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Cover Page Footnote

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ORIGINAL ARTICLE

Effect of repeated Shengmai-San administration on nifedipine pharmacokinetics and the risk/benefit under co-treatment

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Abstract

Herbal interactions with nifedipine/felodipine through cytochrome P450 (CYP) 3A inhibition is significant in humans. Shengmai-San (SMS), a three-herbal formula of Chinese medicine, is commonly prescribed in Asia populations for cardiovascular disorders. This study aimed to elucidate the impact of SMS on nifedipine/felodipine treatment by the findings from rat pharmacokinetic study of nifedipine to the retrospective cohort study of patients with hypertension. The 3-week SMS treatment increased the systemic exposure to nifedipine by nearly two-fold and decreased nifedipine clearance by 39% in rats. Among the ingredients of SMS component herbs, schisandrin B, schisantherin A, and methylophiopogonanone A, inhibited the nifedipine oxidation (NFO) activities of rat hepatic and intestinal microsomes, as well as human CYP3A4. Methylophiopogonanone A was identified as a time-dependent inhibitor of CYP3A4. After 1:5 propensity score matching, 4,894 patients with nifedipine/felodipine use were analyzed. In patients receiving nifedipine/felodipine, the subgroup with concurrent SMS treatment had a higher incidence of headache (92.70 per 1,000 person-years) than the subgroup without SMS treatment (51.10 per 1,000 person-years). There was a positive association between headache incidence and cumulative doses of SMS (1−60 g SMS: hazard ratio (HR): 1.39; 95% confidence interval (CI): 1.11−1.74; >60 g SMS: HR: 1.97; 95% CI: 1.62−2.39; $p < 0.0001$). However, patients who had higher cumulative SMS doses had a lower risk of all-cause mortality (1−60 g SMS: HR: 0.67; 95% CI: 0.47−0.94; >60 g SMS: HR: 0.54; 95% CI: 0.37−0.79; $p = 0.001$). Results demonstrated increased rat plasma nifedipine levels after 3-week SMS treatment and increased headache incidence should be noted in nifedipine/felodipine-treated patients with prolonged SMS administration.

Keywords: Clearance, Headache, Methylophiopogonanone A, Nifedipine, Shengmai-San

1. Introduction

The dihydropyridine calcium channel blockers, nifedipine and felodipine, are currently used for the treatment of various cardiovascular disorders, including hypertension and angina [1]. The absorption of nifedipine from the gastrointestinal tract is rapid and almost 100%, but its extensive first-pass metabolism leads to an absolute bioavailability of 40−50% in humans [2]. The

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pharmacokinetic properties of felodipine are similar to those of nifedipine [3]. Both nifedipine and felodipine undergo oxidative metabolism, which is primarily catalyzed by human cytochrome P450 (CYP) 3A4 and rat CYP3A1 to generate inactive metabolites [3–5]. The oxidative metabolite of nifedipine, dehydronifedipine can be further metabolized through hydroxylations and glucuronidation [6]. In humans and rats, CYP3A appears to be the most abundant hepatic and intestinal CYP subfamily [7,8]. Intestinal elimination encompasses 36–52% of the first-pass metabolism of nifedipine in humans [9], and it contributes more to first-pass elimination in rats than hepatic metabolism [10].

The most common side effects of nifedipine/felodipine include headaches and flushing [1]. Much attention has been paid to the interactions of nifedipine/felodipine with herbs and fruits, such as *Ginkgo biloba* and grapefruit. In a study of eight healthy participants, compared to the ingestion of nifedipine alone, two subjects had an approximately 2-fold increase in the peak plasma concentration of nifedipine and severer and longer-lasting headache after concurrent ingestion of nifedipine and *G. biloba* leaf extract [11]. In another study of nine healthy participants, plasma felodipine concentrations increased (with an up to 48% increase of area under the plasma concentration *versus* time curve (AUC)) after pretreatment (1–24 h) with one glass of grapefruit juice [12]. The number of participants experiencing headaches increased from three to nine when felodipine was taken 4 h after grapefruit juice ingestion.

Grapefruit juice has been demonstrated to decrease intestinal, but not hepatic CYP3A activity in humans [3]. The furanocoumarin-type ingredients of grapefruit juice inhibit human intestinal CYP3A4 in a time-dependent manner, leading to irreversible CYP3A inactivation [13]. A typical characteristic of time-dependent CYP inhibitors is that their inhibitory effect can be enhanced by reduced nicotinamide adenine dinucleotide phosphate (NADPH)-fortified pre-incubation of the inhibitor with the CYP, in which the active metabolite of the inhibitor is formed and binds the CYP tightly. The recovery $t_{1/2}$ of the grapefruit juice (2 h prior to midazolam administration)-elevated AUC of midazolam (a 65% increase) was estimated to be 23 h, and complete recovery required 3 days in healthy participants [14]. Moreover, the increases in the maximum concentration (C_{max}) and AUC of plasma felodipine after five consecutive days of grapefruit juice ingestion were significantly greater than those after ingesting a single glass of grapefruit juice [3].

Abbreviations

AMI	acute myocardial infarction
AUC	area under the plasma concentration versus time curve
CI	confidence interval
CL/F	clearance/fraction absorbed
C_{max}	maximum concentration
CPR	NADPH-cytochrome P450 reductase
CYP	cytochrome P450
DMSO	dimethyl sulfoxide
HHF	hospitalization due to heart failure
HPLC	high performance liquid chromatography
HR	hazard ratio
IPTG	isopropyl β-D-1-thiogalactopyranoside
MS/MS	tandem mass spectrometry
$NADP^+$	nicotinamide adenine dinucleotide monosodium phosphate
NADPH	reduced nicotinamide adenine dinucleotide monosodium phosphate
NFO	nifedipine oxidation
PSM	propensity score matching
SJW	St. John's wort
SMS	Shengmai-San
StD	standardized mean difference
$t_{1/2}$	half-life
TCM	traditional Chinese medicine
t_{max}	time to maximum plasma concentration
UPLC	ultra-performance liquid chromatography

In addition to headaches, increased heart rate and enhanced reduction in blood pressure have been reported in participants taking both grapefruit and felodipine [12]. Other relatively uncommon but severe adverse effects of calcium-cand myocardial infarction [15–17]. These reports revealed that elevated plasma levels of nifedipine and felodipine are highly associated with the incidence of adverse effects. Repeated treatments can enhance the pharmacokinetic alterations induced by time-dependent CYP3A inhibitors.

A traditional herbal formula Shengmai-San (SMS) is prepared from Ginseng Radix (roots of *Panax ginseng*), Ophiopogonis Radix (roots of *Ophiopogon japonicus*), and Schisandrae Fructus (fruits of *Schisandra chinensis*), and has been used to treat cardiovascular diseases [18–20]. From 2000 to 2010, sixteen percent of the prescriptions for the treatment of ischemic heart disease in traditional Chinese medical care in Taiwan contained SMS [21]. In TCM therapy, SMS decoction can be used for up to 3 weeks in patients [22]. Similar to CYP3A inhibition by grapefruit juice, SMS extract inhibits CYP3A activity in a time-dependent manner [23]. The 1-h SMS pretreatment decreased nifedipine clearance by 34% and hepatic nifedipine oxidation (NFO) activity by 49%. However, intestinal, but not hepatic

microsomal NFO activity decreased in 3-week SMS-treated rats [24]. These findings indicate the dynamic irreversible inhibition of NFO by SMS *in vivo* and warrant the present studies on the effects of 2- and 3-week SMS treatments on the pharmacokinetics of nifedipine in rats. Ketoconazole and St. John's wort (SJW) were studied as the positive controls of CYP3A inhibitor and inducer, respectively [23,25]. In addition to CYP3A inhibition, ketoconazole inhibited the glucuronidation activity potently [26]. The inhibitory effects of the ingredients of SMS component herbs on the NFO activities of rat hepatic and intestinal microsomes, as well as recombinant human CYP3A4, were determined. In addition, a single-pay mandatory National Health Insurance (NHI) program was implemented in 1995 in Taiwan and covered more than 99% of the 23 million residents. The NHI in Taiwan provides comprehensive health care, including ambulatory visits, hospital admissions, procedures, medications, rehabilitation, and traditional Chinese medicine (TCM). By using the data of NHI claims, the risks and benefits of health outcomes, considering the combined treatments of nifedipine/felodipine and SMS in patients with hypertension, were evaluated.

2. Materials and methods

2.1. SMS, chemicals and solvents

The powdered decoction of SMS (Lot # 15083105; containing 0.56 ± 0.01 mg/g schisandrol A, 0.10 ± 0.001 mg/g schisandrin A, 0.20 ± 0.003 mg/g schisandrin B and 0.01 mg/g ophiopogonin D) [23,24] was purchased from Sun Ten Pharmaceutical Co., Ltd. (New Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme (PIC/S). The content of methylophiopogonanone A was determined using liquid chromatography (LC)-tandem mass spectrometry (MS/MS) (multi-reaction monitoring transition: 341.1 → 178.0) following the method reported previously for the determination of ophiopogonin D [24]. There was 0.02 μg/g methylophiopogonanone A in SMS decoction. The SMS was prepared from Schisandrae Fructus, Ginseng Radix, and Ophiopogonis Radix (1:2:2). The herbal materials and protocols for the preparation of SMS is under the regulation of The Pharmaceutical Affairs Act of Taiwan and the Regulations for Registration of Medicinal Products, Ministry of Health and Welfare, Taiwan. The identification and quality control of dried herbs used for the decoction preparation were carried out according to the Taiwan Herbal Pharmacopeia (Ministry of Health and Welfare, Taipei, Taiwan). Mr. Chang-Ming Cheng (Non-Profit Organization Brion Research Institute of Taiwan, Taiwan) is the specialist responsible for the materials used for decoction preparation. One gram of SMS contained 0.52 g dried decoction and 0.48 g corn starch. The ethanolic (60% ethanol) extract of SJW (*Hypericum perforatum*) (Lot # 133975; 1.3 mg/g hyperforin) [27] was purchased from APOMEDICA Pharmaceutical Products GmbH (Graz, Austria). Ampicillin, glucose-6-phosphate, isopropyl β-D-1-thiogalactopyranoside (IPTG), $NADP^+$, and nifedipine were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Dehydronifedipine (purity >98%) was synthesized and generously provided by Dr. F. Peter Guengerich (Vanderbilt University, Nashville, TN, USA) [4]. The ingredients (PubChem), ginsenoside Rb1, ginsenoside Rd, methylophiopogonanone A, ophiopogonin D, and schisantherin A were purchased from Sun-Hank Technology Co., Ltd. (Tainan, Taiwan) (purity ≥98%). Arctigenin, panaxatriol, protopanaxatriol, and ruscogenin were purchased from MedChemExpress LLC (Monmouth Junction, NJ, USA). Schisandrin B (purity ≥98%) was purchased from Cayman Chem. (Ann Arbor, MI, USA). Acetonitrile, dichloromethane, dimethyl sulfoxide (DMSO) and methanol were purchased from Merck KGaA (Darmstadt, Germany).

2.2. Expression of recombinant human CYP3A4 monooxygenase system

The CYP3A4 constructs with N-terminal modifications were generously provided by Dr. F. Peter Guengerich (Nashville, TN, USA) [28]. Bicistronic human constructs consisting of the CYP coding sequence, followed by that of NADPH-dependent CYP reductase (CPR), were transformed into *Escherichia coli* DH5α by electroporation (Gene Pulser II, Bio-Rad, Hercules, CA, USA). The bacteria were incubated in Luria–Bertani medium containing 0.1% glucose and 100 μg/mL ampicillin. The overnight incubation was further incubated in Terrific Broth containing 1 mM thiamine, a mixture of trace elements, 100 μg/mL ampicillin, and 1 mM IPTG [28]. After 24 h, membrane fractions were prepared by differential centrifugation, and the CYP content of the membranes was determined using CO-difference spectral analysis [29].

2.3. Rat treatments

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Animal Care and Use

Committee (IACUC) of the National Research Institute of Chinese Medicine and National Defense Medical Center (Taipei, Taiwan) for the studies on microsomal activities (IACUC #108-635-1, 110-636-1) and pharmacokinetics (IACUC #18–240), respectively. Male Sprague–Dawley rats (5 weeks old, weight: 90–120 g) were purchased from the Animal Center of National Yang-Ming University (Taipei, Taiwan) or BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan). Before experimentation, the rats were allowed a one-week acclimation period at the animal quarters (23 ± 2 °C, 12-h daylight cycle). The human-equivalent dose of SMS (powdered decoction) in rats was estimated to be 1.2–2.0 g/kg/day, based on a 50–60-kg person (daily dose: 12–16 g) [23]. To assess pharmacokinetic interactions, the rats were treated with 1.9 g powdered SMS decoction/kg/day. The SMS and SJW were ground (using a mortar and pestle), suspended in water, mixed by vortexing, and then administered to the rats by gastrogavage. The same volume of water was administered to the control group.

2.4. Pharmacokinetic analysis

In ketoconazole group, rats were treated with 20 mg/kg ketoconazole together with nifedipine (3 mg/kg, in 50% polyethylene glycol 400). Each morning, the rats were orally administered 0.3 g/kg SJW extract for 2 weeks and 1.9 g/kg powdered SMS decoction for 2 and 3 weeks. To prevent the acute effects of SMS from interfering with the assessment [23], nifedipine was administered 20 h after the last herbal treatment. The control group received the same volume of vehicle. Blood samples (0.25 mL in heparinized tubes) were collected from tail vein using a catheter [23]. After blood sampling, the same volume of 0.9% saline was infused back into the rats for maintaining body fluid volume. Plasma nifedipine and dehydronifedipine concentrations were determined using an ultra high-performance liquid chromatography (UPLC)-MS/MS system (Shimadzu Nexera series LC-40B X3 (Schimadzu corporation, Kyoto, Japan) equipped with a Biosystems-Sciex API 3000 series triple-quadrupole mass spectrometer (SCIEX, Foster City, CA, USA)). Conditions set in the LC and MS/MS systems for the quantitation of nifedipine and dehydronifedipine primarily followed the method reported previously [23] with slight modification. In this study, an ultra high-pressure column (Waters Symmetry C18, 2.1 × 100 mm, 1.7 μm, Waters Corporation, Milford, MA, USA) was used and separation was performed using 40% A (2 mM ammonium formate and 0.1% formic acid in water) and 60% B (2 mM ammonium formate and 0.1% formic acid in acetonitrile) at a flow rate of 0.2 mL/min. Pharmacokinetic parameters were obtained using non-compartmental model analysis (WinNonlin version 5.3, Pharsight Corporation, Mountain View, CA, USA). The C_{max}, AUC, half-life ($t_{1/2}$), and clearance (clearance/fraction absorbed; CL/F) were estimated.

2.5. Preparation of microsomes and activity determinations

Liver and intestinal microsomes were prepared by differential centrifugation as described previously [24], and the microsomal samples were stored at −75 °C. Microsomal UDP-glucuronosyltransferase activity was determined using *p*-nitrophenol as a substrate [24]. The generation of dehydronifedipine in the NFO assay was determined using high-performance liquid chromatography (HPLC) or UPLC as described previously [24]. In the *in vitro* inhibition studies, the rat microsomes were pre-incubated with individual ingredients in the presence of an NADPH-generating system (10 mM glucose-6-phosphate, 0.5 mM $NADP^+$, and 0.5 U/mL glucose-6-phosphate dehydrogenase) for 10 min prior to the initiation of the NFO reaction by the addition of nifedipine [23]. Microsomal activity was normalized to the protein concentration determined using the method reported by Lowry et al. [30]. The concentration causing 50% inhibition (IC_{50}) of activity was estimated using GraFit software (Erithacus Software Ltd., Staines, UK). The inhibitory effects of ingredients on the NFO activity of bacterial membranes expressing human CYP3A4 were determined in the absence and presence of 10-min pre-incubation to obtain the $IC_{50(-)}$ and $IC_{50(+)}$ values, respectively.

2.6. Kinetic analyses and threshold concentration estimation

The inactivation rate (K_{app}) was determined as the slope calculated by linear regression analysis of the plot of the natural logarithm of the percentage of remaining activity *versus* pre-incubation time. The k_{inact} (maximal inactivation rate constant) and K_I (concentration required for half-maximal CYP3A4 inactivation) values were obtained from nonlinear regression of the equation $K_{app} = (k_{inact} \cdot I)/(I + K_I)$, with the initial values calculated from the double reciprocal plot of inactivation rate *versus* methylophiopogonanone A concentration. The irreversible inhibition was further confirmed by determining the remaining NFO activity after dialysis (Supplementary Methods). In the drug–drug interactions, an

AUC changes ≥ 2-fold should be considered to be noted in the clinical application [31]. The predicted threshold of unbound inhibitor concentration causing a 2-fold AUC change was calculated using high-throughput methods or inactivation kinetics (Suppl. Methods).

2.7. Data source

The data used in this study were obtained from the Longitudinal Generation Tracking Database 2010 (LGTD 2010) maintained by the Health and Welfare Science Center. The LGTD 2010 contained medical claims from two million enrollees randomly sampled from the NHI registry in 2010. The NHI enrollees included in the LGTD do not differ significantly from the overall population of NHI enrollees in terms of age, gender, insured salary and household location. The accuracy of the diagnoses of major diseases, including diabetes mellitus and stroke, in the NHI claims database has been validated previously [32,33]. This study was approved by the Joint Institutional Review Board (JIRB No.: 17-S1-017-1).

2.8. Study subjects

This was a retrospective cohort study. Adult patients who had been diagnosed with hypertension from 2015–2017 (International Classification of Diseases, Ninth Revision, Clinical Modification [ICD-9-CM]: 401–405; ICD-10-CM: I10–I15, N26.2) on three or more ambulatory care claims or in an inpatient setting were identified. The patients with hypertension were followed up from the date of initial diagnosis until the incidence of headache, low blood pressure, hospitalization due to acute myocardial infarction (AMI), hospitalization due to heart failure (HHF), death, or study end (December 31, 2018).

In Taiwan, both drugs and TCMs are included in NHI benefits. Patients can choose to have an ambulatory visit to Western medicine doctors (e.g., internal medicine or family medicine) and TCM doctors in the same therapeutic period. Patients receiving nifedipine or felodipine (monotherapy or combination therapy) were defined as the nifedipine/felodipine group (ATC codes: C08CA05, C08GA01, CA08CA55, CA08CA02, C07FB03, C07FB02, and C09BB05). Patients who received nifedipine/felodipine combined with SMS (powdered decoction) prescribed by a TCM doctor were defined as the nifedipine/felodipine + SMS group. Patients who had been prescribed nifedipine/felodipine for less than 28 cumulative days and those who had taken SMS for less than 7 cumulative days were excluded. The cumulative dose (g) of SMS was determined by aggregating the total amount dispensed by the prescribed dose for each patient from the first date that patients received nifedipine/felodipine to the incidence of undesired health outcome or study end (December 31, 2018). The median cumulative dose of SMS was 60 g. Among the included patients, SMS use was subdivided into cumulative dosages of 1–60 g and >60 g to examine if there was a statistically significant trend in dose-dependence.

2.9. Adverse health outcomes and mortality

The primary outcomes of this study were the occurrence of adverse events, including headaches, low blood pressure, hospitalization due to AMI, and HHF. Headaches and low blood pressure were defined as ambulatory or hospital admission for headache or low blood pressure after taking nifedipine/felodipine. If a patient was hospitalized with AMI or HHF as the primary diagnosis, this was defined as hospitalization due to AMI or HHF. Details of the case definitions for the primary outcomes are listed in Table S1. The secondary endpoint of the study was mortality, and we linked the LGTD to the Cause of Death data using scrambled identification to obtain the vital status of each patient.

2.10. Statistical analyses

Patient baseline characteristics (including age and sex), year of cohort entry (hypertension), comorbidities in the prior year, prior medications (including antihypertensive drugs, statins, non-steroidal anti-inflammatory drugs, metformin, and insulin) were collected. We employed propensity score matching (PSM) to minimize selection bias. We matched the nifedipine/felodipine and nifedipine/felodipine + SMS groups on the logit of the propensity score using a caliper width of 0.2, the standard deviation of the logit. A matching ratio of 1:5 was used. The standardized mean difference (StD) between the covariates of these two groups after PSM was less than 10%, indicating good balance among the groups. The hazard ratio (HR) for the occurrence of adverse health effects was determined using Cox proportional hazard regression with robust variance estimation, and the trend in dose-dependent effects (p-value for trend) was also examined. A negative control outcome, bone fracture due to a vehicle accident, was used to detect and adjust for residual systematic bias [34]. All statistical analyses were performed using SAS

version 9.4 for Windows (SAS Institute, Cary, NC, USA), and a two-sided *p*-value of <0.05 was considered statistically significant. Differences among >2 data sets were analyzed by one-way analysis of variance (ANOVA) followed by a *post-hoc* Dunnett's test (for comparisons with the control group). A two-tailed *p* value of <0.05 was considered statistically significant.

3. Results

3.1. Effects of repeated SJW and SMS treatments on pharmacokinetics of nifedipine and dehydronifedipine in rats

While ketoconazole-treatment caused an 81% decrease of nifedipine clearance, the C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ of nifedipine increased by 135%, 233%, and 550%, respectively (Table 1 and Fig. 1A). Ketoconazole prolonged the half-life of nifedipine by 4-fold. Oral administration of SJW to rats significantly increased hepatic NFO activities by 51% (control group: 1.28 ± 0.08 nmol/min/mg protein; SJW group: 1.93 ± 0.15 nmol/min/mg protein) without affecting the glucuronidation activity of UDP-glucuronosyltransferase (control group: 33.0 ± 3.0 pmol/min/mg protein; SJW group: 33.9 ± 3.4 pmol/min/mg protein). SJW treatment increased the mean clearance of plasma nifedipine by 28% and prolonged the time to maximum plasma concentration (t_{max}) by 97%. In rats treated with SMS for 2 weeks, the change in pharmacokinetic parameters of nifedipine was not statistically significant (Table 1). After treatment with SMS for 3 weeks, the mean C_{max} and AUC_{0-t} values of nifedipine were elevated by 101% and 73%, respectively. The $AUC_{0-\infty}$ values of nifedipine increased by 66%, but without statistical significance. The 3-week SMS treatment significantly decreased the clearance of nifedipine by 39% without affecting its half-life.

After nifedipine administration, plasma dehydronifedipine concentrations were also determined in rats. Ketoconazole increased the C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ values of dehydronifedipine by 125%, 270%, and 191%, respectively (Table 1 and Fig. 1B). Ketoconazole-treatment prolonged the half-life of dehydronifedipine by 150%. Administration of SJW to rats prolonged the t_{max} by 209% without affecting the AUC and clearance of dehydronifedipine. The 2- and 3-week SMS treatments did not cause any significant changes in the pharmacokinetic parameters of dehydronifedipine.

3.2. Inhibition of nifedipine oxidation activities of rat microsomes and human CYP3A4 by herbal ingredients of SMS

The inhibitory effects of SMS ingredients on rat microsomes were determined after a 10-min NADPH-fortified pre-incubation with each ingredient. The Schisandrae Fructus ingredients, schisantherin A and schisandrin B, inhibited hepatic and intestinal microsomal NFO activity with IC_{50} values of <10 μM (Table 2). Among the ingredients of Ginseng Radix, only panaxatriol inhibited intestinal NFO activity, and this effect was mild. Methylophiopogonanone A is an ingredient found in Ophiopogonis Radix [35], and it inhibits both hepatic and intestinal NFO activity. By increasing their concentrations to 100 μM, arctigenin and ruscogenin inhibited NFO activity by no more than 50%. Thus,

Table 1. Effects of ketoconazole, SJW and SMS on the pharmacokinetic profiles of nifedipine and dehydronifedipine in rats.

Parameters	Control	Ketoconazole	SJW[a]	SMS[b]	
				2 weeks	3 weeks
Nifedipine					
C_{max} (μg/mL)	1.97 ± 0.08	4.62 ± 1.02**	1.01 ± 0.16	3.01 ± 0.21	3.96 ± 0.53**
t_{max} (h)	0.38 ± 0.06	0.40 ± 0.06	0.75 ± 0.11**	0.33 ± 0.05	0.33 ± 0.05
AUC_{0-t} (h·μg/mL)	3.87 ± 0.27	12.90 ± 2.40**	2.57 ± 0.20	4.33 ± 0.21	6.69 ± 0.68*
$AUC_{0-\infty}$ (h·μg/mL)	4.12 ± 0.30	26.78 ± 5.80**	3.18 ± 0.17	4.42 ± 0.19	6.84 ± 0.67
$t_{1/2}$ (h)	1.42 ± 0.14	5.66 ± 0.42**	2.23 ± 0.48	1.24 ± 0.20	1.12 ± 0.15
CL/F (l/h/kg)	0.75 ± 0.06	0.14 ± 0.03**	0.96 ± 0.05*	0.68 ± 0.03	0.46 ± 0.04**
Dehydronifedipine					
C_{max} (μg/mL)	0.04 ± 0.01	0.09 ± 0.01**	0.02 ± 0.003	0.04 ± 0.01	0.04 ± 0.01
t_{max} (h)	0.54 ± 0.10	0.90 ± 0.19	1.67 ± 0.31*	1.64 ± 0.79	1.46 ± 0.43
AUC_{0-t} (h·μg/mL)	0.10 ± 0.01	0.37 ± 0.03**	0.07 ± 0.01	0.11 ± 0.02	0.12 ± 0.02
$AUC_{0-\infty}$ (h·μg/mL)	0.13 ± 0.01	0.78 ± 0.12**	0.12 ± 0.02	0.12 ± 0.02	0.14 ± 0.02
$t_{1/2}$ (h)	2.36 ± 0.33	5.89 ± 1.01**	4.22 ± 0.82	2.01 ± 0.72	1.82 ± 0.19

Rats (n = 6 in each group) were orally treated with [a]0.3 g/kg/day SJW for 2 weeks or [b]1.9 g/kg/day SMS for 2 or 3 weeks before the administration of nifedipine (3 mg/kg, oral). In ketoconazole group, 5 rats were orally treated with 20 mg/kg ketoconazole together with nifedipine. Results represent the means ± SEM. *$p < 0.05$; **$p < 0.01$, compared to the control group (One-way ANOVA analysis with a *post-hoc* Dunnett's test).

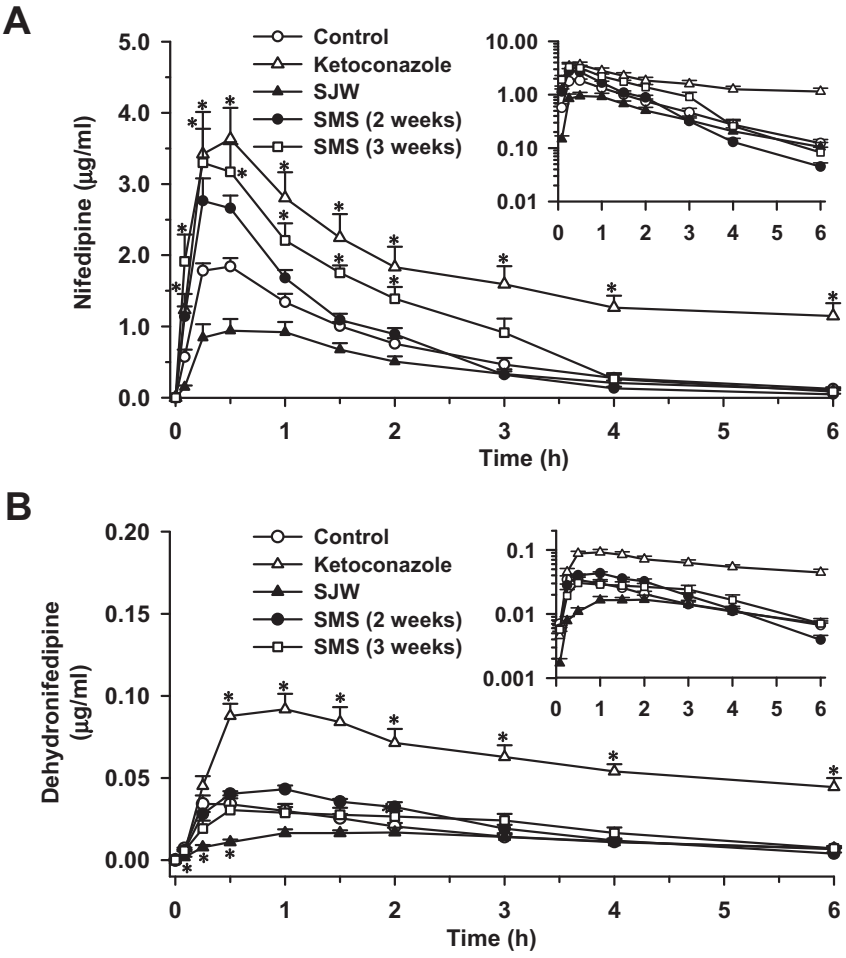

*Fig. 1. Effects of ketoconazole, SJW and SMS treatments on the pharmacokinetics of nifedipine (A) and dehydronifedipine (B) in rats. A single oral dose of ketoconazole (20 mg/kg) was administered together with nifedipine (3 mg/kg, oral) to 5 rats. Rats (6 rats in each group) were orally treated with 0.3 g/kg SJW (suspended in water) for 2 weeks or 1.9 g/kg SMS decoction (suspended in water) for 2 or 3 weeks. The control group (6 rats) received the same volume of water. After water/ketoconazole/herbal treatments, nifedipine was orally administered to the rats as described in the section of Methods. Data represent the means ± SEM. *p < 0.05, compared to the control group.*

in the *in vitro* rat microsome study, schisantherin A, schisandrin B, and methylophiopogonanone A were identified as inhibitors of hepatic and intestinal NFO (Table 2).

In the recombinant human CYP3A4 system, schisantherin A, schisandrin B, and methylophiopogonanone A caused an IC_{50} shift after 10 min of NADPH-fortified pre-incubation (Table 3), indicating time-dependent inhibition. Using the IC_{50} values (high-throughput screening method), the predicted threshold of unbound concentrations of these inhibitors able to increase the AUC 2-fold was <50 nM (Table 2). The results showed that the rat CYP3A inhibitors identified in SMS also inhibited the NFO activity of human CYP3A4. Besides schisandrin B and schisantherin A, methylophiopogonanone A inhibited human CYP3A4 in a time-dependent manner.

3.3. Time-dependent inactivation of human CYP3A4 by methylophiopogonanone A

The methylophiopogonanone A-mediated decrease in NFO activity increased with prolonged NADPH-fortified pre-incubation time (Fig. 2A). After incubating CYP3A4 with increasing concentrations of methylophiopogonanone A for 30 min to ensure complete inactivation, titration of the remaining NFO activity of CYP3A4 generated a partition ratio of 54, which represents the molar ratio of methylophiopogonanone A metabolized per CYP3A4 inactivated (the turnover number appeared to be 55, Fig. 2B). With NADPH-fortified pre-incubation, nonlinear regression analysis of the time-dependent inactivation generated k_{inact} and apparent K_I values of 0.068 ± 0.009 min^{-1} and 13.3 ± 5.9 μM (r = 0.98), respectively (Fig. 2C). Linear regression analysis of the double reciprocal plots for inactivation rate and methylophiopogonanone A

Table 2. Effects of ingredients of SMS component herbs on nifedipine oxidation (NFO) activities in rat hepatic and intestinal microsomes in vitro.

SMS component herbs	Decoction[a] or ingredient	IC_{50} (decoction: mg/mL)[b] (ingredient: μM)	
		Liver	Intestine
SMS	Decoction	4.0 ± 0.5[c]	2.7 ± 0.4[d]
Schisandrae Fructus	Decoction	0.54 ± 0.08	0.12 ± 0.02
	Schisandrin B	3.9 ± 0.5	8.9 ± 1.4
	Schisantherin A	4.4 ± 0.5	2.1 ± 0.7
Ginseng Radix	Decoction	>6.0	>6.0
	Ginsenoside Rb1	>100	>100
	Ginsenoside Rd	80.2 ± 33.3	>100
	Panaxatriol	>100	46.5 ± 9.1
	Protopanaxatriol	>100	>100
Ophiopogonis Radix	Decoction	>15.0	5.2 ± 1.2
	Arctigenin	>100	>100
	Methylophiopogonanone A	16.8 ± 2.6	11.6 ± 0.7
	Ophiopogonin D	>100	>100
	Ruscogenin	>100	>100

In the absence of ingredients, rat hepatic and intestinal microsomal NFO activities were 0.81–1.35 nmol/min/mg protein and 7.1–11.8 pmol/min/mg protein, respectively. The inhibition was determined in the assays with 10-min NADPH-fortified pre-incubation of microsomes with ingredients. Results represent the curve-fitting result with the estimates of variance (denoted by ±) of a set of data of the mean percentage of remaining activities of separated determinations of 3 rats. [a]The ethanolic extracts of herbal decoctions were used in the study of inhibition *in vitro*. [b]The extraction yield was calculated and the IC_{50} value was calculated as mg decoction/mL. The IC_{50} values for the SMS-mediated inhibition were reported in our previous studies [22][c] and [23][d].

concentration generated k_{inact} and apparent K_I values for CYP3A4 of 0.058 min^{-1} and 9.1 μM (r = 0.97), respectively (Fig. 2D). The predicted threshold for causing a 2-fold increase in AUC using inhibition constants was 79.7 nM. With a 30-min NADPH-fortified pre-incubation, the inhibition of NFO by methylophiopogonanone A could not be restored after dialysis. In the recombinant CYP3A4 system, CPR is the electron-transfer partner of CYP, and its activity was not affected by methylophiopogonanone A either before or after dialysis (Fig. 2E). These results demonstrate that methylophiopogonanone A, a component of Ophiopogonis Radix, irreversibly suppresses the NFO activity of human CYP3A4 through time-dependent inhibition.

3.4. Influence of increased cumulative doses of SMS on health outcomes

Baseline characteristics of patients were described in Supplementary materials (Suppl. Fig. 1). The demographic data, including age, sex, year of diagnosis, comorbidities, and prior medication use, are shown in Table 4. There was a good balance in baseline characteristics between the nifedipine/felodipine and nifedipine/felodipine + SMS groups. The incidence rate (IR) of headache was 57.10 per 1,000 person-years in the nifedipine/felodipine group and 92.70 per 1,000 person-years in the nifedipine/felodipine + SMS group. Compared to the nifedipine/felodipine group, patients in the nifedipine/felodipine + SMS group had a significantly higher risk of incident headache (HR: 1.68; 95% confidence interval (CI): 1.44–1.97; $p < 0.0001$) (Table S2). The risks of low blood pressure, hospitalization for AMI, and HHF were similar between the two groups. In addition, there was a significantly lower mortality in the nifedipine/felodipine + SMS group than in the nifedipine/felodipine group (HR: 0.38; 95% CI: 0.12–0.78; $p = 0.0002$). Lastly, the effect of concomitant use of nifedipine/felodipine and SMS on bone fracture due to a vehicle accident was

Table 3. Inhibitory effects of the extract of SMS decoction and herbal ingredients, schisandrin B, schisantherin A, and methylophiopogonanone A, on nifedipine oxidation (NFO) activity of human CYP3A4 in a recombinant enzyme system.

Decoction or herbal ingredient	$IC_{50(-)}$ (μM)	$IC_{50(+)}$ (μM)	Ratio $IC_{50(+)}/IC_{50(-)}$	Predicted threshold (nM)
SMS	>6 (mg/mL)[a]	0.26 ± 0.09 (mg/mL)[a]	<0.04	–
Schisandrin B	6.1 ± 1.4	1.5 ± 0.2	0.25	7.69
Schisantherin A	0.17 ± 0.05	0.06 ± 0.01	0.35	0.34
Methylophiopogonanone A	36.5 ± 7.0	17.4 ± 2.1	0.48	45.0

In the absence of pre-incubation and ingredients, CYP3A4 exhibited NFO activity of 4.13 ± 0.11 nmol/min/nmol cytochrome P450 in the recombinant monooxygenase system. Ethanolic extract of SMS decoction was used in this inhibition study. [a]The extraction yield was calculated and the IC_{50} value was calculated as mg decoction/mL. Results shown in the second and third columns are the mean IC_{50} values ± estimates of variance estimated from curve-fitting analysis (n = 2–3). The predicted threshold of unbound inhibitor concentration causing a 2-fold AUC increase was calculated using the high-throughput method[28]. "–" not calculated.

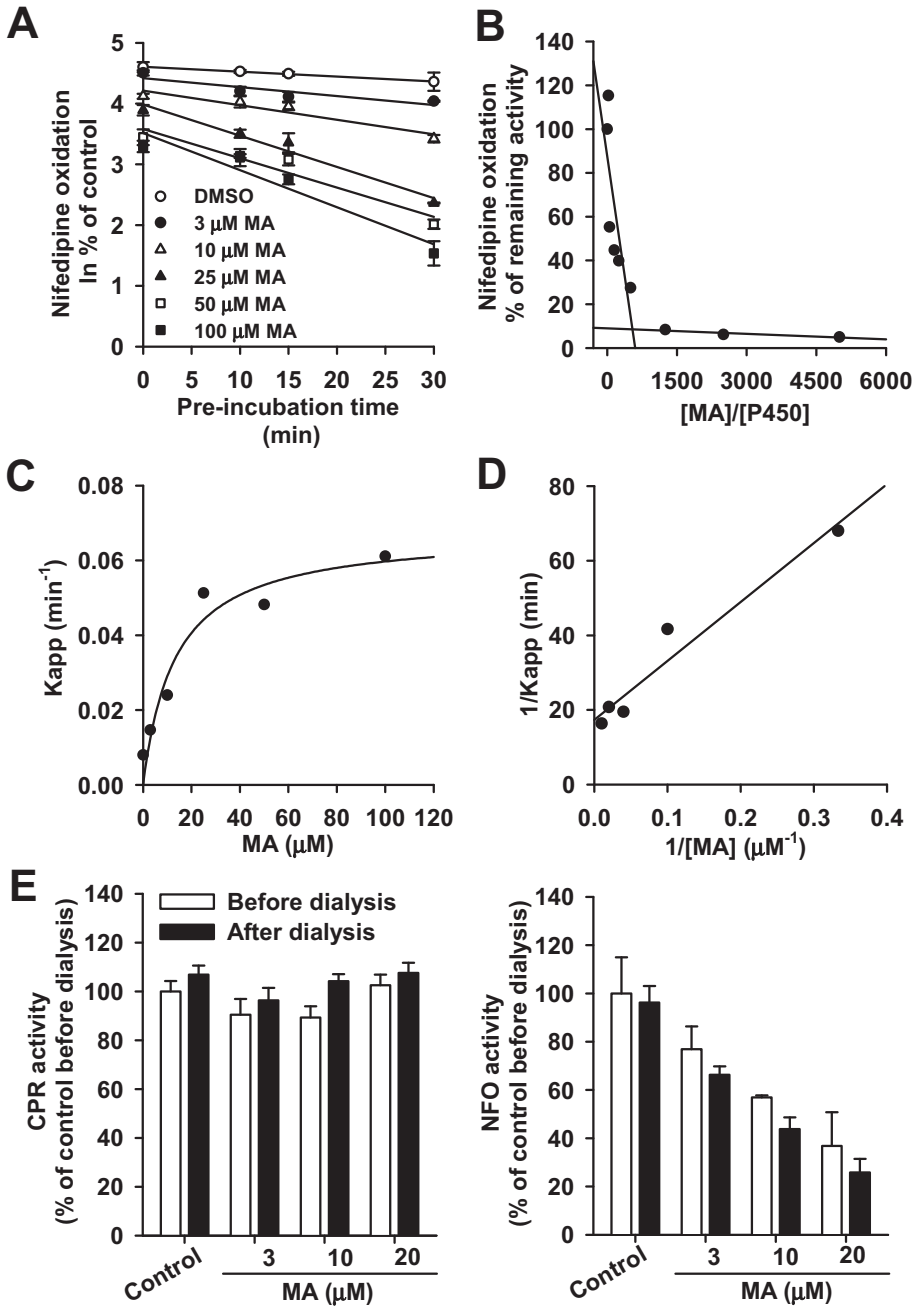

Fig. 2. Irreversible time-dependent inhibition of NFO activity by methylophiopogonanone A (MA) in a recombinant CYP3A4 system. (A) Effects of prolonged pre-incubation on the inhibitory effect of MA (in DMSO) on nifedipine oxidation activity of human CYP3A4. (B) Plot of remaining activity versus the molar ratio of MA to CYP3A4. (C) Plot of inactivation rate versus MA concentration. (D) Double-reciprocal plot of inactivation rate and MA concentration. (E) CPR and NFO activities of MA-inactivated recombinant CYP3A4 system before and after dialysis. In the control assay, the same concentration of DMSO was included. Data represent the means ± SEM (A and E) or means (B–D) of three determinations.

explored. The IRs of bone fracture due to a vehicle accident were 3.18 and 1.17 per 1,000 person-years in the nifedipine/felodipine and nifedipine/felodipine + SMS groups, respectively. Comparing co-treatment with SMS to the use of nifedipine/felodipine alone, there was no significantly different risk of experiencing fractures between the two groups (HR: 0.38; 95% CI: 0.12–1.24; $p = 0.1096$). The results showed that the concurrent use of SMS in nifedipine/felodipine-treated patients increased the risk of headache, but improved survival.

The dose-dependent effect of the concomitant use of SMS on health outcomes is further demonstrated in Table 5. Compared with the non-SMS group, an

Table 4. Baseline characteristics of the hypertensive patients included in this study.

Variable	Nifedipine/Felodipine group	Nifedipine/Felodipine + SMS group	StD
	n (%)	n (%)	
n	4,078	816	
Age (mean ± SD)	64.65 ± 14.18	64.79 ± 12.71	0.011
Sex			
Male	1,595 (39.11)	323 (39.58)	0.010
Female	2,483 (60.89)	493 (60.42)	
Diagnosed year			
2015	2,861 (70.16)	556 (68.14)	−0.006
2016	693 (16.99)	164 (20.10)	
2017	383 (9.39)	80 (9.80)	
2018	141 (3.46)	16 (1.96)	
Prior used medication			
Angiotensin-converting enzyme inhibitor	500 (12.26)	104 (12.75)	−0.015
Angiotensin receptor blocker	1,900 (46.59)	395 (48.41)	−0.036
Other β-blocker	1,789 (43.87)	376 (46.08)	−0.045
Other calcium channel blocker	1,648 (40.41)	337 (41.30)	−0.018
Thiazides	246 (6.03)	50 (6.13)	−0.004
Nitrates	485 (11.89)	98 (12.01)	−0.004
Other anti-hypertension drugs	459 (11.26)	101 (12.38)	−0.034
Statin	1,059 (25.97)	222 (27.21)	−0.028
Non-steroidal anti-inflammation drug	3,189 (78.20)	639 (78.31)	−0.002
Metformin	749 (18.37)	148 (18.14)	0.006
Insulin	224 (5.49)	48 (5.88)	−0.015
Comorbidity			
Diabetes mellitus	1,099 (26.95)	225 (27.57)	−0.014
Ischemic heart disease	787 (19.30)	164 (20.10)	−0.020
Myocardial infarction	10 (0.25)	2 (0.25)	0.000
Stroke	271 (6.65)	57 (6.99)	−0.013
Congestive heart failure	256 (6.28)	53 (6.50)	−0.009
Atrial fibrillation	87 (2.13)	16 (1.96)	0.013

The absolute StD <0.1 means a good balance between two groups.

increased risk of headache was found in the nifedipine/felodipine + SMS group with the use of increased cumulative dosages of SMS (1–60 g SMS, HR: 1.39; 95% CI: 1.11–1.74; >60 g SMS, HR: 1.97; 95% CI: 1.62–2.39; *p*-value for trend <0.0001). The survival benefit was also significant with the cumulative use of SMS; the HR was 0.67 (95% CI: 0.47–0.94) for patients who used nifedipine/felodipine concurrently with SMS at an cumulative dose of 1–60 g and 0.54 (95% CI: 0.37–0.79) for patients who used nifedipine/felodipine concurrently with SMS at an cumulative dose of >60 g (*p*-value for trend = 0.001). The trends in the association of cumulative SMS doses with both headache and survival were statistically significant.

4. Discussion

The changes in the pharmacokinetics of a victim drug by a time-dependent CYP3A4 inhibitor (perpetrator) can be affected by several factors of the perpetrator, including the dosage, administration time period, and tissue susceptibility to the inhibition [13,36]. Hepatic CYP3A has the main

Table 5. Effects of cumulative doses of SMS on health outcomes in patients taking nifedipine/felodipine.

Health outcomes	Hazard ratio (95% CI)			*p* value for trend
	Nifedipine/Felodipine (n = 4,078)	Nifedipine/Felodipine + SMS (1–60 g) (n = 428)	Nifedipine/Felodipine + SMS (>60 g) (n = 388)	
Headache	1.00	1.39 (1.11–1.74)	1.97 (1.62–2.39)	<0.0001
Low blood pressure	1.00	1.10 (0.48–2.54)	1.38 (0.66–2.90)	0.693
Acute myocardial infraction	1.00	1.65 (0.90–3.04)	1.03 (0.50–2.14)	0.267
Hospitalization of heart failure	1.00	1.21 (0.85–1.73)	0.79 (0.52–1.20)	0.274
All-cause mortality	1.00	0.67 (0.47–0.94)	0.54 (0.37–0.79)	0.001
Bone fracture	1.00	0.50 (0.12–2.07)	0.50 (0.12–2.07)	0.275

contribution to the systemic elimination of nifedipine [3,4] and intestinal CYP3A contributes mainly in the first-pass elimination [9,10]. Both grapefruit and SMS contain inhibitors, which inhibit NFO activity in a time-dependent manner [13,23], and decrease intestinal CYP3A function [3,13,24]. The bioavailability of nifedipine has been found to be elevated in humans due to the consumption of grapefruit juice, whereas its half-life, which is associated with systemic elimination, remained unchanged in healthy volunteers [14,37]. Our previous rat study using a 1-h treatment with a single dose of SMS revealed that the increased plasma nifedipine concentration primarily occurred in the elimination phase, resulting in a prolonged half-life and unchanged C_{max} [23]. This may be attributed to factors such as the absorption lag time of SMS ingredients (e.g., t_{max} in SMS (8 g/kg)-treated rats: schisandrin B: 1.6 h; schisantherin A: 2.3 h [38]; t_{max} in Schisandra Fructus extract (5 g/kg)-treated rats: schisandrin B: 4.1 h [39]) during acute treatment. The prolonged half-life was consistent with the decreased hepatic NFO activity by the acute SMS treatment. Our present findings showed that the elevation in the C_{max} and $AUC_{0\text{-}t}$ of nifedipine, together with the reduction in nifedipine clearance by 3-week SMS treatment suggest that the suppression of first-pass metabolism can be a primary factor in the SMS (3-week)-nifedipine interaction. However, the elimination half-life of nifedipine was not significantly affected in rats after 3 weeks of SMS treatment, which is consistent with the unchanged half-life of nifedipine after ingestion of grapefruit juice [14,37]. Like the unchanged hepatic CYP3A function after grapefruit ingestion [3], hepatic CYP3A protein level and activity were not affected by repeated SMS treatments [24]. Compared with the pharmacokinetic changes caused by a single dose of SMS, the 3-week-SMS-treated rats showed a greater increase in C_{max} and $AUC_{0\text{-}t}$ values. These findings reveal the dynamic influence of SMS on the pharmacokinetics of nifedipine after acute and repeated treatments.

Ketoconazole increased the C_{max}, AUC, and half-life of both nifedipine and dehydronifedipine, resulting from its potent inhibitory effects on NFO and glucuronidation activities [23,26]. The 2-week SJW treatment significantly increased liver microsomal activity of nifedipine oxidation to form dehydronifedipine without affecting hepatic glucuronidation activity. The SJW treatment prolonged the t_{max} and increased the clearance of nifedipine. However, the C_{max}, AUC, and clearance of dehydronifedipine were insignificantly affected by this repeated SJW treatment. Compared with ketoconazole-mediated changes, the 3-week SMS-elicited increases in C_{max} and $AUC_{0\text{-}t}$ of nifedipine were moderate. The decrease in nifedipine clearance by SMS was less than that by ketoconazole. The 2- and 3-week SMS treatments did not affect the elimination of dehydronifedipine, consistently with the unchanged hepatic and intestinal glucuronidation activities by SMS treatment [24]. Although that functional inhibition of P-glycoprotein has been suggested to partially contribute to the increased bioavailability of nifedipine by pioglitazone in rats [40], there was no evidence showing that transporters can be a limiting factor for the bioavailability of nifedipine. More studies should be done to reveal the contribution of influx and efflux transporters in the absorption and elimination of nifedipine.

The intestinal CYP3A was more susceptible to SMS-mediated enzyme inhibition in rats after 3 weeks of SMS treatment than hepatic CYP3A [24]. In consistent with that decreased intestinal NFO activity [24], nifedipine clearance significantly decreased after 3-week, but not 2-week, SMS treatment. The decreased intestinal oxidation of nifedipine decreased the first-pass effect and increased plasma concentration. Factors involved in the time-dependent decrease of intestinal NFO activity can comprise the inhibitory behaviors of SMS ingredients, the time-required accumulation of inactivated CYP3A, and the imbalanced degradation/de novo synthesis of CYP3A. The t_{max} of CYP3A inhibitors (>1.6 h) in SMS were longer than that in grapefruit juice, bergamottin, (0.5 h) in rats [41], suggesting that, compared to the events caused by grapefruit-juice [3,14], the delayed absorption of SMS ingredients prolonged the lag time required for CYP3A inactivation. In addition, the half-life of schisandrin B and schisantherin A were 7 and 30 h in rats treated with 8 g/kg lyophilized SMS, respectively [38]. The half-life of methylophiopogonanone A was 8–9 h in rats treated with Qingzao Jiufei decoction (4.6 g/kg) [42]. In our study, SMS were administered to rats once daily. Thus, the time required for accumulating schisantherin A to reach the steady-state was estimated to be at least 8 days. The increase in the AUC of plasma felodipine after five-day grapefruit juice ingestion was greater than that by a single glass of juice [3], indicating that the accumulation of inactivated CYP3A is required for a greater increase of AUC. The time-period for the significantly detected

suppression of CYP function by a time-dependent inhibitor can be longer than that for the plasma level of the inhibitor to reach the steady state [43]. Thus, the time-period required for a significant decrease of CYP3A activity through the accumulation of inactivated CYP3A could be longer than 8 days. On the other hand, our results showed the differential CYP3A modulatory effects of multiple ingredients of SMS component herbs. The interactions between ingredients should also be considered in the drug interaction assessment. Our findings revealed that a 3-week treatment was required for a significant increase of nifedipine AUC_{0-t}. The nearly 2-fold increase in systemic exposure to nifedipine after 3 weeks of SMS treatment in the rats alerted us to the potential herb-drug interactions of SMS with nifedipine or other CYP3A4 drug substrates (oral and first-pass dependent) in patients.

The dosing regimen, complicated composition and multiple modulator effects are determining factors for the metabolic change-mediated drug interactions caused by a compound decoction. For example, to assess the pharmacokinetic interaction, rats were treated with Shoseiryuto at a 10 times higher dose of grapefruit juice due to that Shoseiryuto inhibited rat CYP3A activity with an IC_{50} 10-fold higher than grapefruit juice [44]. The 30-min pre-treatment with grapefruit juice, but not Shoseiryuto, significantly increased the C_{max} of nifedipine. The differences in the absorption, elimination and inhibition kinetics of CYP3A inhibitors in grapefruit juice and Shoseiryuto should be considered when the *in vivo* dose was estimated from the IC_{50} values determined *in vitro*. Accordingly, our previous findings revealed the dose- [23] and time-dependent [24] decrease of CYP3A function in SMS-treated rats. Microsomes resuspended from pellets prepared by differential centrifugation of intestinal mucosa from 3-week SMS-treated rats had similar CYP3A protein levels as the control group but impaired NFO function (a 38% decrease), supporting irreversible inactivation through time-dependent inhibition *in vivo*. Among the component herbs of SMS at their respective doses corresponding to the amounts used for SMS preparation, the decoction of Ophiopogonis Radix irreversibly inactivated intestinal NFO activity. Our current findings further demonstrate that, after 10 min of pre-incubation, schisandrin B, schisantherin A, and methylophiopogonanone A inhibit NFO activity in rat hepatic and intestinal microsomes, as well as human CYP3A4 *in vitro*. The influence of Schisandra Fructus and its lignans on the elimination of CYP3A substrates involves reversible inhibition, time-dependent inhibition, and induction, which could depend on the exposure dose and time of treatment [45,46]. Our previous rat study showed that Schisandra Fructus at its SMS-equivalent dose did not impair hepatic and intestinal microsomal NFO activity after 3 weeks of treatment [24]. However, reversible and partially irreversible inhibitors can be removed during microsomal preparation. Our present findings suggest that we cannot exclude the involvement of the potent inhibitory effects of schisandrin B and schisantherin A on CYP3A activity over repeated treatments. In addition, the 3-day treatment with 8 and 16 mg/kg/day schisandrin B has been reported to increase the AUC of midazolam in rats [47]. In rats orally treated with a single dose of 8 g/kg lyophilized SMS, the C_{max} levels of plasma schisantherin A and schisandrin B were 7.1 and 28.6 nM, respectively [38]. For causing a 2-fold increase in AUC, the predicted threshold of unbound concentrations of schisantherin A and schisandrin B were 0.34 and 7.69 nM in humans, respectively. According to the low threshold levels of these Schisandra lignans, their contribution to the SMS-mediated pharmacokinetic interaction with CYP3A drug substrates should be considered for further human studies. Compared to Schisandra lignans, the predicted threshold of methylophiopogonanone A was higher. The CYP3A4 inactivation by methylophiopogonanone A could not be restored by dialysis, indicating the irreversible inhibition. The oral effect of methylophiopogonanone A needs further studies to show its contribution to the decreased CYP3A function in Ophiopogonis Radix- and SMS-treated rats [24]. Thus, *in vivo*, in addition to impaired intestinal NFO activity *via* time-dependent inactivation [24], the reversible or partially irreversible inhibition of hepatic and intestinal NFO by SMS herbal ingredients or their metabolites could contribute to the decreased clearance of nifedipine in 3-week SMS-treated rats, and possibly in humans.

Headache is the most common side effect of nifedipine/felodipine with high plasma drug concentrations [1]. Consistent with the increased AUC of plasma nifedipine after 3-week SMS treatment in rats, the results of our retrospective study revealed that patients with combined therapy with nifedipine/felodipine and SMS (>7 days) had higher incidence of headaches, without elevating the risks of more severe undesired effects, such as hypotension. SMS treatment was reported to cause headaches in one participant in a study of 15 healthy

volunteers [18]. However, there were no headache and bone fracture in the adverse effects in a study, which reviewed 14 randomized controlled trials (858 patients) of the outcome/adverse effects of SMS [48]. Although the baseline characteristics of the patients were well-balanced between the groups in our study, confounding factors, such as the CYP3A4 genotypes, dosage of nifedipine, formulation of nifedipine/felodipine (e.g., immediate-release capsules and extended-release tablets) and disease development, should be considered.

The results of our studies showed that there were no significant differences in composite cardiovascular outcomes, including hospitalization for AMI and HHF in the nifedipine/felodipine group with and without SMS treatment. However, when patients received both nifedipine/felodipine and SMS treatments, all-cause mortality significantly decreased. This decreased mortality showed a significant association with increased accumulation of SMS dose. Among the patients included in this study, 19–20% of them had ischemic heart disease, 6–7% had congestive heart failure, and 7% had suffered a stroke in both the nifedipine/felodipine and nifedipine/felodipine + SMS treatment groups. The pharmacological activities of SMS are supported by published evidence, such as the amelioration of inflammation in patients who received percutaneous coronary intervention [19] and myocardial protection against ischemic injury in mice [49], suggesting that the therapeutic efficacy of SMS reduces mortality. The reduced mortality by SMS intervention highlights the benefits of herb-drug co-treatment and warrants future clinical trials on patient health outcomes.

In conclusion, the laboratory rat study demonstrated that 3-week daily treatment with SMS decreased the clearance of nifedipine and increased systemic exposure. Rat NFO inhibitors in SMS herbs also inhibited the NFO activity of human CYP3A4. A retrospective population-based study revealed a potential benefit on patient survival and increased risk of headache with the combined use of nifedipine/felodipine and SMS in patients with hypertension.

5. Limitations

The current study has several limitations. First, the population-based claims data analysis that was used does not contain information related to risk factors for death, such as smoking, family history, and obesity. Second, although we employed many methods to prevent possible confounders, potential unmeasurable bias remains due to the observational nature of our study design. However, several factors support the robustness of our findings. We obtained excellent balance in baseline covariates across treatment groups and consistent results using several analytical approaches. Finally, the lack of an observed effect on the negative control outcome of bone fracture due to a vehicle accident suggests that confounding is less likely to be a major concern.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary Materials.

Supplementary Methods

Threshold concentration prediction

According to the high-throughput screening method[1] (Eq. 1) and inactivation kinetic method[2,3] (Eq. 2), the threshold concentration required to produce a doubling of the AUC was estimated. The k_{deg} values for CYP3A4 ranged from 0.000146–0.000825[2-4]. The f_m value of nifedipine and k_{deg} value of CYP3A4 used for the threshold prediction were 0.9 and 0.0003, respectively[2].

$$\frac{AUC_i}{AUC} = \frac{1 + \left\{ 1 + \frac{1 + \left(\frac{IC_{50(-)}}{IC_{50(+)}} \right)}{k_{deg} \times t} \times \ln \left(\frac{2}{1 + \left(\frac{IC_{50(+)}}{IC_{50(-)}} \right)} \right) \right\} \times \frac{[I]}{IC_{50(-)}}}{1 + \frac{[I]}{IC_{50(-)}}} \quad \text{(Eq.1)}$$

$$\frac{AUC_i}{AUC} = \frac{1}{f_m \times \left\{ \frac{k_{deg}}{k_{deg} + \left(\frac{[I] \times k_{inact}}{[I] + K_I} \right)} \right\} + (1 - f_m)} \quad \text{(Eq.2)}$$

f_m: fraction of nifedipine metabolized by CYP3A4
$IC_{50(+)}$: the concentration causing 50% inhibition of activity with NADPH-fortified pre-incubation for a time-period of t
$IC_{50(-)}$: the concentration causing 50% inhibition of activity without pre-incubation
[I]: unbound concentration of the inhibitor
k_{deg}: the rate constant of CYP3A4 degradation
k_{inact}: the maximal inactivation rate constant
K_I: the inhibitor concentration required for half-maximal inactivation
t: pre-incubation time in the assay

Dialysis experiment

Membranes from bacteria expressing human CYP3A4 and CPR (20 nM) were incubated with 3−20 μM methylophiopogonanone A in a final volume of 400 μL (50 mM potassium phosphate buffer (pH 7.4), 10 mM glucose 6-phosphate, 0.5 mM $NADP^+$ (sodium salt), glucose 6-phosphate dehydrogenase, 12.5 mM $MgCl_2$) at 37 °C for 30 min. Incubations containing the same volume of dimethyl sulfoxide, but no methylophiopogonanone A were used as vehicle controls. After pre-incubation, the mixture was placed on ice. A 200 μL aliquot was dialyzed using a mini-dialysis unit (10 kDa cutoff, Thermo Scientific-Pierce Biotechnology, Rockford, IL, USA) against 120 mL of dialysis buffer (50 mM potassium phosphate containing 0.1 mM EDTA at 4 °C) with three buffer changes at 30-min intervals. Aliquots of 10 μL and 75 μL were used for the determination of NADPH-P450 reductase (CPR)[4] and NFO activities. In the determination of NFO activity, the aliquot was added into a reaction mixture containing 29 mM potassium phosphate buffer (pH 7.85), 10 mM glucose 6-phosphate, 0.5 mM $NADP^+$ (sodium salt), 0.5 unit/mL glucose 6-phosphate dehydrogenase, 12.5 mM $MgCl_2$ and 200 μM nifedipine. Oxidation was carried out for 15 min, and the generation of dehydronifedipine was determined as described in the Methods section of the manuscript.

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Table S1. Diagnosis codes of health outcomes.

Health outcomes	ICD-9-CM code	ICD-10-CM code
Headache		R51
Low blood pressure	458, 796.3	I95, R03.1
Acute myocardial infraction (AMI)	410	I21-I22
Heart failure	429, 398.91, 402.x1, 404.x1, 404.x3	I09.81, I11.0, I13.0, I13.2, I50

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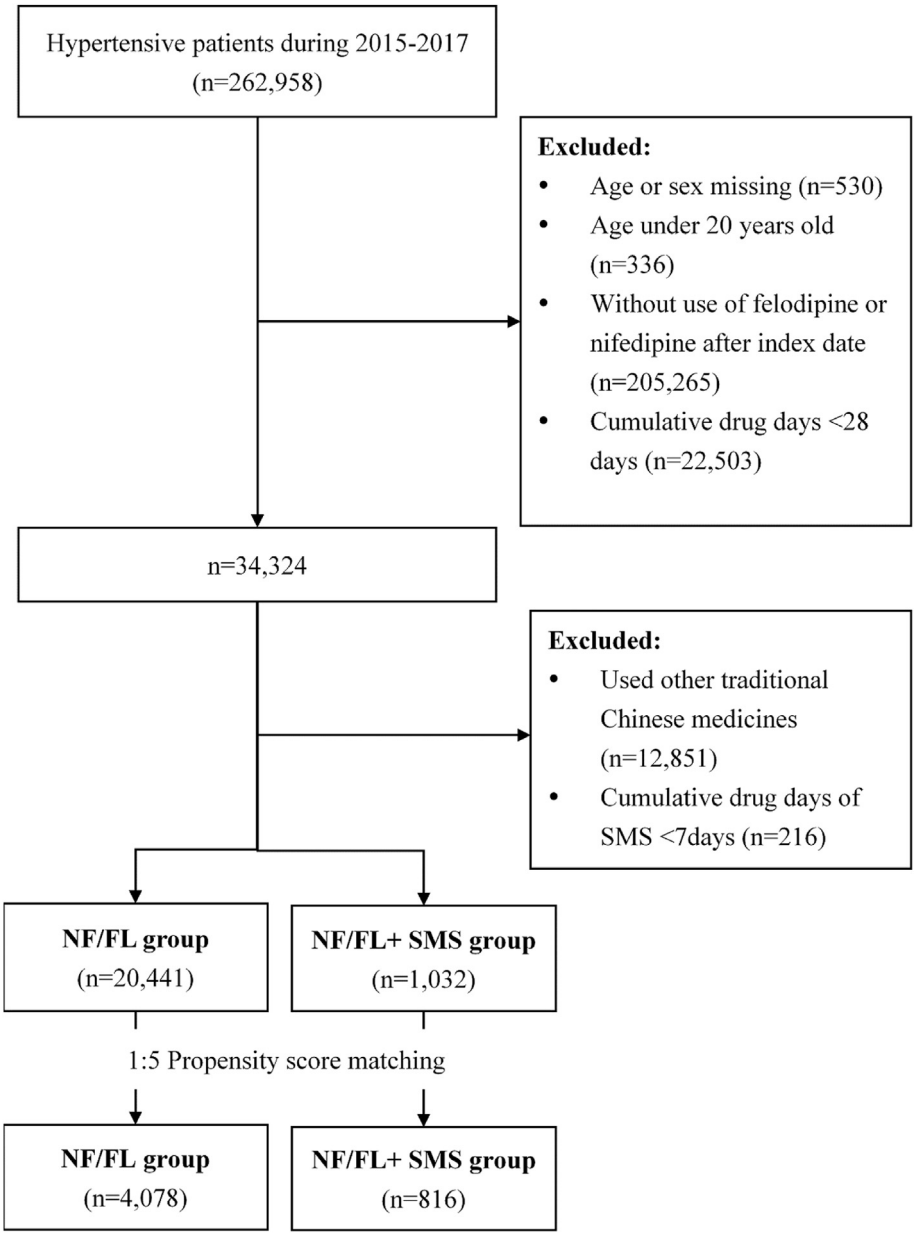
Supplementary Results

Baseline Characteristics of Patients

A total of 34,324 patients with hypertension who used nifedipine or felodipine between 2015 and 2017 were identified. After excluding those who used other TCMs (n=12,851) or had <7 cumulative drug days of SMS usage during the follow-up period (for the nifedipine/felodipine+SMS group) (n=216), 21,473 eligible patients (nifedipine/felodipine: 20,441 patients; nifedipine/felodipine+SMS group: 1,032 patients) were included (Suppl. Fig. 1). After 1:5 PSM, 4,078 patients in the nifedipine/felodipine group and 816 patients in the nifedipine/felodipine+SMS group were analyzed.



Supplementary Fig. 1. Flowchart of patient selection. NF: nifedipine; FL: felodipine; SMS: Shengmai-San.

Table S2. Number of events, incident rates, and hazard ratios (HRs) of health outcomes.

	NF/FL alone			NF/FL + SMS co-treatment			HR (95% CI)	*P* value
	No. of event	Follow-up duration (person-year)	Incident Rate (per 1,000 person-year)	No. of event	Follow-up duration (person-year)	Incident Rate (per 1,000 person-year)		
Headache	638	11,173.72	57.10	205	2,211.36	92.70	1.68 (1.44-1.97)	< 0.0001
LBP	56	12,234.00	4.58	14	2,537.76	5.52	1.24 (0.69-2.23)	0.4678
AMI	75	12,234.00	6.13	20	2,537.76	7.88	1.33 (0.81-2.18)	0.2533
HHF	292	11,907.76	24.52	58	2,472.48	23.46	0.99 (0.75-1.31)	0.9446
All cause death	486	12,315.56	39.46	61	2,570.40	23.73	0.60 (0.46-0.78)	0.0002
Bone fracture	39	12,274.78	3.18	3	2,562.24	1.17	0.38 (0.12-1.24)	0.1096

Abbreviations: LBP, low blood pressure; AMI, acute myocardial infraction; HHF: hospitalization due to heart failure.
There were 4,078 and 816 patients included in the data analyses of groups taking nifedipine/felodipine without (NF/FL alone) and with the co-treatment with Shengmai-San (NF/FL + SMS co-treatment), respectively. Low blood pressure: systolic blood pressure < 90 mm-Hg or diastolic blood pressure <60 mm-Hg.

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