillin at the same concentrations used in the solid medium. The culture was incubated overnight at 37°C and 210 rpm. Subsequently, plasmids were extracted. Plasmids were double-enzyme digested with Hind III and Xho I (Thermo Fisher Scientific, Inc.), and the target gene fragments were inserted into the pYES2/CT plasmid that was digested with the same endonuclease enzyme, and the resultant recombinant plasmids were transformed into E. coli JM109 using the CYP3A5 expression vector and cultured E. coli JM109 at a temperature of 37°C and a revolution of 210 rpm/min in a medium containing 50 µg/ml ampicillin (17). Positive clones were screened for and the recombinant plasmid, CYP3A5-pYES2/CT, was extracted. CYP3A5*4 and CYP3A5*6 mutational expression vectors were constructed.
Stern blotting was performed as described previously (20). The microsomal CYP3A5 yeast proteins were separated on SDS polyacrylamide gels. Proteins were probed with the anti his-V5 (Santa Cruz Biotechnology, Inc., Dallas, TX, USA). The blots were scanned with an Odyssey imager (LI-COR Biosciences, Lincoln, NE, USA).

An in vitro study of amlodipine metabolism by CYP3A5 recombinant enzymes. A primary stock solution of amlodipine was prepared in 50% methanol at a concentration of 100 µg/ml. Estazolam (Beijing Yimin Pharmaceutical Co., Ltd., Beijing, China) was prepared from the primary stock at a concentration of 0.4 µg/ml for use as an internal standard. Working standard solutions of amlodipine were prepared from the primary stock at concentrations of 100, 50, 25, 12.5, 5.25, 3.125, 1.5625 and 0.78125 ng/ml. All solutions were stored at 4°C and warmed to room temperature before use. The constructed CYP3A5 gene polymorphism systems were used to metabolize amlodipine. The amount of drug metabolized was detected using LC-MS and HPLC as previously described (15). Amlodipine solution (50 µl), internal standard (50 µl), 500 µl blank serum (500 µl) and 1 M sodium hydroxide (200 µl) were added to 1.5 ml methyl tert-butyl ether. Following vortexing and centrifugation at a speed of 1,000 x g at 4°C for 10 min, the organic phase was dried at 45°C under nitrogen, reconstituted in methanol (80%, 200 µl) and injected onto a Phenomenex Luna column C18 (150x2 mm; Phenomenex, Inc., Torrance, CA, USA). The mobile phase consisted of methanol (0.1% methanoic acid) and 1 M ammonium acetate (0.1% methanoic acid; methanol/ammonium acetate, 4/1) with a flow rate of 0.2 ml/min. A standard curve was fitted using standard concentrations for the X-axis and the area ratio of standard for the Y-axis. Values for the rate of metabolite formation from 0.2 mg/ml of the recombinant enzyme were derived using the standard curve. K_m, V_max and V_max/K_m values were determined using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) and the Michaelis-Menten equation (6,21).

**Statistical analysis.** Values are presented as the mean ± standard deviation. Statistical comparisons were performed using one-way analysis of variance followed by the Newman-Keuls test (GraphPad Prism 5.0; GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

**Results**

Differences in amlodipine PD among CYP3A5 gene polymorphisms. The present study assessed the genotype of 57 hypertensive patients from the Health Management Center of the Third Xiangya Hospital. The number of patients with each polymorphism were as follows: CYP3A5*1/1 in 7 patients (12.3%); CYP3A5*1/3 in 28 patients (49.1%); CYP3A5*4 in 2 patients (3.5%); and CYP3A5*6 in 2 patients (3.5%). The mean age of the patients (26 males and 13 females) was 45.61±2.33 years and the mean body mass index was 24.15±1.46. Change in blood pressure prior to and following treatment with amlodipine was also recorded (Table IV). Following administration of amlodipine, blood pressure in all patients decreased. The magnitude of the change in blood pressure varied among patients. Individuals with the CYP3A5*1/3 polymorphism exhibited the highest change in blood pressure, followed by CYP3A5*4, CYP3A5*1/1, and CYP3A5*6 (Fig. 1A).

| Table I. Primers used to construct the CYP3A5 gene polymorphism expression vectors in this study. |
|----------------------|-----------------------|
| Primer             | Primer sequence (5’-3’) |
| CYP3A5             | F: aagcttATGGACCTCATCCAAAT (Hind III) |
|                    | R: ctcgagTTCTCCACTTAGGGTTCC (Xho I) |
| CYP3A5*4           | F: ATCCACGAGACCCCTTTGTGGAGAGCAC |
|                    | R: AGGGGTCTCGTGAGATTGTGTAGAGAGA GTG |
| CYP3A5*6           | F: CACTAAAAAGTCTAAATTTGGTTTCTT |
|                    | R: GAACTTTTTAGTGTCTCCACAAAGGGGT |

CYP3A5, cytochrome P450 3A5; F, forward; R, reverse. The lower case letters represent the restriction sites.

| Table II. Polymerase chain reaction system. |
|------------------|----------------|
| Reagents         | volume (µl)   |
| 10x Lamp buffer  | 2.5           |
| dNTP             | 2             |
| 5x C solution    | 5             |
| CYP3A5-CDS-F (10 uM) | 1        |
| CYP3A5-CDS-R (10 uM) | 1        |
| Template         | 1             |
| Lamp DNA polymerase | 0.25     |
| ddH2O            | Up to 25      |

| Table III. Polymerase chain reaction condition. |
|------------------|------------------|
| Temperature (˚C) | Time             |
| 94               | 4 min            |
| 94               | 30 sec           |
| 56               | 30 sec           |
| 72               | 1.30 min         |
| 72               | 5 min            |

| Temperature (˚C) | Time | 35 cycles |
|------------------|------|-----------|
| 94               | 4 min|           |
| 94               | 30 sec|         |
| 56               | 30 sec|         |
| 72               | 1.30 min|       |
| 72               | 5 min|          |

using site-specific mutagenesis of the wild type CYP3A5-pYES2/CT gene (18). The present study screened for positive clones and extracted the recombinant plasmid of CYP3A5 gene polymorphism expression vectors. The recombinant plasmid was transformed into *Saccharomyces cerevisiae* INVSc1 (19). The transformants were selected on Schaeffer's-glucose agar plates at 30˚C for 2-4 days. The microsomal CYP3A5 yeast protein was extracted, western blotting was performed as described previously (20). The microsomal CYP3A5 yeast proteins were separated on SDS polyacrylamide gels. Proteins were probed with the anti his-V5 (Santa Cruz Biotechnology, Inc., Dallas, TX, USA). The blots were scanned with an Odyssey imager (LI-COR Biosciences, Lincoln, NE, USA).
Table IV. Antihypertensive effects of CYP3A5 gene polymorphisms.

| Polymorphism      | Number of patients | Prior to amlodipine administration | Following amlodipine administration | Difference | Diastolic BP (mmHg) | Prior to amlodipine administration | Following amlodipine administration | Difference |
|------------------|--------------------|-----------------------------------|------------------------------------|-----------|--------------------|-----------------------------------|------------------------------------|-----------|
| CYP3A5*1/1       | 7                  | 145.2±19.4                        | 137.5±17.0                        | 7.76±3.2  | 92.94±12.4         | 88.18±11.3                        | 4.76±2.5                          |           |
| CYP3A5*1/3       | 28                 | 144.6±17.6                        | 127.4±8.3                        | 17.27±6.7 | 89.53±12.6         | 78.27±5.9                        | 11.27±8.1                         |           |
| CYP3A5*4         | 2                  | 156.2±19.3                        | 141.1±17.9                       | 15.33±10.4 | 94.17±12.2         | 84.93±12.3                       | 9.38±5.9                          |           |
| CYP3A5*6         | 2                  | 150.2±13.4                        | 143.8±13.1                      | 6.48±4.7  | 93.03±11.4         | 89.52±11.9                       | 3.51±3.4                          |           |

*P<0.05 vs. CYP3A5*1/1 group; **P<0.1 vs. CYP3A5*1/1 group. Data are presented as the mean ± standard deviation. CYP3A5, cytochrome P450 3A5; BP, blood pressure.

Table V. Amlodipine metabolic ratio of CYP3A5 gene polymorphisms.

| Polymorphism      | Number of patients | V max (ng/ml/min) |
|------------------|--------------------|-------------------|
| CYP3A5*1/1       | 7                  | 0.6727±0.028      |
| CYP3A5*1/3       | 28                 | 0.2584±0.013      |
| CYP3A5*4         | 2                  | 0.3347±0.029      |
| CYP3A5*6         | 2                  | 0.7174±0.053      |

Data are presented as the mean ± standard deviation. *P<0.05 vs. CYP3A5*1/1. CYP3A5, cytochrome P450 3A5.

In vivo drug metabolism of amlodipine. The differences in amlodipine PK among different CYP3A5 gene polymorphisms were compared. As presented in Table V, CYP3A5 recombinant enzymes influenced the PK of amlodipine in hypertensive patients. There were differences in the metabolic rate among the polymorphisms; individuals with the CYP3A5*6 polymorphism exhibited the highest metabolic ratio of amlodipine, followed by CYP3A5*1/1, CYP3A5*4 and CYP3A5*1/3. Compared with CYP3A5*1/1, there were significant differences in CYP3A5*1/3 and CYP3A5*4 (P<0.05). There was no statistically significant difference between CYP3A5*6 and CYP3A5*1/1 (P=0.5; Fig. 1B). These results may explain the differences in the antihypertensive effect of amlodipine in individuals with different CYP3A5 polymorphisms.

Construction and verification of the Saccharomyces cerevisiae expression system for CYP3A5 gene polymorphisms. CYP3A5 gene polymorphism expression vectors were successfully constructed for CYP3A5*1/1, CYP3A5*4 and CYP3A5*6, however, this was not achieved for CYP3A5*1/3. The site-specific mutagenesis failed in the CYP3A5*1/3 intron. The introduction of the nucleotides required to form each CYP3A5 polymorphism was confirmed with a PCR electrophoretogram (Fig. 2A). Expression vectors constructed by the PCR method were used as a template for PCR amplification. PCR amplification products were subjected to agarose gel electrophoresis. In addition, agarose gel electrophoresis was performed to analyze the constructed expression vector. The results demonstrated that the expression vector was successfully constructed (Fig. 2B). Double digestion of the expression vectors was performed to further confirm that the expression vectors were correct. The digestion products were assessed using agarose gel electrophoresis. The results demonstrated that the expression vectors were successfully constructed (Fig. 2C). Additionally, Saccharomyces cerevisiae formed into monoclonal cells, indicating that the CYP3A5 gene polymorphism expression vectors were successfully transformed into the INVSc1 yeast cells (Fig. 2D). Bidirectional sequencing comparisons were performed using positive bacterial clones of the double digestion vectors (Fig. 3). CYP3A5*4, the constructed gene fragment exhibited a base group mutation from A to G at bp 599, without the presence of other mutations, indicating that the fragment was CYP3A5*4, CYP3A5*6, the constructed gene fragment exhibited base group mutation from G to A at bp 624, without the presence of other mutations, indicating that the fragment was CYP3A5*6. The results demonstrated that the present study successfully constructed recombinant yeast expression systems for three single nucleotide mutants of human cytochrome CYP3A5, including CYP3A5*1/1, CYP3A5*4 and CYP3A5*6. No additional mutations, apart from the desired point mutations created in the expression vectors, were identified. The gene sequencing results demonstrated that there were mutations in the required base locus while other bases were normal (Fig. 3A-C). These results confirmed that the CYP3A5 gene polymorphism vectors were successfully constructed.

PK prediction model for CYP3A5 gene polymorphisms. To assess the effects of CYP3A5 gene polymorphisms on drug metabolism, the present study constructed a PK prediction model by expressing CYP3A5 gene polymorphism vectors in Saccharomyces cerevisiae. After the CYP3A5 gene polymorphism expression vectors were transformed into yeast, the transformants were selected on Schaeffer’s-glucose agar plates. Subsequently, western blotting was performed to detect protein expression. The results (Fig. 3D) demonstrated that CYP3A5*1/1 and CYP3A5*6 proteins were detected.
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at 54 kDa; whereas CYP3A5*4 was detected at 18 kDa. These results demonstrate that the CYP3A5 gene polymorphism sequences were successfully transformed into the Saccharomyces cerevisiae expression vectors.

In vitro drug metabolism of amlodipine by CYP3A5 recombinant enzymes. The standard curve was fitted using standard concentrations for the X-axis and the area ratio of standard/ internal standard for the Y-axis (Fig. 4A and B).

Values for the rate of metabolite formation using 0.2 mg/ml of the recombinant enzymes were derived using the standard curve. The $V_{\text{max}}$ of amlodipine metabolized by CYP3A5*1/*1, CYP3A5*4 and CYP3A5*6 was $0.61\pm0.02, 0.42\pm0.02$ and $0.66\pm0.04$ pmol/min/pmol P450, respectively. The $K_{\text{m}}$ of CYP3A5*1/*1, CYP3A5*4 and CYP3A5*6 was $0.32\pm0.12 \mu\text{mol/l}, 0.31\pm0.05 \mu\text{mol/l}$ and $0.32\pm0.08 \mu\text{mol/l}$, respectively. The drug clearance ($V_{\text{max}}/K_{\text{m}}$) for CYP3A5*1/*1, CYP3A5*4 and CYP3A5*6 was $1.91\pm0.05, 1.35\pm0.15$ and

Figure 1. In vivo drug metabolism of amlodipine. (A) The antihypertensive effects of amlodipine for each CYP3A5 gene polymorphism. (B) Amlodipine metabolism ratio for each CYP3A5 gene polymorphism. Data are presented as the mean ± standard deviation. *P<0.05 vs. the CYP3A5*1/*1 group. CYP3A5, cytochrome P450 3A5.

Figure 2. Construction and verification of Saccharomyces cerevisiae expression systems for CYP3A5 gene polymorphisms. (A) Electrophoretogram of expression plasmids for the CYP3A5 gene polymorphisms. (B) Polymerase chain reaction results of CYP3A5*1/*1, CYP3A5*4 and CYP3A5*6 expression vector transformation. (C) Electrophoretogram of double digests of the expression vector. (D) Saccharomyces cerevisiae INVSc1 and the transformants of different CYP3A5 alleles. CYP3A5, cytochrome P450 3A5; WT, wildtype.
2.06±0.05 µl/min/pmol P450, respectively (Fig. 4C). The results demonstrated that CYP3A5 recombinant enzymes influenced the PK of amlodipine. There was a significant difference in the drug clearance between CYP3A5*4 and CYP3A5*1/*1 (P<0.05). The difference between CYP3A5*6 and CYP3A5*1/*1 was not statistically significant (P>0.05).

Discussion

CYP3A5 has an important role in the metabolism of various drugs. The major polymorphisms of CYP3A5 are CYP3A5*1, CYP3A5*2, CYP3A5*3, CYP3A5*4, CYP3A5*6 and CYP3A5*7 (22,23). Previous studies have reported the successful construction of an in vitro Saccharomyces cerevisiae expression systems for CYP3A4, CYP2D6 and CYP2C9 (22,24). To the best of our knowledge, the present study is the first to construct the in vitro CYP3A5 gene polymorphism Saccharomyces cerevisiae expression systems for CYP3A5*1/*1, CYP3A5*4 and CYP3A5*6, and to compare the in vitro metabolic rate with in vivo rates.

Amlodipine is a CYP3A5 substrate that is widely used for the treatment of hypertension. Numerous studies have demonstrated that the CYP3A5 gene polymorphisms affect the PK and PD of amlodipine (24-27). The in vitro drug metabolism results of amlodipine by CYP3A5 recombinase in the present study demonstrated that there were significant differences between CYP3A5*4 and CYP3A5*1/*1 (CYP3A5*1/*1 > CYP3A5*6 > CYP3A5*4), while the in vivo PK results for amlodipine demonstrated that CYP3A5*6 exhibited the highest metabolic ratio of amlodipine, followed by CYP3A5*1/*1 and CYP3A5*4. There was a significant difference between the in vitro and in vivo PK results obtained for CYP3A5*1/*1 and CYP3A5*4 (P<0.05) however no significant difference between CYP3A5*1/*1 and CYP3A5*6.

The reaction ratio of in vivo CYP3A5*6 was much larger compared with CYP3A5*1/*1 and CYP3A5*4 polymorphisms, which is in contrast to the in vitro drug metabolism results. This may be due to the fact that there were 2 patients (3.51%) with the CYP3A5*6 allele, which is higher than the normal CYP3A5*6 allele frequency. The CYP3A5*1 allele frequency
is 25% in Chinese populations, and the CYP3A5*3 allele frequency is >25%. However, the CYP3A5*6 allele is not as common in Asian populations, including Chinese and Japanese populations (5,28-30). Further studies that focus on the mechanisms and frequency of the CYP3A5*6 allele are required. Large clinical trials should be conducted to clarify the difference between the PK effects of these three gene polymorphisms. The antihypertensive effects of amlodipine among different patients were determined. CYP3A5*1/3 exhibited the largest antihypertensive effect, followed by CYP3A5*4, CYP3A5*1/1 and CYP3A5*6. The in vitro PK data demonstrated that CYP3A5*1/1 had the largest reaction ratio. Thus, the antihypertensive results are consistent with the PK results. The current study had certain limitations that must be stated. The current study had certain limitations that must be stated.

4. Additionally, only 2 patients were included in the polymorphism study. The current study failed to construct the in vitro CYP3A5*1/3 polymorphism Saccharomyces cerevisiae expression vector as the CYP3A5*1/3 mutation occurs in the CYP3A5 gene intron and it was difficult to mutate the intron. In future studies, we hope to identify another method to solve this problem. Additionally, only 2 patients were included in CYP3A5*4 and CYP3A5*6 groups, and the small patient population and the retrospective nature of the study do not allow any conclusions to be drawn about the effectiveness of this prediction method.

5. Studies consisting of larger groups of patients with long-term follow-up are required to confirm these results.

6. In conclusion, the present study indicates that the CYP3A5 gene polymorphism Saccharomyces cerevisiae expression system is a potential tool for the prediction of drug metabolism, particularly for novel drugs prior to their application in clinical trials. This expression system may also aid in the development of individualized clinical medications.

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