Department of Pharmacy, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People's Republic of China

Capsaicin induces metabolism of simvasatin in rat: involvement of upregulating expression of Ugt1a1

Chaoran Zhu, Xuejia Zhai, Fen Chen, Nanxi Wang, Xinlin Zhang, Yongning Lu

Received November 23, 2105, accepted December 22, 2015

Yongning Lu, Department of Pharmacy, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1277, Jiefang Avenue, Wuhan, Hubei Province, China
luyn_union@163.com

Pharmazie 71: 269–273 (2016) *doi: 10.1691/ph.2016.5861*

Capsaicin (CAP, trans-8-methyl-N-vanillyl-6-nonenamide) is a major pungent substance in hot pepper. However, little is known about the interactions between CAP and clinically used drugs. This study investigated the effect of acute and chronic ingestion of CAP on pharmacokinetics of simvastatin (SV) and the mechanism of this CAP – drug intercation. CAP was orally administered at doses of 3 and 8 mg·kg^{-1} for seven consecutive days once daily and on the 1st day and the 7th day, SV (8 mg·kg^{-1}) was injected intravenously. Plasma concentrations of SV were determined using LC/MS/MS and expression of Ugt1a1 was analyzed by RT-qPCR and Western Blotting. We found that there were 78.0 % ($P< 0.05$) and 81.2% ($P< 0.05$) reduction in the $AUC_{(0-\infty)}$ of SV, respectively, following pretreatment with two doses of CAP. The $AUC_{(0-\infty)}$ of SV in the two dose group pretreated with CAP for 7 days were decreased significantly, compared to the group for 1 day. Both the RT-qPCR and Western Blotting data indicated that 7 days pretreatment with CAP increased the expression level of Ugt1a1 in liver. In conclusion, chronic ingestion of CAP enhanced the expression level of Ugt1a1 in liver, causing the food -drug interaction and decrease in SV exposure in rats to a significant extent.

1. Introduction

Hot pepper fruits are among the most heavily consumed spices throughout the world, and belong to the plant genus Capsicum. The primary pungent principle, capsaicin (CAP) is responsible for spiciness of pepper fruit (Bode and Dong 2011). In green and red pepper, the content of CAP ranges from 0.1% to 1% (Hayman and Kam 2008). Many sources for human exposure to CAP include ingestion of spicy foods and inhalation of cooking fumes and pepper spray aerosols (Szallasi and Blumberg 1993a, 1999b; Robbins 2000; Reilly et al. 2001a, b; Szallasi and Appendino 2004).

Simvastatin (SV), a highly potent inhibitor of 3-hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase, is a lactone prodrug used for the treatment of hypercholesterolemia, hyperlipidemia and to reduce the risk of cardiovascular events (Lee 2015). SV is mainly metabolized by CYP3A4 (Becker et al. 2010). Some studies also revealed that UDP-glucuronosyltransferase 1-1 (UGT1A1) was capable of forming the glucuronide conjugates and the corresponding lactones for statins (Prueksaritanont et al. 2002). Moreover, Tuteja found that ezetimibe may have inhibited the glucuronidation of simvastatin hydroxy acid, resulting in increased SV exposure and subsequent hepatotoxicity (Tuteja et al. 2008).

CAP evokes numerous biological effects and thus has been the target of extensive investigation (Díaz-Laviada 2010; Gooding et al 2010; Reyes-Escogido et al. 2011). Previous studies manifested that CAP can increase clearance and decrease AUC of SV after oral administration of SV (Zhai et al 2013a), which is not satisfactorily explained by the fact that CAP moderately inhibited Cyp3a1/2 (Zhai et al. 2013b). Moreover, one study indicated that systemic exposure to CAP which follows from either the ingestion of CAP-containing foods such as chili peppers or topical administration of a high-concentration cutaneous patch is highly unlikely to inhibit or induce the activities of the CYP enzymes (Babbar et al. 2010). In consequence, the decrease in SV exposure might be attributed to an induction of glucuronidation metabolism, the another important metabolism pathway of SV, following CAP pretreatment. A previous study demonstrated that glucuronidation was also an important pathway for CAP clearance; UGT1A1 and 2B7 are the key enzymes contributing to metabolism of CAP (Sun et al. 2014). However, it remains poorly understood whether CAP might react on the UGTs causing the interaction with SV and other drugs. Considering the frequent consumption of CAP and its current therapeutic application, correct assessment of this compound is important from a public health standpoint.

In this study, we investigated effects between acute and chronic pretreatment of CAP at two dose levels on pharmacokinetics of SV. Moreover, we present the changes in expression of Ugt1a1 isoform after treatment with CAP to determine whether CAP regulates Ugt1a1 in the liver as the inducer of SV metabolizing enzymes.

2. Investigations and results

2.1. Pharmacokinetic studies in rats

After 7 days of treatment, there were no significant differences in mean body weight between the control and CAP pretreatment groups. The plasma concentration–time curves of SV after intravenous injection at the dose of 8 mg·kg^{-1}, with different pretreatments, are shown in Fig. 1. Table 1 lists the pharmacokinetic data of SV for different groups suffering 1 day and 7 days pretreatment.

Upon seven consecutive days pretreatment with CAP, the $AUC_{(0-t)}$ of of SV after administration, were down to 162.8±104.7 and 139.5±69.5 mg/L·h, respectively. All were significantly ($P< 0.05$) lower than the group pretreated with vehicle (668.3±506.5 mg/L·h). Likewise the $AUC_{(0-\infty)}$ values were also decreased. Compared to the

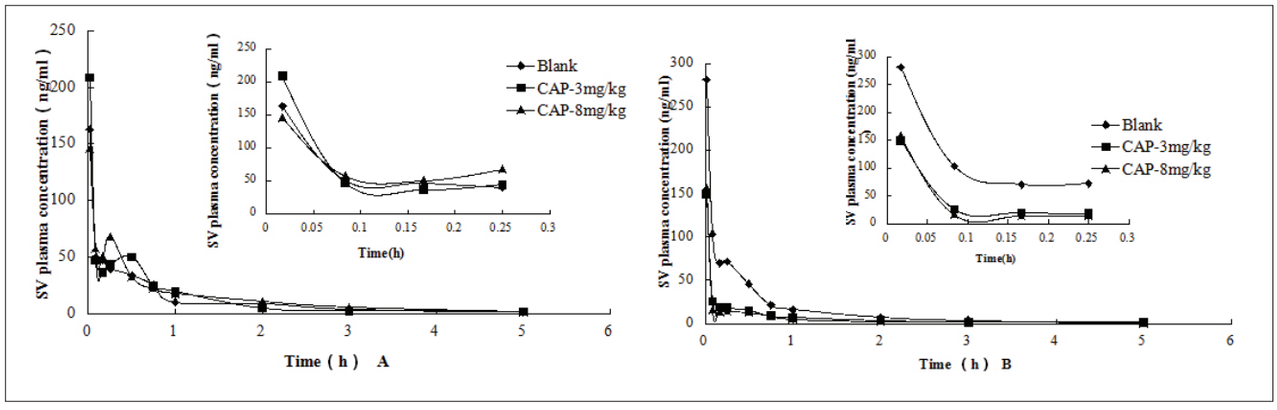

Fig. 1: Plasma concentrations of SV in rats assigned to the different pretreated groups after intravenous injection of SV (8 mg·kg^{-1}) on the 1st day (A) and the 7th day (B).

Table 1: Pharmacokinetic parameters of simvastain in rats assigned to the different pretreated groups Rats received oral once daily 0.5% CMC-Na or CAP for 7 consecutive days. On the 1st day and the 7th day rats were intravenous injected SV (8 mg/kg)

		Control	CAP-3mg/kg	CAP-8mg/kg
1 day pretreatment				
$AUC_{(0-t)}$	ug/L·h	611.9±461.7	372.2±108.2	540.4±194.1
$AUC_{(0-\infty)}$	ug/L·h	667.7±483.2	399±126.1	576.3±187.7
$MRT_{(0-t)}$	h	0.82±0.31	0.80±0.17	1.13±0.29
$MRT_{(0-\infty)}$	h	1.03±0.43	1.12±0.29	1.40±0.44
$T_{1/2}$	h	1.6±0.7	1.7±1.1	1.2±0.3
CL/F	L/h/kg	85.7±104.1	67.7±26.6	48.2±20.3
7 days pretreatment				
$AUC_{(0-t)}$	ug/L·h	668.3±506.5	162.8±104.7*+	139.5±69.5*++
$AUC_{(0-\infty)}$	ug/L·h	750.4±581	165.3±108.3*+	140.7±69*++
$MRT_{(0-t)}$	h	0.79±0.28	0.66±0.20	0.68±0.24
$MRT_{(0-\infty)}$	h	1.03±0.55	0.72±0.24	0.75±0.25
$T_{1/2}$	h	1.6±0.8	0.8±0.2	0.9±0.2
CL/F	L/h/kg	55.7±41	177.9±78.7*+	189.8±63.6*++

Rats received oral once daily 0.5% CMC-Na or CAP for 7 consecutive days. On the 1st day and the 7th day rats were intravenous injected SV (8 mg/kg)
Each value represents the mean ± S.D. of six rats.
* Significantly different with *P< 0.05* versus parameters of control group (0.5% CMC-Na).
+ Significantly different with *P< 0.05* versus parameters of 1 day pretreatment
++ Significantly different with *P< 0.01* versus parameters of 1 day pretreatment

control group, $T_{1/2}$ and MRT in all kind of pretreatment groups for seven consecutive days did not quite reach statistical significance but CL/F (177.9±78.7 and 189.8±63.6 L/h/kg) were significantly (*P< 0.05*) increased. However, all pharmacokinetic parameters of 1 day CAP pretreated groups had no significant difference compared to control group.

The pharmacokinetic parameters of SV between the groups with 1 day pretreatment and 7 days pretreatment showed obvious differences. The AUC values of SV of low dose CAP group and high dose CAP group with 7 days pretreatment, compared to the group under 1 day pretreating condition, were significantly decreased. Moreover, CL/F of low dose group and high does group with 7 days pretreatment are significantly higher than the groups under 1 day pretreating condition.

2.2. RT-qPCR analysis of Ugt1a1 mRNA in rat liver

By the use of specific primers toward mRNA encoding the rat liver Ugt1a1 isoform, we were able to observe that in rats assigned to the CAP (3 and 8 mg·kg^{-1}) groups, the expression of hepatic Ugt1a1, which were elevated to comparable levels, were 2.8 and 2.4-fold that of control, respectively (Fig. 2).

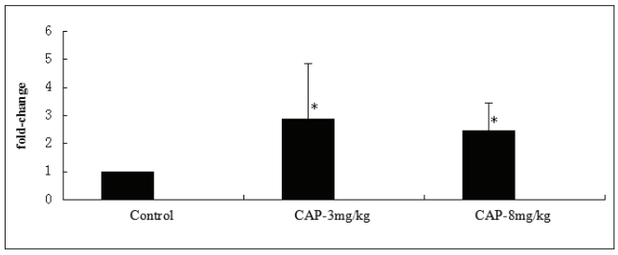

Fig. 2: Effect of multiple administrations of CAP once daily for 7 days at the doses of 3 and 8 mg·kg^{-1} on mRNA expressions of hepatic Ugt1a1 in rats. Each column represents the mean of six determinations from six rats. * Significantly different with P< 0.05 versus parameters of control group (0.5% CMC-Na).

2.3. Western blotting analysis of Ugt1a1 protein in rat liver

Ugt1a1 protein in rat liver was determined by Western Blotting. As shown in Fig. 3, CAP significantly upregulated the Ugt1a1 expression level following 7 days pretreatment with CAP. When pretreated with CAP, significant induction of the Ugt1a1 protein was seen at the 3 and 8 mg·kg^{-1}of CAP, which produced a Ugt1a1 protein level equivalent to 1.5 and 1.7-fold that of control.

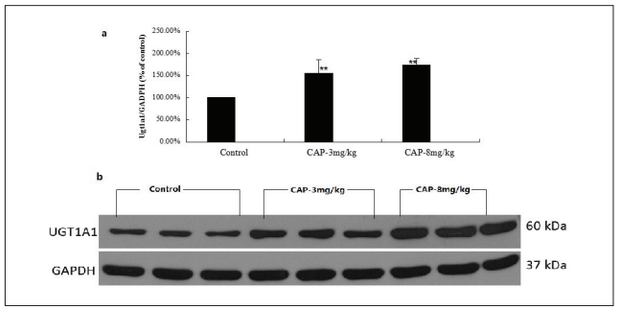

Fig. 3: Effect of multiple administrations of CAP once daily for 7 days at the doses of 3 and 8 mg·kg^{-1} on protein expressions of hepatic Ugt1a1 in rats. a, data analyses of Western Blotting results. b, one of image of Western Blotting results. Each column represents the mean of six determinations from six rats. ** Significantly different with P< 0.01 versus parameters of control group (0.5% CMC-Na).

3. Discussion

CAP might change the P-gp-mediated efflux on intestinal absorption and alters the uptake of other drugs co-administered topically or orally by causing mucosal vasodilation (Hayman and Kan 2008). To exclude influencing factors, we attempted to evaluate the effect of CAP on the pharmacokinetics of SV in the aspect of metabolism and excretion by giving SV intravenously. Furthermore, some herbal medicines, such as St. John's wort (Rengelshausen et al. 2005), showed differential effects toward cytochrome P450 isoforms between short-term and long-term treatment. This is likely to occur in the CAP-drug interactions in various clinical practices. Thus, we delineated the acute and chronic effects of CAP on SV metabolism *in vivo.*

As mentioned above, Zhai employed female rats to study the effect of CAP on the pharmacokinetics of SV, due to the drug interaction mediated by CYP3A4 in human could be reproduced in study with female rats (Ishigami et al. 2001). Based on that, we carried out a further study about the key pathway to alter the pharmacokinetics of SV. In order to keeping consistent with the original experiments, we still chose female rats as the experimental subjects. Furthermore, we found that there was no significant difference between pretreatment with CAP for 7 days and for longer pretreatment period, such as 28 days, which is not shown in detail here. According to the results, it is suitable to choose 7 days to acts as the chronic pretreatment model.

The present study was a detailed investigation of the effect of CAP on the pharmacokinetics of SV. The pharmacokinetic results revealed that groups pretreated with CAP for 7 days increased ($P< 0.05$) the CL/F of SV by 3.2 and 3.4-fold significantly. Besides, there were 78.0 % ($P< 0.05$) and 81.2% ($P< 0.05$) reduction in the $AUC_{(0-\infty)}$ of SV, respectively, following pretreatment with CAP at two dose levels. It was clearly consistent with Zhai's results of oral administration of SV, pretreated with CAP for one week, which caused a decrease by 67.06 % (3 mg/kg $P < 0.05$), 73.21 % (8 mg/kg $P < 0.01$) and 77.49 % (25 mg/kg $P < 0.01$) in the $AUC_{(0-\infty)}$ of SV, respectively compared with the control group (Zhai et al. 2013a). It can certainly be said that CAP enhances the elimination of SV. In the comparison of single and multiple pretreatment, pretreatment with CAP cause a significant increase ($P< 0.05$) in clearance and decrease in AUC of SV. This results indicated that the effect appeared to rely on the pretreatment period. There is a slight decrease in MRT and $T_{1/2}$ in all CAP pretreated groups, compared to the control group. This might be caused by an induction metabolism of SV. According to present study, we could illustrate that the change in pharmacokinetics of SV *in vivo* was due to the frequent consumption of CAP rather than of a single dose.

UGTs are enzymes involved in detoxification of endobiotics xenobiotics, and are encoded by a complex multigene family (Koster et al. 1986; Tephly and Burchell 1990; Conner et al. 1997). SV is substrate of UGT. Tea can act as a chemopreventive agent through the induction of the microsomal detoxification enzyme, UDP-GT (Embola et al. 2002). Gemfibrozil increased the mean total area under the plasma concentration–time curve of SV [$AUC_{(0-\infty)}$] by 35% ($P< 0.01$) (Backman et al. 2000). Similarly, pretreatment increaseed C_{max} of SV by two fold, because gemfibrozil is known to reduce the glucuronidation and elimination of statins (Kellick et al. 2014). It was interesting to note that the expression of UGT was higher in the low dose group than in the high dose group. However, this difference did not reach statistical significance. Therefore, the induction of Ugt1a1 enzyme level by CAP did not show a relationship with the dosage.

For this analysis, we employed RT-qPCR and Western Blotting to assess the changes of Ugt1a1 in mRNA and protein level. The data confirmed that the mRNA expression of level Ugt1a1 was increased ($P< 0.05$) in the liver of rats consumed with CAP compared the controls. The inccrease in Ugt1a1 gene expression was consistent with results obtained from Western Blotting in which two doses of CAP pretreatment induced Ugt1a1 protein expression. The enzyme Ugt1a1 in the liver of rats was induced by ingestion of the CAP for one week, which was obviously coincident with the result of pharmacokinetics, indicating that the change in pharmacokinetics of SV was related to that process.

In summary, CAP certainly alters the pharmacokinetics of SV in the rat. The CL/F of SV was inccreased significantly after dietary pretreament with CAP. At the same time, Ugt1a1 in the liver was increased at both the mRNA and protein levels. These findings proved that chronic ingestion of CAP upregulated the expression level of Ugt1a1 in rat liver, resulting in a decrease of the exposure of SV in rats. As a consequence, since the elimination of drug was altered when CAP was co-administered to rats, the possibility of metabolic interactions between CAP and drug that are substrates to Ugt1a1 enzymes must be considered.

4. Experimental

4.1. Chemicals and reagents

CAP, SV and lovastatin (LV) were purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile (HPLC-grade) was provided by Merck (Darmstadt, Germany). Formic acid (analytical-grade) was obtained from Aladdin Industrial Corporation (Shanghai, China). The water used for HPLC was purified by use of a Milli-Q system (Millipore, Milford, MA, USA). All other chemicals were of analytical grade and were commercially available.

4.2. Animal experiments

Female Sprague-Dawley rats, 226±1.6 g, were obtained from the Laboratory Animal Center of the Tongji Medical College of Huazhong University of Science and Technology (Wuhan, Hubei, China). Before and throughout the study, the rats were housed six per cage and in a temperature and humidity controlled room (approximately 24 °C and 45±5 %) and 12 h night/12 h day conditions. Food and water were available ad libitum, no other restrictions were made. All animal experimentation procedures were carried out according to the guidelines for the care and use of laboratory animal and were approved by the local Animal Ethic Committee.

The 18 rats were randomly divided into three groups: control group (pretreated with 0.5% CMC-Na, p.o., *n=6*); low dose group (pretreated with repeated doses of 3 $mg{\cdot}kg^{-1}$ CAP in 0.5% CMC-Na, p.o., *n=6*); high dose group (pretreated with repeated doses of 8 $mg{\cdot}kg^{-1}$ CAP in 0.5% CMC-Na, p.o., *n=6*). All the rats were given vehicle (control group) or drug (other groups) daily by oral gavage for 7 days. Twelve hours before intravenous administration of SV, access to diet was removed and only water was provided. On the 1st and the 7th day, 30 min after pretreatment with CAP, 8 $mg{\cdot}kg^{-1}$ SV (dissolved in saline containing 2 % DMSO) were injected to rats. Blood samples (300 µL) were collected from orbital veins at 0, 1, 5, 10, 15, 30, 45, 60, 120, 180, 300 min. The blood samples were harvested in a centrifuge tube with heparin. Then, the plasma samples were obtained by immediate centrifugation at 3000 rpm for 10 min. The rats were killed by cervical dislocation, necropsied and the livers obtained. The plasma samples and liver samples were stored at -80 °C.

4.3. Analytical methods

SV was assayed using a rapid and sensitive LC/MS/MS method. Briefly, SV and LV (internal standard) were extracted from 100 µL plasma with 600 µL tert-butyl methyl ether/hexane (7:2; v/v) by liquid-liquid extraction. The separation work was carried out on an Agilent 1200series HPLC system. Chromatographic analysis was conducted using an isocratic elution on a Welch Ultimate® XB-C18 column (5 µm, 2.1 × 100 mm, Shanghai, China) with a mobile phase of with acetonitrile-water (containing 0.1 % formic acid) (88:12; *v/v*) at a flow rate of 0.3 mL/min. An API 4000 MS/MS system (Applied Biosystems, Foster City, CA, USA) with a TurboIonspray source operated in positive electrospray ionization (ESI) mode with selected reaction monitoring (SRM) for LC/MS/MS analyses. The precursor to product ion transitions were m/z 419.3 ≥ 199.2 for SV and m/z 405.3 ≥ 199.2 for LV (IS) with dwell times of 200 ms. The optimal ESI-MS/MS parameters were as follows: the ion spray voltage and source temperature were 5000 V and 400 °C; the gas rates for nebulizing gas (GAS1), turbo gas (GAS2), curtain gas (CUR) and collision gas (CAD) were set to 40, 40, 25 and 6 psi, respectively; the declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP) were set to 80, 8, 16 and 10 V, respectively, for SV and 74, 6, 14 and 11 V, respectively, for LV. The total run time was 3.0 min per sample, the retention times were 1.93, 1.70 for SV and LV (Fig. 4). Calibration curves were linear over the range of 0.5 to 500 ng/mL for SV in plasma. The lower limit of quantification was 0.5 ng/mL in plasma. Accuracy evaluated at three concentration levels (1.5 ng/mL, 150 ng/mL and 300 ng/mL) was acceptable for SV (ranged from 86.8 to 113.7%). Precision (R.S.D.%) of the method ranged from 4.5 to 13.3%. Stability of the samples under the assay conditions was secured. The

CV of the IS-normalised matrix factor calculated from the six batches of matrix was smaller than 15% (7.6% and 0.3%). The extract recovery of SV, determined at three concentration levels, were 70.1%, 67.3% and 61.0%, respectively. The mean extraction recovery of IS was 135.7%. Data acquisition, peak integration and calculations were performed using Analyst 1.6.1 system.

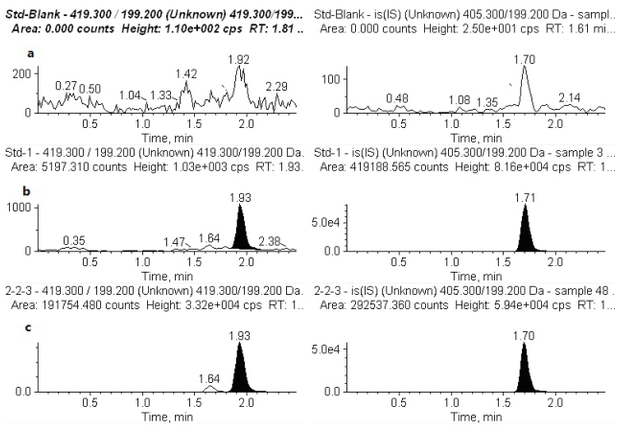

Fig. 4: Representative chromatograms of SV (the left shaded peaks) and LV (the right shaded peaks) in rat plasma. (a) blank sample; (b) plasma spiked with SV (1.5 ng/mL) and LV (50 ng/mL); (c) plasma sample from a rat receiving 8 mg·kg^{-1} SV.

4.4. Pharmacokinetic analysis

Pharmacokinetic analysis was performed basing on a noncompartmental analysis using "The Drug and Statistics" (DAS) 3.2.7 pharmacokinetics fitting software. Pharmacokinetic parameters, such as the area under the plasma concentration–time curve (AUC), the clearance (CL/F), the half-life ($T_{1/2}$) and mean residence time(MRT) were determined.

4.5. Real-time quantitative PCR (RT-qPCR) analysis

Total RNA was extracted from liver samples using the RNAiso Plus (TaKaRa Biotechnology, Dalian, China, Cat. #D9108) following the manufacturer´s instructions and then reversely transcribed to cDNA using PrimeScriptTM RT reagent kit with gDNA Eraser (TaKaRa Biotechnology, Cat. #RR047A). The RT-qPCR was carried out to determine the level of Ugt1a1 mRNA expression by using SYBR® Premix ExTaq™ (TaKaRa Biotechnology, Cat. #RR420A) in a 20 μL reaction system. The reaction system contained 2 μL of cDNA, 10 μL of SYBR Premix Ex Taq™ and 0.2 μM each of the forward and reverse primers (Table.2). The samples were initially denatured at 95 °C for 30 s and amplified using 40 cycles of 95 °C for 5 s, 60 °C for 30 s and dissociated at 95 °C for 15 s, 60 °C for 60 s, 95 °C for 15 s on a StepOnePlus RT-qPCR System (Life Technologies, Carlsbad, CA). Primers used in this study were designed and synthesized by TaKaRa. GAPDH was used as an internal control.

Table 2: PCR Primers for Ugt1a1 and GADPH

Gene	Primers(5´–3´)	Product size (bp)	GenBank Acc.
Ugt1a1	F:TTGGTGGGATAAACTGCCTTCA	165	NM_012683.2
	R:GAATTCTGCCCAAAGCCTCA		
GADPH	F:GGCACAGTCAAGGCTGAGAATG	87	NM_013105.2
	R:ATGGTGGTGAAGACGCCAGTA		

4.6. Western blotting analysis

Total protein was prepared by homogenizing the liver samples in 10 volumes of RIPA Lysis Buffer (ASPEN, Hubei, China). The tube was submersed in a small bucket of ice and water during all homogenization, followed by centrifugation (12000 rpm) for 5 min. The supernatant was the total protein of livers. Protein concentrations were measured using a BCA Protein Assay Kit (Goodbio, Hubei, China) according to the manufacturer´s instructions. Western Blotting for the immunodetection of Ugt1a1 was obtained using 40 μg of extracted membrane proteins in loading buffer (0.25 M Tris base pH 6.8, 50% glycerol, 10% SDS, 0.5% bromophenol blue and 5% ⊠-mercaptoethanol). Protein samples were subject to SDS-polyacrylamide gel electrophoresis on a 8 – 20% polyacrylamide gel at 120 V for 2 h and transferred onto a PVDF membrane. The membrane was blocked by incubating for 1 h at room temperature with Tris buffered saline containing 0.1% Tween 20 and 5% dried skim milk, then incubated with the anti-Ugt1a1 and anti-GADPH (Abcam, Shanghai, China) overnight at 4 °C. The membrane was washed (4×5 min), incubated with secondary antibody (ASPEN, Hubei, China) horseradish peroxidase conjugate (1:10000) for 30 min, and washed again (4×5 min). The protein of interest was detected with enhanced chemiluminescence substrate (Millipore) and imaged using a Kodak film. Then the bar graph shows the quantification of band intensity; data are expressed as mean ± S.D. (n= 6).

4.7. Statistical analysis

The pharmacokinetic parameters (AUC, CL/F, and $T_{1/2}$) of control and test groups were compared by one-way ANOVA followed by Fisher's Protected Least Significance Difference (PLSD) test. The different parameters of SV were also compared between the group pretreated for 1 day and 7 days. All PCR data was analyzed using the $2^{-\Delta\Delta CT}$ method as described previously (Livak et al 2001). The level of significance was $P < 0.05$. All statistical tests were performed with SPSS Statistics version 17 (IBM Inc., Armonk, USA).

Acknowledgement: Funding for this study was provided by the National Nature Science Foundation of China (Grants#81403012 and 81473287).

Conflict of interest: The authors declare that there are no conflicts of interest.

Ethical approval: The study was approved by the local Animal Ethic Committee.

References

Babbar S, Chanda S, Bley K (2010) Inhibition and induction of human cytochrome P450 enzymes in vitro by capsaicin. Xenobiotica 40: 807-816.

Backman JT, Kyrklund C, Kivistö KT, Wang JS, Neuvonen PJ (2000) Plasma concentrations of active simvastatin acid are increased by gemfibrozil. Clin Pharmacol Ther 68: 122-129.

Becker ML, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, Stricker BH (2010) Influence of genetic variation in CYP3A4 and ABCB1 on dose decrease or switching during simvastatin and atorvastatin therapy. Pharmacoepidemiol Drug Saf 19: 75-81.

Bode AM, Dong Z (2011) The two faces of capsaicin. Cancer Res 71: 2809-2814.

Conner CE, Burchell B, Hume R (1997) UDP-glucuronyl transferase enzyme family in both human and Balb/c mouse lung. Biochem Soc Trans 25:181S.

Díaz-Laviada I (2010) Effect of capsaicin on prostate cancer cells. Future Oncol 6: 1545-1550.

Embola CW, Sohn OS, Fiala ES, Weisburger JH (2002) Induction of UDP-glucuronosyltransferase 1 (UDP-GT1) gene complex by green tea in male F344 rats. Food Chem Toxicol 40: 841-844.

Gooding SM, Canter PH, Coelho HF, Boddy K, Ernst E (2010) Systematic review of topical capsaicin in the treatment of pruritus. Int. J. Dermatol 49: 858-865.

Hayman M, Kam PCA (2008) Capsaicin: a review of its pharmacology and clinical applications. Curr Anaesth Crit Care19: 338-343.

Ishigami M, Kawabata K., Takasaki W, Ikeda T, Komai T, Ito K, Sugiyama Y (2001) Drug interaction between simvastatin and itraconazole in male and female rats. Drug Metab Dispos 29: 1068-1072.

Kellick KA, Bottorff M, Toth PP(2014) A clinician's guide to statin drug-drug interactions. J Clin Lipidol 8: S30-S46.

Koster AS, Schirmer G, Bock KW (1986) Immunochemical and functional characterization of UDP-glucuronosyltransferases from rat liver, intestine and kidney. Biochem Pharmacol 35: 3971-3975.

Lee CK, Choi JS, Choi DH (2015) Effects of HMG-CoA reductase inhibitors on the pharmacokinetics of nifedipine in rats: Possible role of P-gp and CYP3A4 inhibition by HMG-CoA reductase inhibitors. Pharmacol Rep 67: 44-51.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using RT-PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408.

Prueksaritanont T, Subramanian R, Fang X, Ma B, Qiu Y, Lin JH, Pearson PG, Baillie TA (2002) Glucuronidation of statins in animals and humans: a novel mechanism of statin lactonization. Drug Metab Dispos 30: 505-512.

Reilly CA, Crouch DJ, Yost GS (2001) Quantitative analysis of capsaicinoids in fresh peppers, oleoresin capsicum and pepper spray products. J Forensic Sci 46: 502-509.

Reilly CA, Crouch DJ, Yost GS Fatah AA (2001) Determination of capsaicin, dihydrocapsaicin, and nonivamide in self-defense weapons by liquid chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. J Chromatogr A 912: 259-267.

Rengelshausen J, Banfield M, Riedel KD, Burhenne J, Weiss J, Thomsen T, Walter-Sack I, Haefeli WE, Mikus G (2005) Opposite effects of short-term and long-term St John's wort intake on voriconazole pharmacokinetics. Clin Pharmacol Ther 78: 25-33.

Reyes-Escogido ML, Gonzalez-Mondragon EG, Vazquez-Tzompantzi E (2011) Chemical and pharmacological aspects of capsaicin. Molecules 16: 1253-1270.

Robbins W (2000) Clinical applications of capsaicinoids. Clin J Pain 16 :S86-S89.

Sun H, Wang H, Liu H, Zhang X, Wu B (2014) Glucuronidation of capsaicin by liver microsomes and expressed UGT enzymes: reaction kinetics, contribution of individual enzymes and marked species differences. Expert Opin Drug Metab Toxicol 10: 1325-1336.

Szallasi A, Appendino G (2004) Vanilloid receptor TRPV1 antagonists as the next generation of painkillers. Are we putting the cart before the horse? 47: 2717-2723.

Szallasi A, Blumberg PM (1993) Mechanisms and therapeutic potential of vanilloids (capsaicin-like molecules). Adv Pharmacol 24:123-155.

Szallasi A, Blumberg PM (1999) Vanilloid (capsaicin) receptors and mechanisms. Pharmacol Rev 51:159-212.

Tephly TR, Burchell B (1990) UDP-glucuronosyltransferases: a family of detoxifying enzymes. Trends Pharmacol Sci 11: 276-279.

Tuteja S, Pyrsopoulos NT, Wolowich WR, Khanmoradi K, Levi DM, Selvaggi G, Weisbaum G, Tzakis AG, Schiff ER (2008) Simvastatin-ezetimibe-induced hepatic failure necessitating liver transplantation. Pharmacotherapy 28: 1188-1193.

Zhai XJ, Shi F, Chen F, Lu YN (2013) Capsaicin pretreatment increased the bioavailability of cyclosporin in rats: involvement of P-glycoprotein and CYP 3A inhibition. Food Chem Toxicol 62: 323-328.

Zhai XJ, Chen JG, Liu JM, Shi F, Lu YN (2013) Food-drug interactions: effect of capsaicin on the pharmacokinetics of simvastatin and its active metabolite in rats. Food Chem Toxicol 53: 168-173.