Drug-Drug Interaction Potential of SH-1028, a Third-generation EGFR-TKI: In Vitro and Clinical Trials

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Abstract

SH-1028 is an irreversible third-generation EGFR tyrosine kinase inhibitor (EGFR-TKI) for the treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC). Considering the possibility of combination therapy in patients with NSCLC, we investigated the drug-drug interaction (DDI) potential of SH-1028 both in vitro and in clinical trials. The in vitro studies were conducted to determine the potential of SH-1028 as a substrate, inducer, or inhibitor of cytochrome P450 (CYP) subtypes. A phase I drug-drug interaction study in healthy volunteers was performed to evaluate the impact of co-administering rifampicin (a strong CYP3A4 inducer) and itraconazole (a strong CYP3A4 inhibitor) on the pharmacokinetics of SH-1028. The in vitro experiments showed that SH-1028 was mainly metabolized by CYP3A4. The activities of CYP1A2, 2B6, 2C19, 2D6 and 3A4 enzymes were slightly inhibited in vitro with SH-1028. SH-1028 has no obvious induction effect on CYP1A2 and CYP2B6 activities, but has potential induction effect on CYP3A4 mRNA expression. However, SH-1028 may not induce or inhibit human CYPs significantly at the clinically expected dose (200 mg). It is speculated that itraconazole and rifampicin affect the metabolism of SH-1028. In the clinical application of SH-1028, special attention should be paid to the interaction between SH-1028 and drugs or foods that affect the activity of CYP3A4.

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Abstract

SH-1028 is an irreversible third-generation EGFR tyrosine kinase inhibitor (EGFR-TKI) for the treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC). Considering the possibility of combination therapy in patients with NSCLC, we investigated the drug-drug interaction (DDI) potential of SH-1028 both *in vitro*and in clinical trials. The *in vitro* studies were conducted to determine the potential of SH-1028 as a substrate, inducer, or inhibitor of cytochrome P450 (CYP) subtypes. A phase I drug-drug interaction study in healthy volunteers was performed to evaluate the impact of co-administering rifampicin (a strong CYP3A4 inducer) and itraconazole (a strong CYP3A4 inhibitor) on the pharmacokinetics of SH-1028. The*in vitro* experiments showed that SH-1028 was mainly metabolized by CYP3A4. The activities of CYP1A2, 2B6, 2C19, 2D6 and 3A4 enzymes were slightly inhibited *in vitro* with SH-1028. SH-1028 has no obvious induction effect on CYP1A2 and CYP2B6 activities, but has potential induction effect on CYP3A4 mRNA expression. However, SH-1028 may not induce or inhibit human CYPs significantly at the clinically expected dose (200 mg). The geometric mean ratios of pharmacokinetic parameters and their corresponding 90% confidence intervals for SH-1028 in combination and alone did not fall within the range of 80% to 125%. It is speculated that itraconazole and rifampicin affect the metabolism of SH-1028. In the clinical application of SH-1028, special attention should be paid to the interaction between SH-1028 and drugs or foods that affect the activity of CYP3A4.

Key words : SH-1028, drug metabolism, CYP3A4, drug-drug interaction, pharmacokinetics

1. Introduction

As one of the malignant tumors, lung cancer is with the fastest growth in terms of both morbidity and mortality worldwide, posing a great threat to human health and life. Non-small cell lung cancer (NSCLC) is the most common histological type of lung cancer, accounting for about 80-85% of lung cancers[1-3]. In recent years, with the remarkable efficacy of new molecular targeted drugs in clinical practice, the correctness and feasibility of molecular targeted therapy theory has been demonstrated, and cancer treatment has been pushed to an unprecedented new stage. Small-molecule targeted drugs are usually signal transduction inhibitors, which can specifically block the signal transduction pathways necessary for tumor growth and proliferation[4-6]. Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) provide favorable therapeutic outcomes for NSCLC patients with positive EGFR mutation. However, after 9-13 months of EGFR-TKIs treatment, many patients developed resistance to EGFR-TKIs, with the EGFR-T790M mutation being the most common, accounting for about 50% of resistant mutations[7-13].

SH-1028 is a third-generation EGFR-TKI that irreversibly binds to mutant forms of EGFR (T790M, L858R and deletions in exon 19). The *in vitro* studies showed that SH-1028 was 80 times more potent against the EGFR-T790M/L858R mutations than wild-type EGFR. And *in vitro*SH-1028 was eliminated mainly through phase I metabolism such as demethylation, oxidation and dealkylation. A total of 21 metabolites were detected in human liver microsomes, among which the demethylated metabolite Imp3 (active metabolite) was one of the main metabolites. Importantly, SH-1028 and Imp3 were still active for wild-type EGFR, being

distinct from the main metabolite of osimertinib (AZ5104). Hence, SH-1028 is proposed as a treatment for NSCLC patients with resistance to first-generation EGFR TKIs or as an alternative medicine for advanced NSCLC patients with positive EGFR mutation[14].

Drug exposure may be affected in drug combination clinically. With the increase or decrease of drug exposure, the probability of adverse reactions in patients increases correspondingly, and /or the efficacy is affected, therefore it is necessary to carry out research on drug-drug interaction[15]. So, *in vitro* and Phase I clinical evaluations have been conducted to clarify whether SH-1028 is a substrate, inhibitor, or inducer of relevant drug metabolism enzymes, with the Phase I clinical trial (CTR20210558) evaluating the effects of CYP3A4 inhibitor itraconazole and CYP3A4 inducer rifampicin on the pharmacokinetics of SH-1028 in healthy Chinese male adults. Since Imp3 has been identified as the main active metabolite in human plasma and Imp2 is a metabolite with high levels of drug exposure in human, the pharmacokinetics of both Imp2 and Imp3 were also investigated in this study.

Materials and Methods

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies[16].

2.1. *In Vitro* Studies

2.1.1. Chemicals and Materials

All SH-1028 used in the studies were prepared at Nanjing Sanhome Pharmaceutical Co. Ltd. Details of other materials and reagents used*in vitro* studies are shown in **Table S1** .

2.1.2. Metabolism Studies

The prepared 200 μM SH-1028 working solution was incubated with human recombinant enzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4) in NADPH solution (2 mM) and phosphoric acid buffer solution (50 mM, pH = 7.4), respectively. Methanol:acetonitrile (1:1) solution containing 5 ng/mL Buspirone (internal standard) was added at 0 min and 60 min to stop the reaction. Samples were tested using a validated LC-MS/MS method. The main enzymes related to SH-1028 metabolism were determined according to the residual drug content after reaction.

2.1.3. Inhibitory Potential of SH-1028 for CYPs

The experiment included a test group, a negative control (NC) group and a positive control (PC) group. After the liver microsomes were pre-incubated with different concentrations of SH-1028 (0.16, 0.8, 4, 20 and 100 μM) or blank buffer solution (NC group) or selective inhibitor (PC group) for 15 minutes, probe substrate and NADPH were added to start the enzyme reaction. After further incubation for 30 minutes, methanol was added to stop the reaction. The metabolite production of each probe substrate was determined by LC-MS/MS. The relative activity of each sub-enzyme was calculated according to the amount of metabolite production there of, so as to judge whether SH-1028 inhibited the activity of the sub-enzyme.

2.1.4. Induction Potential of SH-1028 for CYPs

In this experiment, enzyme activity and mRNA expression levels were used to evaluate whether SH-1028 could induce drug metabolism enzymes CYP1A2, 2B6 and 3A4 in primary human hepatocytes. After resuscitation, primary human hepatocytes from three donors were pre-incubated at 37°C, 5% CO_2 for 24 hours, and then added to medium containing different concentrations of SH-1028 (3, 1 and 0.1 μM) or the positive inducer. The blank control group was treated with blank incubation matrix containing the same concentrations of organic solvent as the test group. The samples and liver cells of each group were cultured at 37°C and 5% CO_2 for 3 days. There were 3 parallel samples in each group. After 3 days of continuous administration, the culture medium was then discarded. The enzyme activity was detected, and each probe substrate (final concentration at 100 μM) was added to the solution and incubated at 37°C with 5% CO_2 for

60 minutes. After extraction, metabolite generation was detected by LC-MS/MS to detect the sub-enzyme activity. The mRNA expression of each sub-enzyme was analyzed by quantitative fluorescence (QF) - PCR.

2.2. Clinical Trials

2.2.1. Study Design

This study was a phase I, single center, open-label trial in Chinese healthy male adults. The purpose of this study was to evaluate the drug-drug interaction between SH-1028 tablets and itraconazole or rifampicin capsules, as well as the pharmacokinetic characteristics of SH-1028 tablets in healthy subjects, so as to provide a basis for the design of drug administration regimen for subsequent clinical trials. SH-1028 tablets were manufactured by Nanjing Sanhome Pharmaceutical Co. Ltd. (Nanjing, China). Rifampicin capsules were purchased from Shenyang Hongqi Pharmaceutical Co. Ltd. (Shenyang, China) and itraconazole capsules were supplied by Xi'an Janssen Pharmaceutical Co. Ltd. (Xi'an, China). This study was conducted in accordance with the guidelines for Good Clinical Practice (GCP) of National Medical Products Administration (NMPA,China) and International Council for Harmonisation (ICH). The protocol and related materials were approved by the Clinical Medical Research Ethics Committee of the First Affiliated Hospital of Bengbu Medical College.

A total of 40 male subjects were enrolled in this study, which were assigned into Group A and Group B, with 20 subjects in each group. All subjects provided informed consent before any study procedures or assessments. To be eligible, subjects were required to be healthy male, aged 18–45 years, with a body mass index (BMI) between [?]19.0 and [?]26.0 kg/m^2 and the weight between [?]50 and [?]80 kg. In Group A, subjects received a single oral dose of SH-1028 at 200 mg on Day 1; then received itraconazole 200 mg twice daily for 7 days (Days 8–14); in the morning of Day 12, received itraconazole 200 mg together with a single dose of SH-1028 at 200 mg. On Days 1 and 12, blood samples were collected intensively after administration of SH-1028 tablets for pharmacokinetic analysis. While in Group B, subjects received a single oral dose of SH-1028 at 200 mg on Day 1, then rifampicin 600 mg once daily for 9 days (Days 8–16); in the morning of Day 14, rifampicin 600 mg together with a single dose of SH-1028 at 200 mg. On Days 1 and 14, intensive blood sampling was performed after giving SH-1028 tablets for pharmacokinetic (PK) analysis. Water was forbidden within 1 hr before and 2 hrs after SH-1028 tablets administration, and fasting was required for 4 hrs after SH-1028 tablets dosing in both groups.

2.2.2. Pharmacokinetic Analyses

Group A had blood collection after Day 1 and Day 12 administration. Group B had blood samples collected intensively after Day 1 and Day 14 administration. The collection time points of Groups A and B were within 1 hr before medication and 0.5, 1, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 48, 72 and 96 hrs after medication. A total of 3 mL of whole blood samples were collected at each time point. The concentrations of SH-1028 and its metabolites Imp2 and Imp3 in human plasma samples were quantified by CR Medicon's bioanalytical laboratory (Nanjing, China) using a validated LC-MS/MS method. The linearity range of SH-1028 and Imp3 were 0.200˜100 ng/mL, compared to 0.400˜200 ng/mL for Imp2. Further details are included in the Supplementary Methods section of the Supplementary Materials.

2.2.3. Statistical Methods

PK analysis was performed based on the Pharmacokinetics Concentration Set (PKCS) and Pharmacokinetics Parameter Set (PKPS). PKCS included all the subjects who received the study drugs at least once and had at least one valid plasma concentration data for the analytes, while PKPS included all the subjects who received the study drugs at least once and had at least one pharmacokinetic parameter data. The PK parameters were calculated by noncompartmental analysis (WinNonlin, v8.0, Pharsight Corp, USA). The primary PK parameters included maximum plasma concentration (C_{max}), AUC of the time from 0 to t ($AUC_{0\text{-}t}$) and AUC from 0 extrapolated to infinity ($AUC_{0\text{-}inf}$). The secondary PK parameters included but were not limited to time to reach maximum plasma concentration (T_{max}), clearance (CL/F), and mean residence time (MRT). If a subject's $AUC_{0\text{-}inf}$extrapolated percentage ($AUC_{\%Extrap}$)>20%, the subject's $AUC_{0\text{-}inf}$, $t_{1/2}$, and

$AUC_{\%Extrap}$ did not undergo descriptive statistical analysis. The 90% confidence interval of geometric mean ratios (GMRs) of the primary PK parameters of SH-1028 and its main metabolites (Imp2 and Imp3) were calculated between SH-1028 combined with itraconazole or rifampicin and SH-1028 alone. If the confidence interval fell within 80.00%˜125.00%, no significant difference was considered in the primary pharmacokinetic parameters between the combination of two drugs and the single drug, indicating no drug-drug interaction between SH-1028 and itraconazole or rifampicin.

2.2.4. Safety

All adverse events (AEs) observed during the clinical trial were recorded, including those based on abnormal clinical symptoms and vital signs, laboratory examination and ECG. The grades of the adverse events were judged according to CTCAE5.0, and their relevance to the trial drug were determined by investigators.

3. Results

3.1. Metabolizing Enzymes

The stability of SH-1028 in 7 recombinant human sub-enzymes are shown in**Table 1** . The residual percentages (RPs) of SH-1028 in CYP1A2, CYP2B6, CYP2C9, CYP2C19 and CYP2D6 incubated for 60 minutes were all >80%, indicating stable metabolism. The RP of SH-1028 in CYP2C8 for 60 minutes was 68.9%, indicating slight metabolism. As to incubation in CYP3A4 for 60 min, the RP was 0.381%, indicating metabolic instability and that CYP3A4 plays a major role in SH-1028 metabolism. The metabolic phenotypic data of the quality control substances Phenacetin, Diclofenac, Bupropion, Amodiaquine, Omeprazole, Dextromethorphan and Testosterone in human recombinases in the assay were all within the range of the verified historical data.

3.2. Inhibition of Enzymatic Activity by SH-1028

The enzyme activities of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4 measured with standard substrates were not affected or slightly inhibited *in vitro* at SH-1028 concentrations in the range of 0.16, 0.8, 4, 20, and 100 μM (**Table 2**). When midazolam and testosterone were used as substrates for CYP3A4, the IC_{50} of CYP3A4 were observed to be 13.8 and 22.3 μM, indicating that CYP3A4 is the sub-enzyme most sensitive to the inhibition by SH-1028. Based on the proposed clinical dose of SH-1028 (200 mg, approximately 5.3 mg/kg for dogs based on body surface area), and the plasma C_{max} in dogs at 0.302 μM (163.1 ng/mL), clinically relevant pharmacokinetic DDIs through inhibition of CYP sub-enzymes were not thought to be possible at plasma concentrations expected for SH-1028.

3.3. Induction of CYP1A2, CYP2B6 and CYP3A4

Three batches of primary human hepatocytes (HNN, RMH and DJJ) were co-incubated with SH-1028 at 0.1, 1 and 3 μM, respectively, and the activity values of both CYP1A2 and CYP2B6 sub-enzymes were lower than 40% from the positive control (PC) group (**Figure S1**). The mRNA expression levels of CYP1A2 and CYP2B6 were lower than the 4-fold of that in the NC group, indicating that SH-1028 has no induction effect on the enzyme activities and mRNA expression of CYP1A2 and CYP2B6 in the above concentration range. After co-incubation with SH-1028 at the above concentrations, the activity values of CYP3A4 sub-enzyme in primary human hepatocytes of the three donors were lower than 40% in the PC group, indicating that SH-1028 has no induction effect on the activity of CYP3A4 sub-enzyme in this concentration range. At the concentration of 0.1 μM, the mRNA expression level of CYP3A4 in primary human hepatocytes was lower than the 4-fold of that in the NC. For the concentrations at 1 and 3 μM, the mRNA expression levels of CYP3A4 in one of the three donors were 4.61-fold and 7.31-fold of that in the NC (**Figure S1**), respectively. Considering that individuals have different sensitivity to drugs and the expected maximum plasma drug concentration of SH-1028 is 0.302 μM < 1 μM, SH-1028 is unlikely to have DDI due to CYP induction in clinical practice. The enzyme activity in the PC group was more than or equal to 2 times of that in the NC group, eliminating the possibility of false negativity. Roxithromycin does not induce CYP450 mRNA expression, so it was used as a negative control drug in the experiment. The mRNA expression levels of roxithromycin in the primary hepatocytes of the three donors were all lower than the 4-fold of that in the

NC group, showing consistence with the quality standard and exclusion of the possibility of false positivity caused by PCR non-specific amplification.

3.4. Clinical Trials

3.4.1. Subjects

A total of 40 subjects were enrolled in this study, with all these subjects completed the study. All the subjects (20 in Group A and 20 in Group B) were included in the Full Analysis Set (FAS), Safety Set (SS), Pharmacokinetics Concentration Set (PKCS), and Pharmacokinetics Parameter Set (PKPS). Baseline demographics of the subjects are shown in**Table S2** .

3.4.2. Pharmacokinetics in Itraconazole Study

The mean plasma concentration–time profile for SH-1028 tablets administered alone or in combination with itraconazole (200 mg, bid) in Group A (N=20) are shown in **Figure 1** and the corresponding PK parameters are listed in **Table 3** .

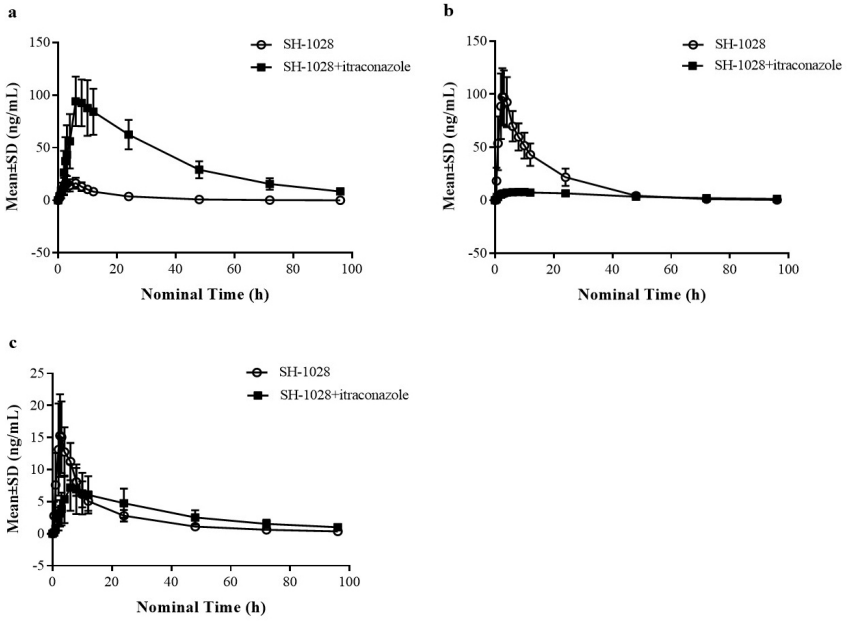

Figure 1. Mean plasma concentration–nominal time profiles of SH-1028, Imp2 and Imp3 following administration of 200 mg SH-1028 with or without itraconazole in healthy adults. a, SH-1028; b, Imp2; c, Imp3; data shown as Mean ± SD (n = 20).

The plasma concentrations of SH-1028 were elevated at all the time points after administration of itraconazole compared to SH-1028 alone. The median T_{max} of SH-1028 was observed at 6.00 and 5.00 h with or without itraconazole, respectively. The $C_{max}$and AUC_{0-t} values of SH-1028 were increased by 5.68-fold and 13.47-fold when SH-1028 was co-administered with itraconazole. The mean clearance (CL/F) of SH-1028 was decreased by ˜93.2% relative to SH-1028 alone. The GMRs and their corresponding 90% CIs for the comparison of SH-1028 with and without itraconazole did not fall within the 80%–125% boundary. Meanwhile, the median T_{max} of the active metabolite Imp3 was also extended from 2.75 to 6.00 h (without vs. with itraconazole). And the elimination rate of Imp3 was decreased in the presence of itraconazole. In addition, the C_{max} of Imp3 for SH-1028 combined with itraconazole was only 44.12% of that for SH-1028 alone, and AUC_{0-t} value of Imp3 increased slightly by 17.40% in the presence of itraconazole.

In contrast, SH-1028 combined with itraconazole significantly reduced Imp2 exposure levels. The C_{max} and $AUC_{0\text{-}t}$ values of Imp2 reduced by 91.89% and 75.52% compared with that for SH-1028 alone. The mean CL/F increased ˜3.58-fold when SH-1028 was co-administered with itraconazole. This suggests that itraconazole inhibited the production of Imp2 *in vivo* .

3.4.3. Pharmacokinetics in Rifampicin Study

After administration of SH-1028 either alone or in combination with rifampicin, mean plasma concentration–time profiles and PK parameters were measured (**Figure 2 and Table 4**). Rifampicin significantly reduced the plasma concentrations of SH-1028 and shortened the T_{max} (3.0 vs. 2.5 h). When SH-1028 was co-administered with rifampicin, the C_{max} and AUC_{0-t}values of SH-1028 were decreased by 89.77% and 96.02%, and the CL/F of SH-1028 was 20.6-fold higher, compared with the administration of SH-1028 alone. The 90% CI for GMRs (with vs. without rifampicin) of C_{max} and AUC_{0-t} of SH-1028 did not fall within the 80%–125% boundary, suggesting that rifampicin affected the pharmacokinetics of SH-1028 by inducing CYP3A4. After combined administration with rifampicin, the C_{max} and $AUC_{0\text{-}t}$ of the active metabolite Imp3 decreased by 73.23% and 88.96%. Simultaneous CL/F increased by 6-fold.

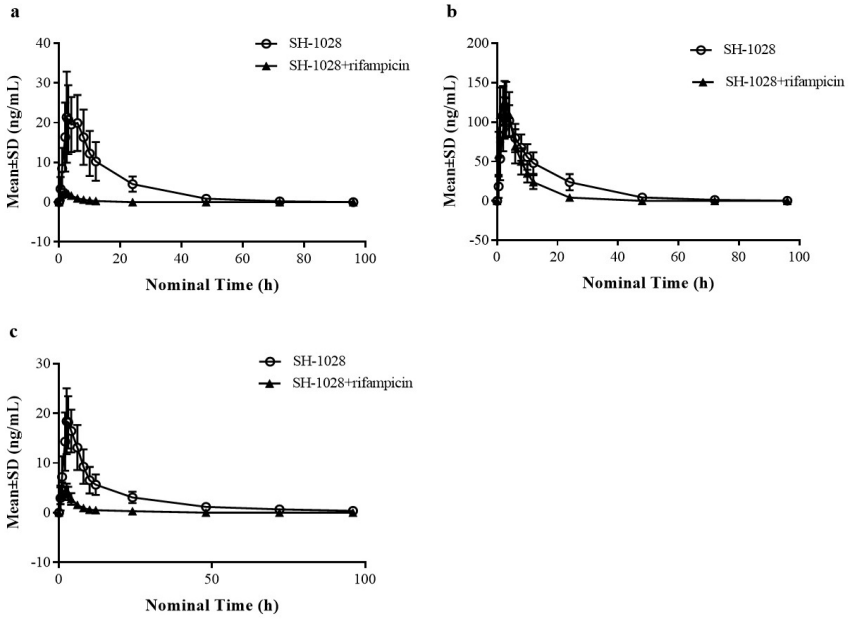

Figure 2. Mean plasma concentration–nominal time profiles of SH-1028, Imp2 and Imp3 following administration of 200 mg SH-1028 with or without rifampicin in healthy adults. a, SH-1028; b, Imp2; c, Imp3; data shown as Mean ± SD (n = 20).

It is interesting to note that the C_{max} value of the metabolite Imp2 increased slightly (21.36%) after combination with rifampicin. Moreover, the AUC_{0-t} value of Imp2 was reduced by 39.0%. It could be observed from the mean plasma concentration–time profiles of Imp2 that the elimination rate of Imp2 was increased in the presence of rifampicin.

3.4.4. Safety

A total of 40 subjects were enrolled in the safety set (SS). SH-1028 was well tolerated when administered alone or in combination with itraconazole or rifampicin (**Table S3**). All adverse events were of grade I-II, and no deaths or serious AEs were reported throughout the study.

4. Discussion

SH-1028 is a third-generation EGFR-TKI treatment for NSCLC. The patients with NSCLC may have concomitant diseases requiring multiple medications. The possibility of change in PK profile of SH-028 in combination with other medications, consequently affecting the efficacy and/or safety of SH-1028, makes it necessary to study the DDI potential of SH-1028.

The *in vitro* metabolism studies have showed that SH-1028 is mainly metabolized by CYP3A4. Meanwhile, under the expected clinical dose, SH-1028 may not have significant induction or inhibition effect on human CYPs. In preclinical experiments, SH-1028 was found to be eliminated mainly through phase I metabolic reactions such as demethylation, oxidation and dealkylation, with demethylation metabolite Imp3 as one of the main metabolites. As an active metabolite of SH-1028, Imp3 has also been shown good selectivity for wild-type EGFR kinase.[14] Interestingly, Imp2 was not found *in vitro* , while it is a unique metabolite in human body. Speculatively, Imp2 may be related to the hepatoenteric circulation, and the specific mechanism needs further exploration. Based on the results of *in vitro* experiments, the clinical trials investigated the effects of inhibitor and inducer of CYP3A4 on the pharmacokinetics of SH-1028 and its metabolites (Imp3 and Imp2).

In the clinical trials, itraconazole was administered 200 mg twice daily for 7 days. SH-1028 was co-administered on the 5^{th} day of itraconazole administration with the latter continued for another 2 days to maintain complete inhibition of CYP3A4. Previous studies have reported that itraconazole 200 mg once daily for 4-5 days is sufficient to result in drug interaction effects on CYP3A substrates. And administration of 600 mg rifampicin capsules once daily for 7 consecutive days could induce the maximum activity of CYP3A4 enzyme[17-19]. This study was designed requiring subjects to take 600 mg rifampicin capsules once daily for 7 days, and to take SH-1028 tablets on the 7^{th} day of rifampicin administration with PK samples collected. During PK sample collection, it was necessary to maintain the maximum induction level of CYP3A4 enzyme. So subjects were given 600 mg rifampicin capsules once daily for another 2 days, totally 9 days.

In the study of the interaction between itraconazole and SH-1028, with the presence of itraconazole, the C_{max} and AUC_{0-t} values of Imp2 decreased, while the C_{max} and AUC_{0-t} values of SH-1028 increased. Itraconazole also slightly reduced the C_{max}value of the active metabolite Imp3 (90% CI of GMR of each parameter did not fall within the interval of 80%-125%). Itraconazole significantly reduced the exposure ratio of plasma metabolites to the parent drug, while total exposure of SH-1028 and active metabolite Imp3 increased after co-administration. This indicated that itraconazole affected the first-pass metabolism of SH-1028.

In comparison, rifampicin co-administration with SH-1028 significantly decreased the C_{max} and AUC_{0-t} of SH-1028, as well as the overall exposure level of Imp3, but significantly increased the C_{max} ratio of Imp3 to parent drug (from 77.8% to 206%) and the AUC_{0-t} ratio (from 76.5% to 210%). This may be caused by rifampicin inducing both primary and secondary metabolisms of SH-1028. Rifampicin also reduced AUC_{0-t} of Imp2, leading to the C_{max}ratio (Imp2 to SH-1028) increased from 423% to 5,100% and AUC_{0-t} ratio increased from 490% to 7,470%, presumably because the induction of secondary metabolism of Imp2 was insufficient to offset the induction of SH-1028 metabolism.

5. Conclusions

In summary, SH-1028 is mainly metabolized through CYP3A4 *in vitro* . And it is unlikely to cause inhibition or induction of CYPs at the intended clinical dose. This clinical study was designed and performed to evaluate the drug-drug interactions between SH-1028 and itraconazole or rifampicin. All subjects tolerated the study drug well in the study. Both itraconazole and rifampicin had significant effects on SH-1028 exposure in humans. SH-1028 has been shown to be a moderately sensitive CYP3A4 substrate *in vivo* . Based on our findings, special attention should be paid to interactions with drugs or foods that affect the activity of CYP3A4 when using SH-1028 clinically. In addition, it is not recommended to use SH-1028 in combination with CYP3A4 inhibitors or inducers.

Clinical trial registration number

CTR20210558

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

HT-L, H-Z and ZQ-W contributed to the conception and design. XL-L and YY-L provided medical supervision. YY-L and YY-X drafted the manuscript, and YY-L also conducted the analysis and interpretation of the data. S-Y designed figures and tables, and reviewed the grammar. JX-D, CX-H, Y-W and MH-Z contributed to the management of drug and biological sample disposition. S-Y, XD-Z and RF-S contributed to quality control throughout the study. BY-L and YZ-D contributed to the study organization and implementation. J-X and F-G participated in the sample collection. All authors were involved in revising the paper critically for intellectual content and the final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

Conflicts of interest

We confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Table 1. The stability of SH-1028 in 7 recombinant human sub-enzymes

Sub-enzymes	**with NADPH (60min)**	**with NADPH (60min)**	**with NADPH (60min)**	**without NADPH**
	RP(Mean)(%)	**SD**	**CV%**	**RP(Mean)(%)**
CYP1A2	92.9	3.84	4.14	101
CYP2B6	94.0	5.30	5.64	102
CYP2C8	68.9	6.56	9.51	94.0
CYP2C9	103	12.8	12.5	100
CYP2C19	92.5	3.93	4.24	100
CYP2D6	84.2	6.41	7.61	102
CYP3A4	0.381	0.0140	3.69	100

RP: residual percentage.

Table 2. The effect of SH-1028 on CYP sub-enzyme activity (%NC group)

Sub-enzymes	**NC (%NC)**	**PC (%NC)**	**0.16 (%NC)**	**0.8 (%NC)**	**4 (%NC)**	**20 (%NC)**	**100 (%NC)**	**IC_{50} (μM)**
CYP1A2	100±2.10	35.6±1.25	98.8±4.98	97.8±1.38	86.5±0.69	67.9±1.24	24.8±0.57	36.7
CYP2B6	100±2.16	4.65±0.39	109±3.71	106±3.08	93.6±3.90	86.7±3.69	42.2±2.42	79.1
CYP2C8	100±5.26	18.0±1.85	94.3±3.13	101±1.75	97.4±1.15	102±4.70	87.6±2.76	NA
CYP2C9	100±2.23	13.1±0.80	92.5±2.07	97.0±3.69	98.2±3.42	92.8±2.97	62.3±0.69	NA
CYP2C19	100±8.91	0.00±0.00	98.4±3.35	113±6.14	90.3±2.97	77.5±4.07	28.5±3.99	49.6
CYP2D6	100±3.68	3.08±0.04	98.2±2.86	97.3±0.87	93.8±1.80	82.3±1.47	37.1±0.42	65.7
CYP3A4 (Testosterone)	100±8.41	0.576±0.22	101±7.47	102±1.93	73.7±1.27	43.8±3.99	7.59±0.28	13.8
CYP3A4 (Midazolam)	100±2.02	2.72±0.23	98.3±2.72	95.3±8.98	86.3±2.65	55.6±1.21	12.0±0.51	22.3

NC: negative control, PC: positive control; data shown as Mean ± SD.

Table 3. Pharmacokinetics of SH-1028

Analyte	Parameters	SH-1028 (N=20)	SH-1028+Itraconazloe (N=20)	GMR	90% CI of GMR
SH-1028	**C_{max} (ng/mL)**	19.24 ± 7.1	105.2 ± 23	568.20	(479.45, 673.37)
	AUC_{0-t} (h*ng/mL)	2775.6 ± 95	3596 ± 849	1346.99	(1143.74, 1586.37)
	AUC_{0-inf} (h*ng/mL)	2832.4 ± 94	3922 ± 971	1426.14	(1213.09, 1676.60)
	T_{max} (h)	5.00(2.00,8.00)	6.00(6.00,12.00)		
	CL/F (L/h)	793.3 ± 277	54.15 ± 14		
Imp2	**C_{max} (ng/mL)**	102.4 ± 26	8.277 ± 2.3	8.11	(7.00, 9.39)
	AUC_{0-t} (h*ng/mL)	1556 ± 422	379.9 ± 107	24.48	(21.14, 28.36)
	AUC_{0-inf} (h*ng/mL)	1616 ± 421	437.3 ± 129	27.82	(23.85, 32.45)
	T_{max} (h)	2.50(2.00,6.00)	8.00(2.50,24.00)		
	CL/F (L/h)	137.4 ± 41	492.3 ± 133		
Imp3	**C_{max} (ng/mL)**	16.46 ±5.9	7.701 ± 4.0	44.12	(35.04, 55.55)
	AUC_{0-t} (h*ng/mL)	287.9 ± 67	294.5 ± 134	117.40	(96.14, 143.37)
	AUC_{0-inf} (h*ng/mL)	313.7 ± 71	352.8 ± 153	132.64	(108.88, 161.58)
	T_{max} (h)	2.75(2.00,6.00)	6.00(6.00,12.00)		
	CL/F (L/h)	856.5 ± 277	671.7 ± 274		

Imp2 and Imp3 following administration of 200 mg SH-1028 with or without itraconazole (200 mg, bid), T_{max} shown as Median (Min, Max) and others shown as Mean ± SD.

Table 4. Pharmacokinetics of SH-1028

Aalyte	Parameters	SH-1028 (N=20)	SH-1028+Rifampicin (N=19[a])	GMR	90% CI of GMR
SH-1028	**C_{max} (ng/mL)**	26.31 ± 10	2.637 ±0.9	10.23	(8.29, 12.61)
	AUC_{0-t} (h*ng/mL)	349.7 ± 129	14.01 ± 5.5	3.98	(3.22, 4.91)
	AUC_{0-inf} (h*ng/mL)	355.9 ± 129	16.54 ± 5.8	4.72	(3.90, 5.73)
	T_{max} (h)	3.00(2.50,8.00)	2.50(1.00,4.00)		
	CL/F (L/h)	638.1 ± 233	13170 ±3433		
Imp2	**C_{max} (ng/mL)**	111.4 ± 24	134.5 ± 24	121.36	(108.93, 135.21)
	AUC_{0-t} (h*ng/mL)	1714 ± 491	1046 ± 287	61.00	(52.27, 71.19)
	AUC_{0-inf} (h*ng/mL)	1772 ± 493	1062 ±286	61.52	(52.78, 71.71)
	T_{max} (h)	3.00(2.00,4.00)	2.50(1.00,4.00)		
	CL/F (L/h)	124.8 ± 35	203.5 ± 64		
Imp3	**C_{max} (ng/mL)**	20.46 ± 5.5	5.442 ± 1.4	26.77	(22.98, 31.20)
	AUC_{0-t} (h*ng/mL)	267.4 ± 76	29.39 ± 7.3	11.04	(9.52, 12.80)
	AUC_{0-inf} (h*ng/mL)	284.6 ± 81	39.13 ±7.5	14.02	(11.69, 16.82)
	T_{max} (h)	2.76(2.00,6.00)	2.500(0.50,4.00)		
	CL/F (L/h)	754.5 ± 204	5284 ± 1038		

Imp2 and Imp3 following administration of 200 mg SH-1028 without or with rifampicin (600 mg, qd). a: One subject vomited 1.55 h after combined administration (Day 14), occurring within twice of the median T_{max} (4.00 h). Therefore, the PKCS and PKPS did not included his plasma concentration data of Days 14-18

before and after Day14 administration, but the PKCS and PKPS still included his plasma concentration data of Days 1-5. T_{max} shown as Median (Min, Max) and others shown as Mean ± SD.

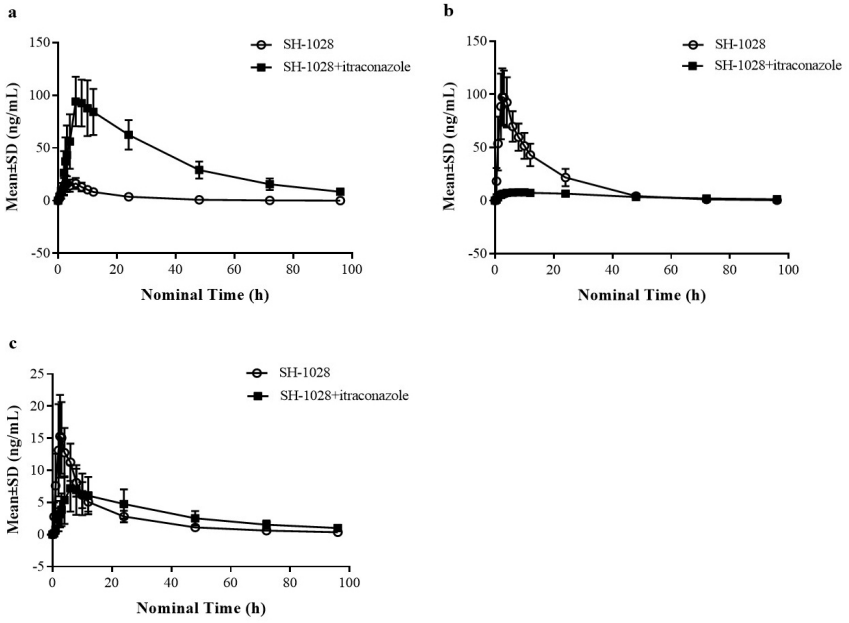

Figure 1. Mean plasma concentration–nominal time profiles of SH-1028, Imp2 and Imp3 following administration of 200 mg SH-1028 with or without itraconazole in healthy adults. a, SH-1028; b, Imp2; c, Imp3; data shown as Mean ± SD (n = 20).

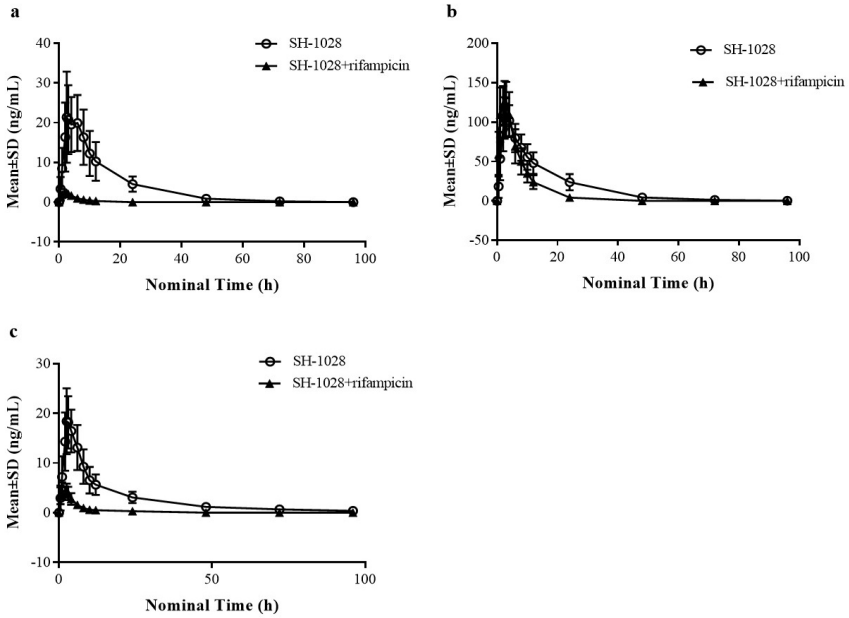

Figure 2. Mean plasma concentration–nominal time profiles of SH-1028, Imp2 and Imp3 following admin-

istration of 200 mg SH-1028 with or without rifampicin in healthy adults. a, SH-1028; b, Imp2; c, Imp3; data shown as Mean ± SD (n = 20).

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