doi.org/10.36721/PJPS.2020.33.6.REG.2543-2546.1

Inhibitory effect of sesamin on ivabradine metabolism in rats

Ren-ai Xu[1], Wei Sun[2], Ruimin Chen[2], Naihua Liu[3,4] and Chengke Huang[2]*

[1]The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China
[2]The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, China
[3]State Key Laboratory of Pharmaceutical Biotechnology, The University of Hong Kong, Hong Kong, China
[4]Department of Medicine, The University of Hong Kong, Hong Kong, China

Abstract: In this work, the aim of our study was to assess whether sesamin could influence the pharmacokinetics of ivabradine and its active metabolite N-desmethylivabradine in rats. At the begining, 12 healthy male Sprague-Dawley rats were randomly divided into two groups: The rats were received an oral administration of 1.0mg/kg ivabradine alone (the control group), and the rats were given 1.0mg/kg ivabradine co-administered with 50mg/kg sesamin by gavage (the test group). After that, blood samples were collected from the tail vein of rats, and ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) were used for determing the plasma concentrations of ivabradine and N-desmethylivabradine in rats. Finally, the pharmacokinetic parameters were estimated using DAS 2.0 software. As the results, the pharmacokinetic parameters ($t_{1/2}$, C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$) of ivabradine in the control group were significantly lower than those in the test group ($P<0.05$). Moreover, sesamin significantly decreased $t_{1/2}$, C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of N-desmethylivabradine when compared to the control. These results demonstrated that sesamin increases plasma concentration of ivabradine and decreases N-desmethylivabradine conversely. Hence, our data indicated sesamin could influence the pharmacokinetic profile of ivabradine in rats, which might cause food-drug interaction in humans.

Keywords: Ivabradine, N-desmethylivabradine, sesamin, drug interaction, pharmacokinetics.

INTRODUCTION

Ivabradine, a novel heart rate-lowering agent, specifically inhibits the depolarizing cardiac pacemaker I_f current in the sinus node. The activity of ivabradine provides pure heart rate reduction at rest and during exercise, which increases coronary perfusion and improves myocardial oxygen balance without any significant influence. Recently, ivabradine has been efficacious and safe for anti-ischemic in patients with stable angina pectoris (Khawaja *et al.*, 2009; Lattuca *et al.*, 2015; Milliez *et al.*, 2009; Nar *et al.*, 2015; Prasad *et al.*, 2009; Tardif *et al.*, 2009).

The metabolic clearance of ivabradine possesses for ~80% of its total clearance and renal clearance accounts for the other 20%. In addition, CYP3A4 is the main enzyme in the metabolism of ivabradine, therefore, numerous potential interactions can arise with CYP3A4 inhibitors and inducers. Its main metabolite, N-desmethylivabradine, has been shown to block I_f in a similar way and is also a CYP3A4 substrate (Riesen *et al.*, 2011; Riesen *et al.*, 2010).

Sesamin is a major lignan in sesame, and its biological effects, such as suppression of hypertension (Miyawaki *et al.*, 2009), anticarcinogenic effects (Miyahara *et al.*, 2000), and antioxidant effect (Ikeda *et al.*, 2003; Nakai *et al.*, 2003), have been extensively studied by many researchers. Thus, sesamin seems to be one of the most reliable food factors, the physiological effects of which can be expected by individuals taking it as a supplement or remedy. Meanwhile, some food factors are known to affect the xenobiotic metabolism. For example, sesamin shows potent inhibitions of CYP3A4 and interferes with the metabolism of therapeutic drugs (Lim *et al.*, 2012). Therefore, there are potent food-drug interactions when using sesamin in combination with ivabradine. However, it is not presently clear whether sesamin has the capacity to affect the pharmacokinetics of ivabradine and N-desmethylivabradine.

In this study, we had used an ultra-high performance liquid chromatography-mass spectrometry method (UPLC-MS/MS) to determine the concentrations of ivabradine and N-desmethylivabradine in rat plasma and investigate the pharmacokinetic changes of ivabradine and N-desmethylivabradine in rats after oral administration of sesamin.

MATERIALS AND METHODS

Chemicals materials

Ivabradine (purity >98%) and N-desmethylivabradine (purity >98%) were obtained from Sigma-Aldrich Company (St. Louis, MO). Carbamazepine (purity >98%, IS) was purchased from the Nation Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Sesamin (purity >98%) was purchased from commercial sources (INDOFINE Chemical

**Corresponding author:* e-mail: huangchk@163.com

Company, Inc., Somerville, NJ, USA). Acetonitrile and methanol were HPLC grade and purchased from Merck Company (Darmstadt, Germany). HPLC grade water was obtained using a Milli Q system (Millipore, Bedford, USA).

UPLC-MS/MS conditions

Chromatographic separation was performed on an Acquity BEH C18 column (2.1mm × 50mm, 1.7μm) by employing the Acquity ultra performance liquid chromatography (UPLC) unit (Waters Corp., Milford, MA, USA) (Chen *et al.*, 2015). The mobile phase combined solvent A (acetonitrile) and solvent B (0.1% formic acid in water), and a gradient program was employed with the flow rate of 0.40mL/min as follows: 0-1.0 min linear increased from 10% to 90% A, 1.0-1.9 min maintained at 90% A, 1.9-2.0 min linear decreased to 10% A, and 2.0-3.0 min maintained at 10% A. The overall run time was 3.0 min. The mass spectrometric detection was operated using a XEVO TQD triple quadruple mass spectrometer equipped with an electro-spray ionization (ESI) source under the multiple reaction monitoring (MRM) mode. The MRM transitions were monitored at *m/z* 469.2 → 177.2 for ivabradine, *m/z* 455.2 → 262.2 for N-desmethylivabradine and *m/z* 237.1 → 194.2 for IS, respectively. Data acquisition and instrument control were obtained by the Masslynx 4.1 software (Waters Corp., Milford, MA, USA).

Sample preparation

Protein precipitation was used as sample pre-treatment for the plasma samples. To 100μL of plasma sample in a 1.5 mL centrifuge tube, 200μL acetonitrile (IS in acetonitrile 30ng/mL) was added. After vortexing for 1min, the tubes were centrifugated for 10min at 15,000g. Transfer 100μL supernatant to a new tube, 100μL water was added and mixed, then 2μL was injected into the UPLC-MS/MS system for analysis.

Pharmacokinetic study

Male Sprague-Dawley rats with body weights of 220±20 g were obtained from Laboratory Animal Center of Wenzhou Medical University. Twelve rats were randomly divided into two groups: The control group and the test group, which were received oral 1.0mg/kg ivabradine alone and co-administered with 50mg/kg sesamin, respectively. Diet was prohibited for 12h before the experiment but water was freely available. After oral administration, blood samples (0.3mL) of Sprague-Dawley rats were collected from the tail vein into heparinized 1.5mL polythene tubes at 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 12h. After immediately centrifugation at 4000g for 8 min, the supernatant plasma was obtained and stored at -20°C until analysis.

STATISTICAL ANALYSIS

The non-compartmental analysis was used to calculate the pharmacokinetic parameters by DAS (Drug and statistics) software (Version 2.0, Shanghai University of Traditional Chinese Medicine, China). The statistical analyses were evaluated by unpaired *t*-test (SPSS 19.0, Chicago, IL). A value of $P<0.05$ was statistically significant.

RESULTS

To investigate the effect of sesamin on the pharmacokinetics of ivabradine and its active metabolite N-desmethylivabradine in rats, we applied non-compartmental model to analyze the changes of the main pharmacokinetic parameters. The mean pharmacokinetic parameters and mean plasma concentration-time curves of ivabradine in two groups were showed in table 1 and fig. 1, respectively. In addition, the mean pharmacokinetic parameters and mean plasma concentration-time curves of N-desmethylivabradine in two groups were presented in table 2 and fig. 2, respectively.

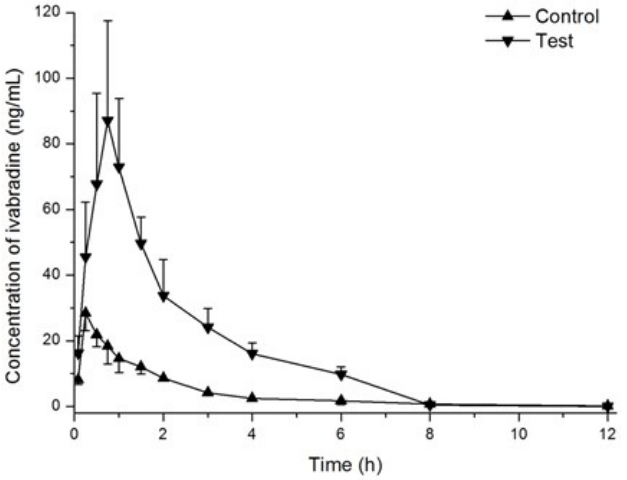

Fig. 1: Mean plasma concentration-time curve of ivabradine after single oral administration of ivabradine 1.0mg/kg alone or co-administration with 50mg/kg sesamin in rats.

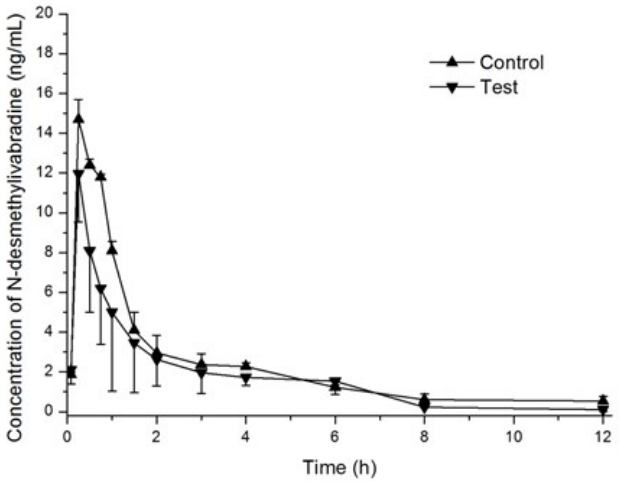

Fig. 2: Mean plasma concentration-time curve of N-desmethylivabradine after single oral administration of

Table 1: The main pharmacokinetic parameters of ivabradine in two groups (n=6).

Parameters	Control	Test
$t_{1/2}$ (h)	1.70 ± 0.56	1.92 ± 0.79 *
T_{max} (h)	0.27 ± 0.07	0.40 ± 0.14
CL_z/F (L/h/kg)	20.20 ± 3.32	6.28 ± 3.24 **
C_{max} (ng/mL)	28.40 ± 5.26	95.84 ± 66.25 **
$AUC_{(0-t)}$ (ng·h/mL)	48.76 ± 5.27	210.26 ± 139.57 **
$AUC_{(0-\infty)}$ (ng·h/mL)	49.91 ± 5.22	210.35 ± 139.67 **

Table 2: The main pharmacokinetic parameters of N-desmethylivabradine in two groups (n=6).

Parameters	Control	Test
$t_{1/2}$ (h)	2.44 ± 0.35	1.57 ± 0.49 *
T_{max} (h)	0.26 ± 0.04	0.20 ± 0.09
CL_z/F (L/h/kg)	35.13 ± 2.65	76.15 ± 11.50 **
C_{max} (ng/mL)	14.70 ± 0.99	12.19 ± 1.20 *
$AUC_{(0-t)}$ (ng·h/mL)	27.76 ± 2.04	20.45 ± 6.48 *
$AUC_{(0-\infty)}$ (ng·h/mL)	28.59 ± 2.17	20.66 ± 6.68 *

*Significantly different from control, $P < 0.05$; ** Significantly different from control, $P < 0.01$.

ivabradine 1.0 mg/kg alone or co-administration with 50 mg/kg sesamin in rats.

When compared with the control group in this study, our results showed that co-administration with sesamin exhibited significant increase in the pharmacokinetic parameters ($t_{1/2}$, C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$) of ivabradine. Furthermore, CL_z/F of ivabradine was significantly decreased in sesamin co-administration group in comparison to the control group. Besides, co-administration with sesamin had no significant effect on the T_{max} of ivabradine as compared to the control group (table 1).

As for the metabolite of ivabradine, co-administration with sesamin significantly decreased the pharmacokinetic parameters ($t_{1/2}$, C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$) of N-desmethylivabradine when compared to the control. Furthermore, CL_z/F of N-desmethylivabradine was significantly increased in sesamin co-administration group in comparison to the control group (table 2).

DISCUSSION

As we known, CYP3A4 is the most abundant P450 enzyme in humans, accounting for an average 30-40% of total P450 protein in the liver (Yuan *et al.*, 2002). Ivabradine is metabolized to N-desmethylivabradine mainly by CYP3A4 in humans (Portoles *et al.*, 2006). Thus, it is important to evaluate the potential pharmacokinetic interaction based on the inhibition of ivabradine metabolism mediated by CYP3A4. In this study, we investigated the influence of sesamin on the pharmacokinetics of ivabradine and N-desmethylivabradine in rats.

In pharmacokinetic study, co-administration of a single dose of sesamin significantly increased the $AUC_{(0-t)}$ of ivabradine, suggesting that oral administration of sesamin increased the oral bioavailability of ivabradine and may increase the anti-ischemic effect. The $t_{1/2}$ of ivabradine was significantly increased, in contrast N-desmethylivabradine was decreased. It has been reported that ivabradine is mainly metabolized via hepatic CYP3A4. It could be concluded that sesamin inhibited CYP3A4 which metabolized ivabradine to N-desmethylivabradine. Meanwhile, it has been proved that sesamin inhibited the expression of CYP3A4 in vitro (Jan *et al.*, 2012; Lim *et al.*, 2012; Parker *et al.*, 2000), but the impact of sesamin on CYP3A4 has not been reported in vivo. Therefore, the result should suggest that sesamin may mainly inhibit CYP3A4 in vivo, but it also needs to be studied further.

Similar results were also found by other researches. As reported, clopidogrel, a thienopyridine anti-platelet agent, could inhibit the metabolism of ivabradine to N-desmethylivabradine in rats, which may be related to its competitive inhibition effect on CYP3A4 (Sun *et al.*, 2015). In addition, another research also indicated that silibinin could change the pharmacokinetics of ivabradine, not the metabolite N-desmethylivabradine in rats (Chen *et al.*, 2015). Although the mechanism may be involved the inhibition of CYP3A4-mediated metabolism, further research is needed to confirm.

CONCLUSION

Up to now, there are few reports about the effects of food factors on ivabradine metabolism. In our study, this is first time to assess the effect of sesamin on the pharmacokinetics of ivabradine and N-

desmethylivabradine, and these results indicated that sesamin may have a potential interaction with ivabradine. Thus, when sesamin and ivabradine are administrated concurrently, more attention should be paid in order to reduce the adverse reaction. All above results showed preponderance of the evidence for clinical rational use of ivabradine, and provided a new perspective for the understanding of sesamin.

REFERENCES

Chen XP, Zheng HT, Cai WW, Li MK, Zhang JW and Hu J (2015). The effect of silibinin on the pharmacokinetics of ivabradine and N-desmethylivabradine in rats. *Pharmacology*, **96**(3-4): 107-111.

Ikeda T, Nishijima Y, Shibata H, Kiso Y, Ohnuki K and Moritani T (2003). Protective effect of sesamin administration on exercise-induced lipid peroxidation. *Int. J. Sports Med.*, **24**(7): 530-534.

Jan KC, Chang YW, Hwang LS and Ho CT (2012). Tissue distribution and cytochrome P450 inhibition of sesaminol and its tetrahydrofuranoid metabolites. *J. Agric. Food Chem.,* **60**(35): 8616-8623.

Khawaja MZ and Walker DM (2009). Ivabradine - the beauty of a slow heart rate. *Int. J. Clin. Pract.,* **63**(4): 542-546.

Lattuca B and Roubille F (2015). Ivabradine: A promising drug in cardiogenic shock to prevent the undesirable sinus tachycardia induced by dobutamine? *Int. J. Cardiol.,* **178**: 308-310.

Lim YP, Ma CY, Liu CL, Lin YH, Hu ML, Chen JJ, Hung DZ, Hsieh WT and Huang JD (2012). Sesamin: A naturally occurring lignan inhibits CYP3A4 by antagonizing the pregnane x receptor activation. *Evid-Based Compl Alt*. 2012: Article ID 242810.

Milliez P, Messaoudi S, Nehme J, Rodriguez C, Samuel JL and Delcayre C (2009). Beneficial effects of delayed ivabradine treatment on cardiac anatomical and electrical remodeling in rat severe chronic heart failure. *Am. J. Physiol-Heart C,* **296**(2): H435-H441.

Miyahara Y, Komiya T, Katsuzaki H, Imai K, Nakagawa M, Ishii Y and Hibasami H (2000). Sesamin and episesamin induce apoptosis in human lymphoid leukemia Molt 4B cells. *Int. J. Mol. Med.*, **6**(1): 43-46.

Miyawaki T, Aono H, Toyoda-Ono Y, Maeda H, Kiso Y and Moriyama K (2009). Antihypertensive effects of sesamin in humans. *J. Nutr. Sci. Vitaminol.*, **55**(1): 87-91.

Nakai M, Harada M, Nakahara K, Akimoto K, Shibata H, Miki W and Kiso Y (2003). Novel antioxidative metabolites in rat liver with ingested sesamin. *J. Agric. Food Chem.,* **51**(6): 1666-1670.

Nar G, Inci S, Aksan G, Demirelli S, Soylu K, Yuksel S, Gulel O and Icli A (2015). Ivabradine on aortic stiffness in patients with heart failure. *J. Invest Med.*, **63**(4): 620-625.

Parker RS, Sontag TJ and Swanson JE (2000). Cytochrome P4503A-dependent metabolism of tocopherols and inhibition by sesamin. *Biochem. Biophys. Res. Commun.,* **277**(3): 531-534.

Portoles A, Terleira A, Calvo A, Martinez I and Resplandy G (2006). Effects of Hypericum perforatum on ivabradine pharmacokinetics in healthy volunteers: an open-label, pharmacokinetic interaction clinical trial. *J. Clin. Pharmacol.,* **46**(10): 1188-1194.

Prasad UK, Gray D and Purcell H (2009). Review of the I-f selective channel inhibitor ivabradine in the treatment of chronic stable angina. *Advances in Therapy*, **26**(2): 127-137.

Riesen SC, Ni W, Carnes CA, Lindsey KJ, Phelps MA and Schober KE (2011). Pharmacokinetics of oral ivabradine in healthy cats. *J. Vet. Pharmacol. Ther.*, **34**(5): 469-475.

Riesen SC, Schober KE, Lindsey KJ, Carnes CA, Ni W and Phelps MA (2010). Pharmacokinetics of Oral Ivabradine in Healthy Cats. *J. Vet. Intern Med.*, **24**(3): 732-732.

Sun W, Wang Z, Chen H, Zhang XD, Huang CK, Lian QQ, Shang-Guan WN, Zhu GH, Hu GX and Wang ZS (2015). The effect of clopidogrel on pharmacokinetics of ivabradine and its metabolite in rats. *Drug Dev. Ind. Pharm*., **41**(9): 1512-1517.

Tardif JC, Ponikowski P, Kahan T and Investigators AS (2009). Efficacy of the I-f current inhibitor ivabradine in patients with chronic stable angina receiving beta-blocker therapy: A 4-month, randomized, placebo-controlled trial. *Eur. Heart J.,* **30**(5): 540-548.

Yuan R, Madani S, Wei XX, Reynolds K and Huang SM (2002). Evaluation of cytochrome P450 probe substrates commonly used by the pharmaceutical industry to study *in vitro* drug interactions. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, **30**(12): 1311-1319.