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ORIGINAL ARTICLE

A safety, tolerability, and pharmacokinetic study of a novel simvastatin silica-lipid hybrid formulation in healthy male participants

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Abstract

Simvastatin (SIM) is a commonly used cholesterol-lowering drug that can reduce the risk of major cardiovascular events. However, due to its poor intrinsic water solubility, the drug is poorly absorbed from the gastrointestinal tract and exhibits a low oral bioavailability of approximately 5%. The aim of this study was to fabricate and optimize SIM encapsulated silica-lipid hybrids (SLH) as a solid-state lipid-based formulation to enhance absorption and bioavailability during a human in vivo pharmacokinetic study. SLH formulations were formulated by spray drying a submicron emulsion with either Aerosil® 300 fumed silica nanoparticles (SLH-A) or Syloid® 244 amorphous micronized silica (SLH-B). A cross-over, double-blinded study design was implemented to evaluate the performance of SLH formulations compared with a commercially available formulation in 12 healthy male participants after oral administration under fasting conditions. SLH formulations enhanced the bioavailability of SIM up to 1.6-fold and more importantly the active simvastatin acid (SIMA), 3.5-fold when compared with an equivalent dose of commercial formulation. The results demonstrate that the porous nanostructure of SLH impact systemic SIM and SIMA concentrations and may serve as a novel approach to enhance the bioavailability of specifically the parent or metabolite. No significant difference was observed in exposure when SLH formulations were administered at 10 mg in comparison with 20 mg of the commercial formulation, suggesting the potential for dose reduction. The study indicated that SLH formulations were safe and well-tolerated when administered to healthy males, confirming the commercial potential of SLH to enhance the bioavailability of poorly water-soluble drugs.

Keywords Simvastatin · Clinical trial · Oral delivery · Bioavailability · Lipid formulation · Silica

Abbreviations

AUC	Area under the curve
C_{max}	Maximum concentration
DSC	Differential scanning calorimetry
HbsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HMG-CoA	3-hydroxy-3-methyl-glutaryl-coenzyme A
HPLC	High-performance liquid chromatography
k_e	Apparent terminal elimination rate constant
LBFs	Lipid-based formulations
LCMS	Liquid chromatography-mass spectrometry
SIM	Simvastatin
SIMA	Simvastatin acid
SLH	Silica-lipid hybrid
SSF	Simvastatin Sandoz formulation
$T_{1/2}$	Apparent terminal half-life
T_{max}	Time to maximum concentration

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Introduction

Lipid-modifying interventions, specifically the use of statins, are believed to be the most beneficial tool in the prevention of coronary heart disease, reducing the risk of cardiovascular

events by up to 30% in patients. Statins are among the top-selling medications in both Europe and the USA with the market expected to continually rise as the population continues to age [1, 2]. Additionally, in a society where dietary fat intake is in excess, statins exist as suitable therapeutics to those who make a panoply of negative choices regarding living a healthy lifestyle [3].

Statins act by competitive inhibition of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase in the liver, causing an interruption in the mevalonate pathway leading to a reduction in cholesterol synthesis [4, 5]. Owing to its efficacy and safety profile, the currently available simvastatin (SIM) is listed on the World Health Organization's List of Essential Medicines to treat priority conditions [6]. SIM is a lipophilic drug (log P = 4.7) and classified as a Biopharmaceutics Classification System (BCS) Class II compound with poor aqueous solubility and high intestinal permeability [7, 8]. Due to its poor aqueous solubility, SIM exhibits low oral bioavailability of approximately 5% when administered as a conventional tablet [9]. Additionally, SIM is subject to high pre-systemic metabolism. However, from a pharmacological point of view, this is desired as SIM exerts its action after metabolic conversion to the active simvastatin acid (SIMA), a process mediated by cytochrome P4503A (CYP3A) [10, 11]. The limited solubility of SIM in aqueous media means that to solubilize a therapeutic dose of 20 mg, more than 10 L of water must be consumed which is impractical for patients [9]; thus, the majority of the SIM dose is expelled.

Several drug delivery systems have been used to enhance solubilization and in vitro dissolution rate of SIM, most of which are lipid-based formulations (LBFs), including solid lipid nanoparticles [12, 13] and self-emulsifying systems [10, 14]. However, conventional LBFs suffer from fundamental limitations that prevent their clinical and commercial translation; of most significance, LBFs are typically wet systems, such as simple lipid solutions, emulsions, and self-emulsifying systems, with low physicochemical stabilities. Our research has established an innovative solid-state LBF that provides food-mimicking solubilization effects of poorly soluble drugs, through confining lipid nano-emulsions within a porous silica matrix. Importantly, owing to their nanostructured porous matrix, silica-lipid hybrids (SLH) outperform their precursor lipid nano-emulsions by promoting the rapid dispersion of solubilizing lipid species during lipase-mediated hydrolysis, which enhances drug solubilization and absorption across the gastrointestinal tract [15–17].

SIM SLH systems have previously been well-characterized in vitro and in vivo, where a 3.2-fold improvement in metabolite exposure was observed in rodents, compared with the commercially available Simvastatin Sandoz® [18]. Additionally, the effect of silica particle size, shape, and porosity was investigated by formulating SLH particles with either mesoporous silica (Syloid® 244, with pore sizes of 19 nm and a specific surface area of 311 m^2g^{-1}) [19] or fumed silica nanoparticles (Aerosil® 300, which aggregates to form a silica network with pores approximately 2–7 nm in size and has a specific surface of 300 m^2g^{-1}) [20, 21]. In comparison with the commercial formulation, enhancement in SIM and SIMA exposure was greatest for SLH containing Syloid® 244, with a 2.9-fold and 3.2-fold improvement, respectively. Although no statistical difference was observed in exposure between SLH formulations, it was postulated that the difference in drug solubilization was attributed to the difference in silica geometry [18]. Although SLH was revealed to be a promising approach to increase SIM solubilization, limited reports exist with respect to cross-species (rodent to human) pharmacokinetics for LBFs, specifically solid-state formulations, and thus the ability for translation to humans is unknown. Thus, this is of strong interest and will be explored extensively within this investigation.

One previous phase I human clinical study has investigated the influence of oral SLH administration (with ibuprofen encapsulated as the model drug) to healthy male participants [22]. The primary aim of the study was to determine whether the delivery system was associated with any toxicological concerns and secondarily to examine the effect of SLH on pharmacokinetic parameters of ibuprofen. The bioavailability of ibuprofen encapsulated SLH was enhanced 1.95-fold compared with the commercially available Nurofen®, and review of safety assessments confirmed negligible acute side effects. However, the commercial translation of an ibuprofen SLH formulation was not feasible, owing to a high clinical dose (400 mg) which would significantly contribute to the pill burden commonly associated with poorly water-soluble drugs [23]. Nevertheless, it is hypothesized that SLH technology can be applied to various alternative BSC class II drugs with lower dosing requirements to maximize drugs' bioavailability in humans.

Given the lack of reported information on human pharmacokinetics for nanostructured hybrid silica-lipid formulations and the promising solubilization and oral bioavailability data for SIM SLH during in vitro and in vivo animal studies, we seek to determine if these preclinical findings translate into improved human clinical absorption and thus enhance knowledge of cross-species (rodent to human) pharmacokinetics. The current research presents a proof-of-concept phase I clinical trial to evaluate the safety, tolerability, and pharmacokinetics of orally administered SIM encapsulated SLH when manufactured with either silica nanoparticles or mesoporous silica. Therefore, we will explore the hypothesis that SIM encapsulated SLH provides improved bioavailability, compared with the already marketed Simvastatin Sandoz® when administered to healthy volunteers as a single oral dose under fasted conditions, and examine the correlation between cross-species pharmacokinetics. Additionally, we aim to elucidate

whether silica geometry influences drug solubilization and oral bioavailability in humans. The insights gained from this research will provide key insights into the fundamental properties of solid-state LBFs that enhance oral drug absorption in humans, while aiding in the translation of future LBF development from bench-top to bedside.

Methods

Materials

Simvastatin was purchased from Hangzhou Dayangchem Co., Ltd (Hangzhou, China). Lovastatin, simvastatin hydroxy acid ammonium salt, and lovastatin hydroxy acid sodium salt were purchased from Toronto Research Chemicals (Ontario, Canada). Simvastatin Sandoz® 20 mg tablets were purchased through a local pharmacy (Royal Adelaide Hospital, Adelaide, Australia). Capmul® MCM was kindly provided by ABITEC Corporation (Columbus, USA). Fumed hydrophilic silica nanoparticles with the specific surface area 300 m^2g^{-1} (Aerosil® 300 Pharma) and amorphous hydrophilic silica nanoparticles with the specific surface area of 311 m^2g^{-1} (Syloid® 244FP) were generously provided by Evonik Degussa (Essen, Germany) and Grace Davison Discovery Sciences (Rowville, Australia), respectively. Soybean lecithin was purchased from Merck (Bayswater, Australia). All chemicals and solvents were of analytical grade. High purity water was acquired from a Merck Milli-Q water purification system (Bayswater, Australia).

HPLC analysis

HPLC analysis was performed using a Shimadzu Prominence system (Tokyo, Japan) equipped with an Alltech LiChrospher RP C18 analytical column (5 μm × 4.6 mm ID × 250 mm). An isocratic elution method was employed, using a mobile phase consisting of methanol and water containing 0.05% acetic acid in the ratio of 85:15 (v/v). The mobile phase was eluted at a flow rate of 1.2 mL/min, and the eluent was monitored at an ultraviolet detection wavelength of 238 nm. The column temperature was maintained at 40 °C. Linear calibration curves ($R^2 \geq 0.999$) were obtained by plotting the chromatographic peak area against drug concentration over the range of 0.2–10 μg/mL.

Fabrication of SLH formulations

SLH formulations were prepared following a previously established two-step procedure [18, 24]. Briefly, soybean lecithin (6% w/w) was dissolved in Capmul® MCM (5 g) with the aid of sonication. Subsequently, SIM was dissolved in the lipid solution at 14% w/w at 60 °C, prior to the addition of water to form a lipid-in-water emulsion. The lecithin-stabilized emulsion was homogenized at a pressure of 1000 bar for five-volume cycles (Avestin® EmulsiFlex-C5 Homogenizer, Ottawa, Canada). An aqueous dispersion of Aerosil® 300 and Syloid® 244 silica (5% w/v) was prepared by ultrasonication. Separately, each silica dispersion was added to a homogenized emulsion to achieve a lipid: silica ratio of 2:1. The silica-stabilized emulsion was stirred overnight and spray dried to form SLH microparticles (Büchi Mini Spray Dryer B-290 apparatus, Flawil, Switzerland). The following spray drying conditions were used: emulsion flow rate 5 mL/min, aspirator 100%, airflow rate 0.6 m^3/min, and inlet temperature 150 °C. Herein, SLH fabricated with Aerosil® 300 will be referred to SLH-A, and SLH fabricated with Syloid® 244 will be referred to as SLH-B.

Determination of drug content

The drug content of SLH particles was determined using a solvent extraction method. Approximately 10 mg of the formulation was added to 10 mL of methanol. The dispersion was sonicated for 1 h to ensure complete drug extraction, followed by centrifugation at 29066 ×*g* for 20 min at 24 °C. The supernatant was then diluted appropriately with the mobile phase for HPLC analysis.

Drug crystallinity

The crystallinity of SIM was investigated using differential scanning calorimetry (Discovery DSC, TA Instruments, New Castle, DE, USA). Approximately 2 mg of each sample was heated in a hermetically sealed aluminum pan at a rate of 5 °C/min over a temperature range of 25–170 °C, under a flow of dry nitrogen gas (80 mL/min).

Clinical study population and design

A randomized, cross-over, double-blinded study design was used to evaluate the safety and pharmacokinetic profiles of Simvastatin Sandoz® and SIM encapsulated SLH in 12 healthy male participants aged from 19 to 67 years under fasting conditions. From herein, the Simvastatin Sandoz® formulation will be referred to as "SSF." All participants provided written informed consent and were screened for inclusion and exclusion criteria prior to the study. Participants were enrolled into one of two groups, whereby group 1 received SLH-A and group 2 received SLH-B. Each participant was orally administered one of three capsule formulations on each study day in a randomized order: (1) SSF ground to a powder equivalent to 20 mg SIM, (2) SIM SLH powder equivalent to 10 mg SIM, and (3) SIM SLH powder equivalent to 20 mg SIM. Each dose was given with 240 mL of room temperature water following an overnight fast, and no food was consumed

until 4 h after dosing. There was a 7-day wash-out period between each dose. Only upon completion of all sample analysis was the randomization code broken. The study was conducted following an ethical approval from the University of South Australia Human Research Ethics Committee.

Pharmacokinetic evaluation

In each period, blood samples were collected via the cannulated cubital vein at pre-dose (within 1 h before dosing) and 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, and 8 h following dose administration. The blood plasma samples were subjected to pharmacokinetic analysis using a validated LCMS method.

Safety assessments

All participants who received a dose of SIM were included in the safety population. Assessments included physical examinations, recording of vital signs, electrocardiography, clinical laboratory tests (biochemistry, hematology, HbsAg/HCV screening), urinalysis and urine drug, and alcohol breath tests. Each participant was questioned regarding adverse events. The nature, time, duration, and severity of all adverse events were recorded. The relationship between the adverse event and the formulation was categorized as likely related, unlikely related, or not related.

Blood sample collection and preparation

Blood samples for pharmacokinetic analyses were collected into 9 mL ethylenediaminetetraacetic acid (EDTA) containing tubes. Collected blood samples were immediately placed on ice, and plasma was separated by centrifugation at 2000 ×*g* for 10 min at 4 °C. Plasma was transferred to polypropylene containers, immediately frozen using liquid nitrogen, and stored at –80 °C until analysis.

The samples were extracted using liquid-liquid extraction. Briefly, plasma samples (500 μL) were spiked with 25 μL of internal standard and vortexed for 10 s. Ammonium acetate solution 100 mM pH 4.5 (300 μL) was added, and samples were vortexed for a further 10 s prior to the addition of 4 mL methyl tertiary-butyl ether (MTBE). Samples were sonicated for 10 min and centrifuged at 3270 ×*g* for 10 min. The organic layer was collected and evaporated to dryness under nitrogen (GeneVac HT-4X, Ipswich, UK). The resulting precipitates were reconstituted with 200 μL of acetonitrile: ammonium acetate buffer (1 mM pH 4.5) (30:70) and sonicated prior to centrifugation at 29066 ×*g* for 10 min. The supernatant was collected for LCMS analysis.

LCMS analysis

Plasma concentrations of SIM and SIMA were analyzed with an ultra-HPLC system (Shimadzu, Kyoto, Japan) equipped with an electrospray mass spectrometer detector (LC/MS–MS) (Sciex Qtrap 6500+, USA).

Analytes were separated through a Kinetex RP C18 analytical column (2.6 um × 3 mm ID × 50 mm) by gradient elution using mobile phase A (5 mM ammonium acetate containing 5% acetonitrile and 0.1% formic acid) and mobile phase B (acetonitrile containing 5 mM ammonium acetate and 0.1% formic acid) with a total flow rate of 0.3 mL/min. The gradient relating to mobile phase B was as follows: 0–2 min moving from 25 to 100%, 2–3.5 min held at 100%, and 3.5–4 min moving from 100 to 25%. An injection volume of 10 μL was used. The mass spectrometer was operated in positive ionization mode for SIM and negative ionization mode for SIMA. Mass to charge ratios (m/z) in multiple reaction monitoring for SIM and SIMA were 436.3/199.2 and 435.3/115, respectively. Internal standards, lovastatin, and lovastatin acid were monitored at 422.2/199.4 and 421.2/101.1, respectively. The lower and upper limits of quantification for both SIM and SIMA were 0.1 ng/mL and 10 ng/mL, respectively. A calibration curve consisting of a control blank, two zero standards, and six non-zero calibrators for SIM and SIMA was analyzed with every sample batch. Quality control standards were also analyzed. Linear calibration curves were obtained by plotting the internal standard peak area ratio against analyte concentrations. A weighting of 1/x was applied to all samples.

Pharmacokinetic analysis

Non-compartmental analysis (NCA) of the pharmacokinetic data was performed using the PKNCA package [25] in *R* [26]. The following pharmacokinetic parameters were estimated: the maximum observed plasma concentration (C_{max}), the time to C_{max} (T_{max}), the area under the plasma concentration-time curve from time 0 to the last measured concentration ($AUC_{0\text{-}8}$) and from time 0 to time infinity (AUC_{inf}) estimated using the linear-up-log down trapezoidal method, apparent terminal elimination rate constant (k_e) as determined by linear regression of the terminal points of the log-linear concentration-time curve, and the apparent terminal half-life ($t_{1/2}$) calculated as $0.693/K_e$.

Statistical analysis

Experimental data was statistically analyzed using an unpaired Student's *t* test. Data was considered statistically significant when $p < 0.05$.

Results

Physicochemical characterization of test formulations

Capmul® MCM was selected as the lipid phase for SLH due to providing a high solubilizing capacity for SIM, as previously reported in literature [18]. SIM encapsulated SLH formulations were successfully fabricated using Aerosil® 300 silica nanoparticles (SLH-A) and Syloid® 244 mesoporous silica (SLH-B). A free-flowing white powder was obtained after spray drying lipid: silica at a ratio of 2:1. Additionally, this ratio maximized formulation drug loading levels, whereby SLH-A and SLH-B had SIM loading levels of 8.5 and 7.0% w/w, respectively, with a standard deviation of < 0.4% w/w. This corresponded to loading efficiencies > 85%.

The DSC thermograms of pure SIM, a physical mixture (0.5% w/w crystalline SIM in silica), SLH-A, and SLH-B are shown in Fig. 1. Pure SIM and the physical mixture exhibited an endothermic peak at 140 °C corresponding to the melting point range of SIM (138–140 °C) [27]. This endothermic peak was absent from the thermograms of SLH-A and SLH-B, indicating that SIM was encapsulated within both SLH in its amorphous form.

Demographic characteristics of the study population

The study included 12 male participants. Participants enrolled in group 1 (n = 6) had a mean age of 25.5 ± 4.5, compared with a mean age of 32 ± 18 for group 2 (n = 6). Three of the participants were Asian, and nine participants were Caucasian. All of the twelve enrolled participants completed the study. A summary of the demographic characteristics is presented in Table 1.

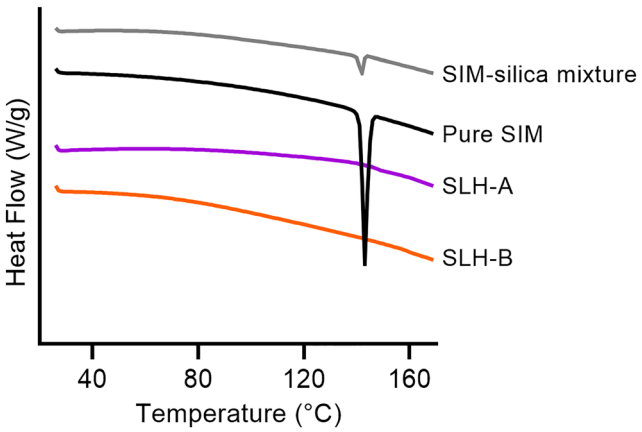

Fig. 1 DSC thermograms of pure SIM, a physical mixture containing SIM and silica, SLH-A, and SLH-B

Table 1 Summary of participants' demographics and baseline characteristics

	Group 1	Group 2
Number of participants	6	6
Age (years)	25.5 ± 4.5	32 ± 18
Height (m)	1.81 ± 0.16	1.74 ± 0.07
Weight (kg)	85.3 ± 32	84.8 ± 22
Race, *N* (%)		
Caucasian	5 (83)	4 (67)
Asian	1 (17)	2 (33)

Values are expressed as mean ± standard deviation, unless otherwise specified

N: number of participants studied

Pharmacokinetic analysis

Simvastatin

The mean SIM plasma concentration-time profiles following administration of a single dose of SSF 20 mg and SLH-A and SLH-B at a 10 and 20 mg dose are summarized in Fig. 2, with corresponding pharmacokinetic parameters summarized in Table 2. No significant difference in exposure metrics ($AUC_{0\text{-}8}$) was observed between participants who received SLH-A and SLH-B, and thus, the data for SSF represents all 12 participants (p = 0.31). Administration of SSF at 20 mg dosage attained a mean C_{max} of 2.52 ng/mL at a median T_{max} of 1 h and an overall exposure ($AUC_{0\text{-}8}$) of 6.15 ng h/mL.

Throughout the 8-h study period, SLH-A displayed significantly higher mean plasma concentrations than SSF when administered at 20 mg equivalent dose (p < 0.05). SLH-A reached a C_{max} of 6.00 ng/mL at a median T_{max} of 0.75 h post-dose, and the $AUC_{0\text{-}8}$ (10.1 ng h/mL) was 1.6-fold higher than SSF (p = 0.03). In comparison, C_{max} and $AUC_{0\text{-}8}$ for the 20 mg dosage of SLH-B were 2.55 ng/mL and 5.48 ng h/mL, respectively, and were not significantly different from that of SSF (p = 0.98).

When administered at 10 mg dose, SLH-A exposure was not statistically different to the 20 mg dose of SSF ($AUC_{0\text{-}8}$ = 4.61 ng h/ml for SLH-A vs 6.15 ng h/ml for SSF). Additionally, although plasma concentrations were lower for SLH-B, the overall exposure was not significantly different when compared with SSF with an $AUC_{0\text{-}8}$ of 3.88 ng h/mL (p = 0.06). No significant difference in SIM exposure was evident between SLH-A and SLH-B when administered at 10 mg (p > 0.05).

Between-participant variability (geometric CV%) was high for all formulations ranging from 27.8 to 103% for $AUC_{0\text{-}8}$ and 63.3 to 128% for C_{max}. A reduction in variability for both $AUC_{0\text{-}8}$ and C_{max} was observed for SLH formulations when administered at 10 mg compared with 20 mg.

Table 2 Summary of the pharmacokinetic parameters for SIM

	SSF 20 mg	SLH-A 10 mg	SLH-A 20 mg	SLH-B 10 mg	SLH-B 20 mg
N	12	6	6	6	6
$AUC_{0\text{-}8}$ (ng h/mL)	6.15 [67.4]	4.61 [27.8]	10.1 [44.6]	3.88 [41.7]	5.48 [103]
AUC_{inf} (ng h/mL)	7.57 [70.9]	4.95 [23.6]	10.9 [40.3]	4.60 [32.2]	5.93 [98.6]
C_{max} (ng/ml)	2.52 [72.3]	2.07 [63.3]	6.00 [71.1]	1.26 [88.6]	2.55 [128]
T_{max} (h)	1.00 [0.750, 3.00]	0.875 [0.750, 1.50]	0.750 [0.500, 2.00]	1.25 [0.750, 4.00]	0.750 [0.500, 4.00]
$T_{1/2}$ (h)	2.25 [0.960]	1.95 [0.557]	2.16 [1.05]	2.74 [1.76]	1.94 [0.419]

Geometric mean [CV%] data is presented. Time is displayed as median [min, max]

N: number of participants studied, $AUC_{0\text{-}8}$: area under the plasma concentration-time curve from time 0 to 8 h post-dose, AUC_{inf}: area under the plasma concentration-time curve from time zero to infinity, C_{max}: the maximum observed plasma concentration, T_{max}: the time to C_{max}, $T_{1/2}$: apparent terminal half-life

Simvastatin acid

The geometric mean plasma concentration-time curve and a summary of the pharmacokinetic parameters for SIMA per treatment are presented in Fig. 3 and Table 3, respectively.

When SIM was administered at 20 mg dose, geometric means for $AUC_{0\text{-}8}$ of the active metabolite, SIMA, were 7.65 and 12.1 ng h/mL for SLH-A and SLH-B, respectively, compared with 3.42 ng h/mL for SSF ($p = 0.07$ for SLH-A and $p < 0.001$ for SLH-B, compared with SSF). Furthermore, SLH-B achieved a C_{max} of 2.46 ng/mL after 3.0 h post-dose, whereas SLH-A achieved C_{max} only 2.0 h post-dose reaching a value of 1.76 ng/mL.

When SLH was administered at a dosage of 10 mg, $AUC_{0\text{-}8}$ for SLH-B was 1.9-fold greater than SLH-A ($AUC_{0\text{-}8}$ = 7.12 ng h/mL for SLH-B and 3.68 ng h/ml for SLH-A, $p = 0.9$). Additionally, no significant difference in exposure was evident between a 20 mg dose of SSF and a 10 mg dose of the SLH formulations ($p > 0.05$).

Between-participant variability (geometric CV%) was rather low for SLH-B at a 10 mg dose (18.6% for $AUC_{0\text{-}8}$ and 23.3% for C_{max}), however high for all other formulations ranging between 64.8 to 92.3% for $AUC_{0\text{-}8}$ and 70.6 to 101% for C_{max}. Variability was similar for SLH formulations at a 20 mg dose, ranging from 57.6 to 64.8% for $AUC_{0\text{-}8}$ and 70.6 to 80.1% for C_{max}.

In vivo (rat)-in vivo (human) correlation

Correlations were performed for SIM and SIMA using $AUC_{0\text{-}10}$ of the in vivo plasma drug concentration-time curves from a previous study of the equivalent formulations in Sprague-Dawley rats [18] and $AUC_{0\text{-}8}$ of the in vivo plasma drug concentration-time curves from humans for the following formulations when dosed at 20 mg: SSF, SLH-A, and SLH-B (Fig. 4). Low IVIVC ($R^2 = 0.0604$) was evident between the animal and human pharmacokinetic data for SIM; however, IVIVC was high ($R^2 = 0.9758$) for SIMA.

Safety and tolerability

All 12 participants who participated in the study were included in the safety evaluation. The administration of SIM was well-tolerated by the healthy participants. A total of five adverse events were recorded in four participants (Table 4). However, all adverse events were categorized as not related or unlikely related to the test formulations. No serious adverse events were reported during the study.

Discussion

A randomized, double-blinded, cross-over study design was employed to assess the safety, tolerability, and pharmacokinetic profile of SIM encapsulated SLH, compared with the commercially available SIM in twelve healthy male participants. All participants completed the study and were included in the pharmacokinetic and safety evaluation. All participants were administered a 20 mg dose of SSF, and either SLH-A or SLH-B at 10 mg and 20 mg dose, under fasting conditions. Since SIM is currently available at therapeutic doses ranging from 5 to 80 mg, a 20 mg dosing regimen was chosen for this study, with the presumption that this dose would allow for adequate pharmacokinetic quantification of both the parent drug and metabolite, while also minimizing potential side effects associated with higher doses including muscle pain, weakness, and damage [28]. Following FDA guidelines, both the parent drug and active metabolite, SIM and SIMA, respectively, were analyzed to allow for the differentiation of different SIM release rates and to explore the rate of metabolite formation and exposure between formulations [29, 30].

All formulations were administered in identical opaque size 00 gelatin capsules. To ensure that the appearance of the investigational products was identical, the commercial tablet, containing various excipients such as pre-gelatinized maize starch, lactose monohydrate, microcrystalline cellulose, citric acid monohydrate, and butylated hydroxyanisole, was ground

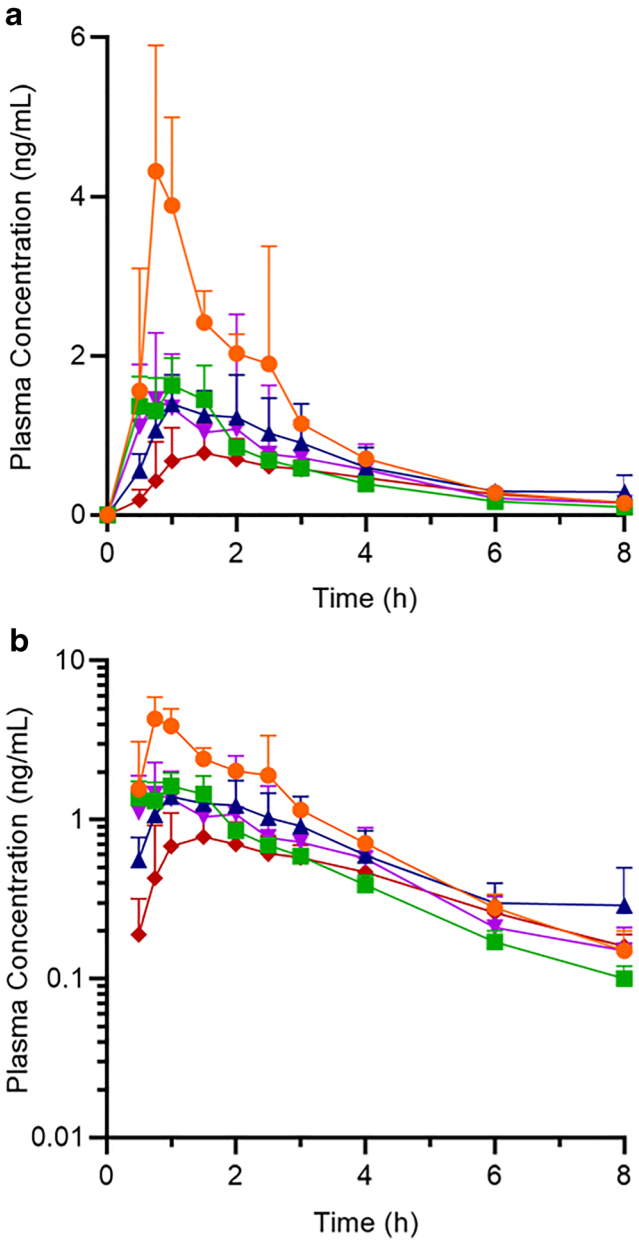

Fig. 2 Geometric mean plasma concentrations of SIM represented as (**a**) linear-linear and (**b**) log-linear: SSF 20 mg (blue-shaded triangle), SLH-A 10 mg (green-shaded square), SLH-A 20 mg (orange-shaded circle), SLH-B 10 mg (red-shaded diamond), and SLH-B 20 mg (purple-shaded inverted triangle). Each value represents the mean ± SE, n = 12 for SSF, and n = 6 for SLH formulations

to a powder prior to filling into a capsule [28]. It is predicted that this may have slightly influenced the pharmacokinetics, e.g. reducing the T_{max} compared with if the unmodified tablet was administered, as the disintegration process was significantly reduced upon crushing the tablet. The SIM prescribing information states that peak concentrations of the active metabolite are attained within 1.3 to 2.4 h post-dose [28]. However, in the current study, SSF reached maximum SIM and SIMA plasma concentrations 1 h and 4 h after dose administration, respectively. Although the prolonged T_{max} was unexpected, the results from this study are comparable with literature whereby a T_{max} for SIMA of 4.5 h post-dose has been reported [31].

The bioavailability of SIM encapsulated SLH was enhanced up to 1.6- and 3.5-fold for SIM and SIMA, respectively, compared with the commercial counterpart (SSF 20 mg dose). We hypothesize that the enhanced performance is attributed to the SLH delivery system, primarily the ability to deliver the drug in a non-crystalline and pre-solubilized state, thus avoiding the rate-limiting dissolution step observed with the crystalline commercial formulation (Fig. 5). Additionally, owing to the nanostructured porous matrix and enhanced lipid surface area that favors lipid-lipase interactions, lipid digestion and thus drug release are facilitated [32, 33]. The lipid component within the SLH formulation consists of mono- and diglyceride medium-chain fatty acids which upon digestion leads to the formation of highly solubilizing colloidal species that promote greater absorption compared with the commercial product. The ability for SLH to improve oral bioavailability has also been investigated by Tan et al. in a single-dose study, whereby ibuprofen encapsulated SLH demonstrated a 1.95-fold greater exposure, compared with the commercially available Nurofen® [22]. Similarly, the authors suggested that the superior ibuprofen bioavailability observed with the SLH formulation was due to the preservation of the drug in a solubilized state and presence of hydrophilic silica which facilitated the dispersion and release of oil droplets and drug into the aqueous environment. This reveals that SLH technology can be applied to various poorly water-soluble drugs in order to maximize bioavailability.

Although statistical significance was limited due to the population size of the study and the high variation observed between participants, interestingly, SIM absorption was greatest for SLH-A, whereas SLH-B demonstrated the greatest metabolic conversion to SIMA. SLH-A was manufactured with Aerosil® 300 silica which consists of 50-nm aggregates of fumed nanoparticles and randomly orientated pores predominately between 2 and 7 nm, whereas SLH-B is composed of Syloid® 244 amorphous silica with a well-defined porous network approximately 19 nm in diameter [20, 21]. A previous study by Gustafsson et al. demonstrated the relationship between silica pore diameter and lipase activity, whereby lipase activity increased with increasing pore size (up to 8.9 nm), due to providing greater space for lipase to fold into its active conformation [34]. Conversely, Joyce et al. reported enhanced lipid digestion with SLH when fabricated with silica comprising of a smaller pore diameter (Aerosil® 300), compared with silica with a larger pore diameter (Syloid® 244) [35]. It was postulated that the pore diameter of Syloid® 244 was greater than the optimal pore size for lipase activity, and therefore, diffusion and activation of lipase into the Aerosil® 300 pores was faster, facilitating lipid digestion. Furthermore, the

Table 3 Summary of the pharmacokinetic parameters for SIMA

	SSF 20 mg	SLH-A 10 mg	SLH-A 20 mg	SLH-B 10 mg	SLH-B 20 mg
N	12	6	6	6	6
$AUC_{0\text{-}8}$ (ng h/mL)	3.42 [79.0]	3.68 [92.3]	7.65 [64.8]	7.12 [18.6]	12.1 [57.6]
AUC_{inf} (ng h/mL)	6.78 [39.6]	8.15 [35.9]	10.5 [62.2]	12.1 [19.6]	21.5 [77.8]
C_{max} (ng/ml)	0.671 [94.2]	0.681 [101]	1.76 [80.1]	1.53 [23.3]	2.46 [70.6]
T_{max} (h)	4.00 [1.00, 8.02]	3.00 [0.750, 8.02]	2.00 [0.500, 6.00]	4.00 [2.50, 6.02]	3.00 [2.00, 4.02]
$T_{1/2}$ (h)	4.69 [3.22]	4.87 [3.27]	4.25 [3.17]	3.99 [1.50]	5.41 [2.74]

Geometric mean [CV%] data is presented. Time is displayed as Median [min, max]

N: number of participants studied, $AUC_{0\text{-}8}$: area under the plasma concentration-time curve from time 0 to 8 h post-dose, AUC_{inf}: area under the plasma concentration-time curve from time zero to infinity, C_{max}: the maximum observed plasma concentration, T_{max}: the time to C_{max}, $T_{1/2}$: apparent terminal half-life

digestion of lipid encapsulated within Aerosil® 300 silica has shown to trigger the rapid release of free fatty acids from the nanostructured matrix into the aqueous phase, which thereby forms fatty acid-rich lamellar phases that enhance the solubilization capacity of poorly water-soluble drugs [32]. Therefore, it is hypothesized that the digestion-promoting behavior of SLH-A enhances the concentration of SIM available for absorption across the intestinal epithelium.

SIM is subject to pre-systemic metabolism by cytochrome P450, specifically CYP 3A4 [11]. CYP 3A4 represents the most abundant enzyme in the human liver and intestine; however, it is a pre-systemic metabolism in the liver which is predominately responsible for the metabolic activation of SIMA [9]. Previous studies have shown that CYP 3A4 enzymes can become saturated when instantaneously exposed to a high dose of SIM, leading to rapid absorption and enhanced bioavailability of the parent compound [36, 37]. Thus, the rapid release of SIM from SLH-A, due to lipase-mediated hydrolysis of lipid confined within the nanostructured silica matrix likely resulted in a greater extent of unchanged SIM to become bioavailable and be absorbed into the systemic bloodstream. In contrast, a reduction in digestion kinetics of medium-chain triglycerides within Syloid® 244 has been reported when compared with Aerosil® 300, and mesoporous silica and silicates have shown to retain key digestion products during lipolysis [32]. Therefore, the mesoporous silica structure of SLH-B may impede the release of lipase-mediated digestion products, which subsequently slows the release and solubilization of SIM, preventing the saturation of CYP3A4 and therefore leading to enhanced SIMA plasma concentrations. Alternatively, it is known that pre-systemic metabolism is avoided when a drug enters the systemic circulation via the intestinal lymph [18]. As SIM is a lipophilic drug (log P = 4.7), it is reasonable to suggest that the dose of SIM released from SLH-A may be absorbed by the lymphatic system, resulting in reduced first-pass metabolism and thus limited conversion to SIMA. However, the impact of porous silica nanostructure on the bioavailability and metabolic conversion of SIMA warrants further investigation, since this approach may serve as a novel approach for enhancing the systemic concentrations of either the parent or the metabolic compound.

Inter-individual variability in the expression of CYP3A4 can vary as much as 40-fold in the liver, thus highly impacting the first-pass metabolism and influencing the bioavailability of orally administered drugs [38]. Specifically, genetic polymorphism of CYP3A4 can lead to an individual being a poor or extensive metabolizer if under- or over-expressed, respectively [39]. Although CYP3A4 expression in study participants was not examined, it is reasonable to suggest that the high between-participant variation observed for SIMA from formulations may be attributed to inter-individual differences in CYP3A4 expression. On the other hand, the between-participant variation observed for SIM may simply reflect variability in absorption due to variation in gastrointestinal physiology such as local pH and gastrointestinal transit time which may vary with age [40].

Arising from the enhanced bioavailability, the results also suggest that the application of SLH technology may lead to a significant reduction in the dose required to achieve equivalent therapeutic concentrations. When SIM encapsulated SLH was administered at a 10 mg dosage, no significant difference in SIM or SIMA exposure was observed compared with the commercial SSF. SLH technology is therefore important when considering alternative drugs, such as pain relief and chemotherapeutic agents, whereby patients experience undesirable and potentially toxic side effects as a consequence of high-dose administration to achieve therapeutic concentrations in the body [23, 41, 42]. Furthermore, a reduction in dose may reduce the pill burden commonly associated with poorly water-soluble drugs [23].

A previous study executed by the same research group evaluated the pharmacokinetic performance of SSF, SLH-A, and SLH-B in Sprague-Dawley rats [18]; therefore, IVIVC was investigated. IVIVC aims to predict the human in vivo drug exposure of an oral formulation based on the in vivo exposure data obtained from an animal pharmacokinetic study. Rats are

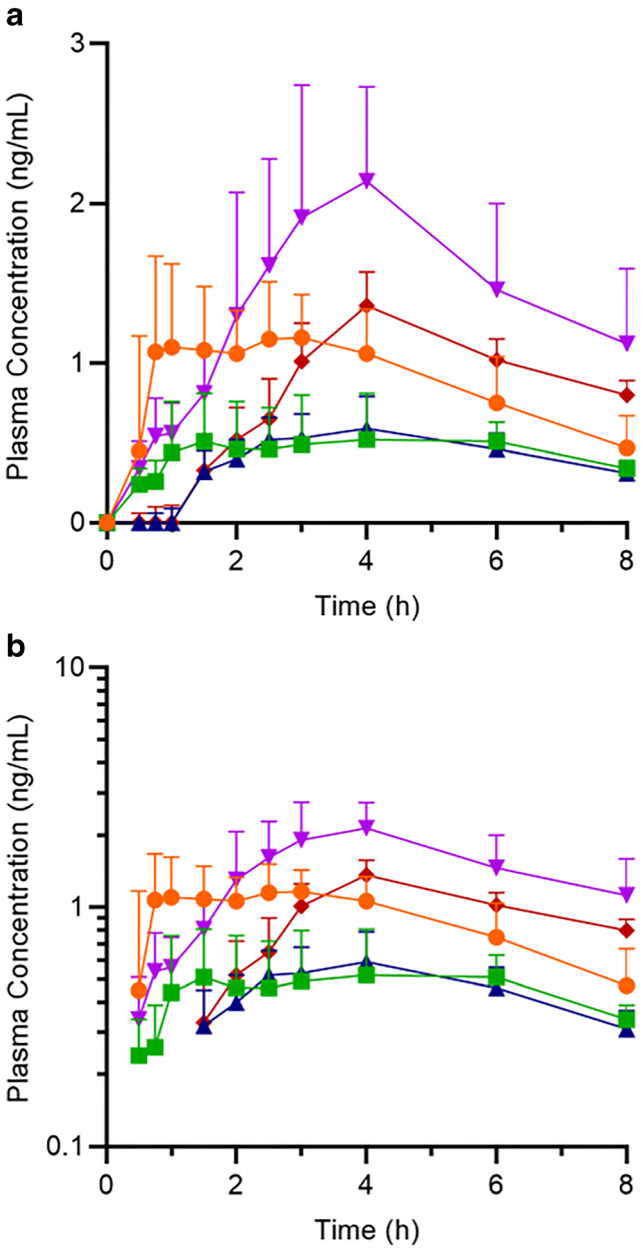

Fig. 3 Geometric mean plasma concentrations for SIMA represented as (**a**) linear-linear and (**b**) log-linear: SSF 20 mg (blue-shaded triangle), SLH-A 10 mg (green-shaded square), SLH-A 20 mg (orange-shaded circle), SLH-B 10 mg (red-shaded diamond), and SLH-B 20 mg (purple-shaded inverted triangle). Each value represents the mean ± SD, n = 12 for SSF and n = 6 for SLH formulations

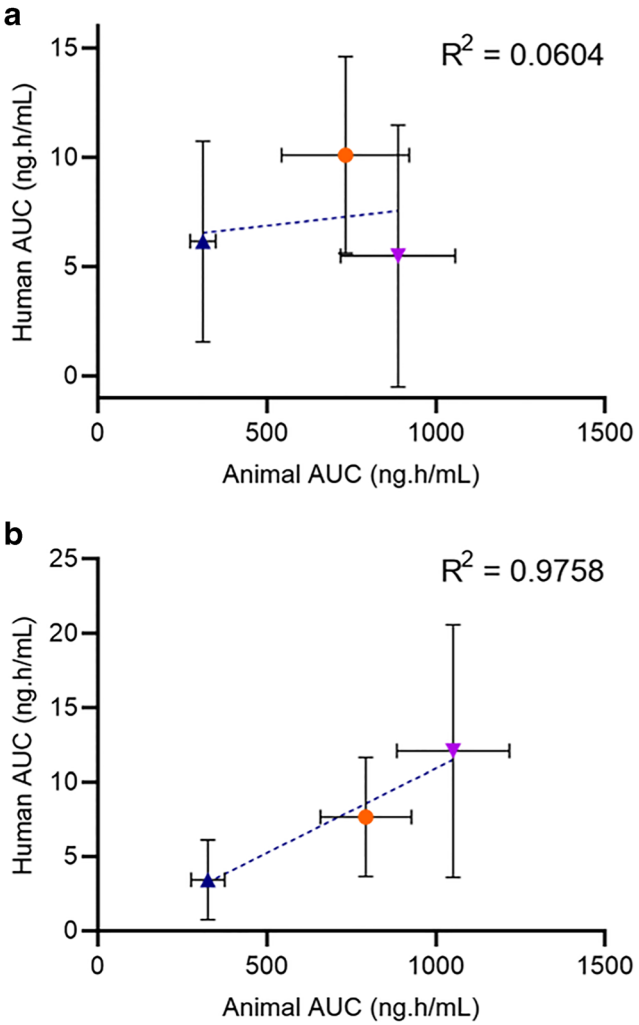

Fig. 4 IVIVC plotted as the area under the plasma concentration-time curve for (**a**) SIM and (**b**) SIMA, after oral administration to fasted male Sprague-Dawley rats and fasted male participants. Formulations include a 20 mg dose of SSF (blue-shaded triangle), SLH-A (orange-shaded circle), and SLH-B (purple-shaded inverted triangle). Values represent geometric mean ± SD (n = 12 for SSF and n = 6 for SLH formulations) for human $AUC_{0\text{-}8}$ values and mean ± SD (n = 4) for animal $AUC_{0\text{-}10}$ values

the most frequently used animal species for investigating the preclinical performance of oral LBFs; however, correlation and comparisons to human bioavailability have long been debated. This is due to a species-specific difference as rats lack a gallbladder and exhibit a continuous bile flow, unlike humans whereby bile secretion is stimulated from the gallbladder in response to the presence of food or lipid in the gastrointestinal tract [43]. Therefore, the constant secretion of bile in rats may reduce the increased solubilizing potential of LBFs. When

Table 4 Classification and number of reported adverse events after administration of SIM formulations

Adverse event	Group 1 (*N*)	Group 2 (*N*)
Participants with at least one AE	2	2
Infection and infestations		
Chlamydial infection	1	0
Inflammation	1	0
Nervous system		
Headache	0	2
Musculoskeletal		
Arthritis	0	1

N number of participants studied

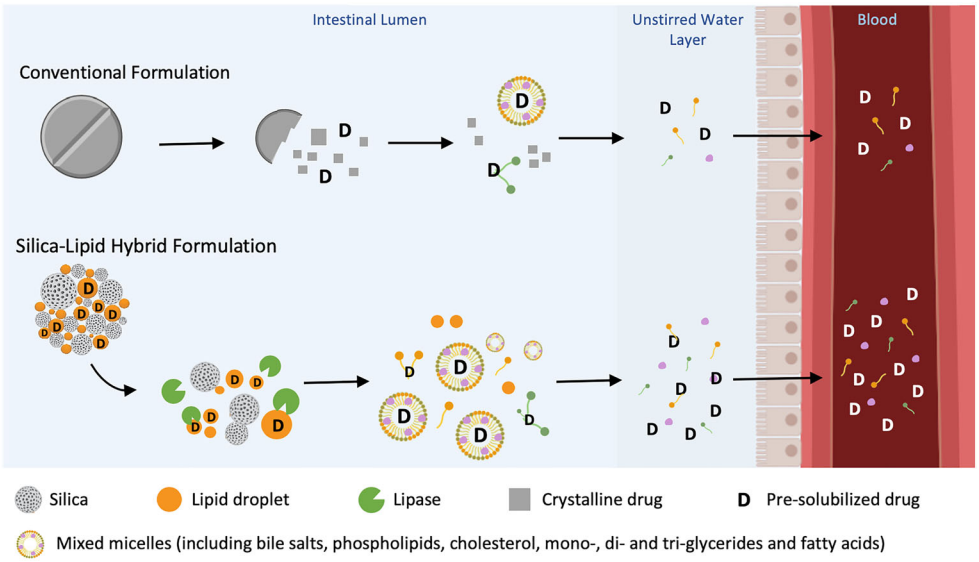

Fig. 5 Schematic representation of the enhanced bioavailability provided by SLH formulations compared with a conventional crystalline drug after oral administration. When administered as an SLH, exogenous lipid is digested by lipase enzymes, facilitating the production of solubilizing colloidal species, such as mixed micelles, which readily incorporate the pre-dissolved drug and promote absorption into the bloodstream. Conversely, crystalline drug exhibits limited dissolution, and only the solubilized portion of the drug can be incorporated into endogenous micelles and undergo absorption, resulting in sub-optimal bioavailability

comparing the $AUC_{0\text{-}10}$ in rats and $AUC_{0\text{-}8}$ for humans in the current study, a low correlation was present for SIM (R^2 = 0.06), signifying that drug release from an LBF may not be readily predicted and translated between species. However, the correlation for the active metabolite, SIMA, was significantly high (R^2 = 0.98). This demonstrates that rats may serve as a valuable species for preclinical testing of novel formulations to determine metabolite formulation, rather than absorption of the parent compound.

All components of the SLH formulation are classified as non-hazardous according to the Occupational Safety and Health Administration (OSHA) and are approved as excipients by the US Food and Drug Administration and the Australian Therapeutic Goods Administration [44]. A review of adverse event data indicated that SLH was safe and well-tolerated when administered to healthy male participants. A total of five adverse events were recorded in four of the twelve participants. Adverse events were categorized based on their relationship to the investigational products. The inflammatory adverse event was considered unlikely related, whereas the chlamydial infection, headaches, and arthritis were categorized as not related to the investigational products. No serious adverse events were reported. Additionally, there was no clinically significant change in vital signs, electrocardiogram measurements, and clinical laboratory tests from baseline to the exit evaluation, suggesting that there was no drug effect. Therefore, the data supports that the application of SLH technology is safe when administered to healthy male participants under fasting conditions.

Acknowledging the study limitations of small sample size and limited blood sampling time points, the combination of safety, tolerability, and pharmacokinetic data strongly demonstrate the safe use and effectiveness of SIM encapsulated SLH in healthy human participants. The results illustrate the solubilizing potential of LBFs, especially SLH, and provide a foundation for future human studies to investigate long-term safety in a multiple-dose study and the application to alternative and challenging poorly water-soluble drugs.

Conclusion

This phase I clinical trial proves that SIM encapsulated SLH is an effective formulation when administered to healthy male participants under fasting conditions as demonstrated by a 3.5-fold enhancement in bioavailability for the active metabolite, SIMA, when compared with a current commercially available product. Additionally, the formulation was safe and well-tolerated exhibiting no significant adverse events. The current clinical study highlights the promising potential of SLH as a solid LBF that can be utilized to enhance the bioavailability of numerous poorly water-soluble drugs and prodrugs, such as SIM which also undergoes extensive metabolism.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethics approval The study was approved by the University of South Australia Human Research Ethics Committee under protocol: 201719.

Consent to participate All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

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