Supersaturated Silica-Lipid Hybrid Oral Drug Delivery Systems: Balancing Drug Loading and In Vivo Performance

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Received November 4, 2018; accepted December 12, 2018

ABSTRACT

Supersaturated silica-lipid hybrid (super-SLH) drug carriers are a recent strategy to improve the drug loading of oral solid lipid based formulations, however they are yet to be studied in vivo. This study investigated the in vivo pharmacokinetics (PK) of super-SLH containing ibuprofen (IBU), as a model Biopharmaceutics Classification Scheme (BCS) class II drug, analyzing the influence of supersaturated drug loading on oral bioavailability and assessing in vitro-in vivo correlation (IVIVC). In addition, super-SLH was directly compared with spray-dried SLH and Nurofen to explore its potential advantages over the well-established and commercial formulations. Fasted male Sprague-Dawley rats were administered formulation suspensions (10 mg/kg IBU) via oral gavage, and blood samples were acquired and plasma was analyzed for IBU concentrations over 24 hours. In vivo, super-SLH with drug loads of 9.5 (99.5% saturated) and 19.3% w/w (227% saturated) achieved bioavailabilities equal to spray-dried SLH and 2.2-fold greater than Nurofen. This effect diminished for super-SLH with a drug load of 29.1% w/w (389% saturated), which exhibited a bioavailability of less than Nurofen due to its greater extent of supersaturation and larger content of crystalline IBU. The super-SLH containing 19.3% w/w IBU provided the greatest PK performance, achieving the same degree of bioavailability enhancement as spray-dried SLH and requiring 63% less formulation. A significant positive IVIVC was observed between the performances of the formulations. These findings indicate the potential of super-SLH as an improved oral solid lipid based formulation strategy for enhancing oral bioavailability of other BCS class II drugs.

Introduction

Solid lipid-based formulations (LBF) continue to emerge as effective oral formulation strategies for the ever-increasing number of Biopharmaceutics Classification Scheme (BCS) class II drugs (i.e., poor solubility, high permeability) arising from the drug discovery pipeline (Mandi et al., 2017; Joyce et al., 2018a). They retain the beneficial properties of conventional lipid LBF by delivering the drug to the gut in a solubilized state, bypassing the rate-limiting step of dissolution, where the lipid component is digested by gastrointestinal lipases, creating a lipophilic and drug solubilizing environment that mimics the postprandial effects of dietary fats and oils improving drug solubilization and hence absorption (Chakraborty et al., 2009; Feeney et al., 2016). Additionally, solid LBF possess the added benefits of existing as powders, which are beneficial for improved stability, dosage precision, and manufacturing (Jannin et al., 2008; Tang et al., 2008). Solidification of LBF as a dry powder can be achieved through the adsorption to a solid carrier, e.g., silica, polymers, nanostructured carbon, carbonates, and aluminosilicates, often up to 50% w/w (Tan et al., 2013; Dening et al., 2016a; Bremmell and Prestidge, 2018). Further dilution of the LBF occurs when additional excipients are incorporated to form powders with suitable properties for tablet compression (Bremmell et al., 2013). Consequently, this leads to reduced drug loading capacities within solid LBF, limiting their clinical use for drugs with high doses and low potency.

SUPPLEMENTAL MATERIAL

This work was supported by The Australian Government Research Training Program for the PhD Scholarship of Hayley Schultz; The Academy of Finland [Grant Number 287625] funding for Miia Kovalainen; and The Australian Research Council’s Centre of Excellence in Convergent Bio-Nano Science and Technology. https://doi.org/10.1124/jpet.118.254466. This article has supplemental material available at jpet.aspetjournals.org.

ABBREVIATIONS: AUC, area under the curve; BCS, Biopharmaceutics Classification Scheme; HPLC, high-performance liquid chromatography; IBU, ibuprofen; ISTD, internal standard; IVIVC, in vitro-in vivo correlation; LBF, lipid based formulation; PK, pharmacokinetic; SEM, scanning electron microscopy; S<sub>eq</sub>, equilibrium solubility; SLH, silica-lipid hybrid; SNEDDS, self nano-emulsifying drug delivery system; super-SLH, supersaturated silica-lipid hybrid.
The maximum LBF drug load is limited by the drug’s solubility in the lipid formulation (Porter et al., 2007; LaFountaine et al., 2016). The drug is commonly loaded below its equilibrium solubility ($S_{eq}$) (e.g., 75%–80%) to ensure the drug remains solubilized within the formulation and avoids recrystallization (Christensen et al., 2004; Thomas et al., 2012). Drug recrystallization is generally regarded as unsuitable for effective oral delivery, due to reintroducing a dissolution step of crystalline drug (Porter et al., 2004; Pouton, 2006), which limits the rate of absorption in vivo, as only the solubilized drug present in the aqueous phase is available for absorption (Khan et al., 2016). Previously, a liquid LBF was supersaturated, loading simvastatin at up to 200% of its $S_{eq}$ by dissolving the drug at an elevated temperature to form supersaturated self nano emulsifying drug delivery systems (super-SNEDDS) (Thomas et al., 2013). It was reported that no drug recrystallization was observed within the super-SNEDDS over 10 months due to the high viscosity of the lipids and that super-SNEDDS demonstrated superior in vivo performance compared with the standard SNEDDS (Thomas et al., 2013).

Recently, we applied a supersaturation method to the silica-lipid hybrid (SLH) solid LBF to load drug above its $S_{eq}$ and form super-SLH to overcome the limitation of low drug loading (Schultz et al., 2018). SLH are a well-established solid LBF, existing as porous microparticulate dry powders, fabricated by spray drying or lyophilizing silica-stabilized Pickering emulsions (Simovic et al., 2009; Tan et al., 2009; Dening et al., 2016b; Meola et al., 2018). Despite their proven effectiveness in enhancing bioavailability of many BCS class II drugs in vivo (Tan et al., 2011; Nguyen et al., 2013; Rao et al., 2014; Joyce et al., 2017), including a phase I human clinical trial (Tan et al., 2014), the low drug loading capacities of SLH (often less than 5%) have limited their broader clinical application to drugs with high doses and low potency. The super-SLH formulation overcomes this limitation by improving drug loading through supersaturation of the model BCS class II drug ibuprofen (IBU). IBU possesses a pKa of $\sim$4.5 (Avdeef et al., 1998) and a Log P of $\sim$4 (Avdeef et al., 1999) and it’s $S_{eq}$ in water is pH dependent, with approximately 46 µg/ml at pH 1.5 (sparingly soluble), while increasing to $>300$ µg/ml above pH 7 (more than slightly soluble) (Potthast et al., 2005). Super-SLH were prepared containing drug loads of 8.7%–44.8% w/w, substantially greater than the spray-dried SLH with an IBU load of 5.6% w/w previously reported (Tan et al., 2014). Super-SLH fabrication involved dissolving drug in the lipid at an elevated temperature (i.e., 60°C) followed by subsequent encapsulation of the lipid-drug mixture within the pores of mesoporous silica particles to inhibit the recrystallization of drug crystals and maintain the supersaturated state at room temperature (Schultz et al., 2018). Portions of drug recrystallized in the formulations, proportional to the drug load that influenced in vitro dissolution kinetics at pH 2.1, where all super-SLH formulations exhibited superior dissolution to the spray-dried SLH and commercial product Nurofen.

Therefore, in the current study, we aim to explore the in vivo pharmacokinetics (PK) of super-SLH containing IBU, analyzing the influence of supersaturated drug loading on oral bioavailability and assessing in vitro-in vivo correlation (IVIVC). In addition, super-SLH was directly compared with spray-dried SLH and Nurofen, exploring the potential advantages of an enhanced drug load on oral bioavailability over the well-established LBF and commercial formulations. This study marks the first in vivo investigation into the oral delivery performance of super-SLH and an important step forward in encouraging the clinical application of solid-state LBF.

### Materials and Methods

#### Materials

The drug of interest, ibuprofen (IBU) (>98%, GC), and internal standard flufenamic acid (ISTD) (analytical grade) were purchased from Sigma Aldrich (Castle Hill, Australia). Commercially available Nurofen tablets (Reckitt Benckiser, Sydney, Australia) were crushed into a uniform powder before use. The lipid, Capmul MCG (propylene glycol caprylate), was sourced from Abitec (Columbus, OH). Glacial acetic acid (analytical grade) was purchased from Chem-Supply (Gillman, Australia). Silica nanoparticles (Aerosil 300 Pharma), with a surface area of 300 ± 30 m²/g were donated by Evonik (Melbourne, Australia). Nanoporous silica microparticles (Parteck SLC 500), with a 9–11 µm particle size and 6 µm pore size, were donated by Merck (Bayswater, Australia). Soybean lecithin and liquid chromatography grade methanol were purchased from Merck. Heparin sodium (5000 IU/5 ml) was purchased from Pfizer (Perth, Australia). High purity Milli-Q water was acquired from a Milli-Q water purification system (Merck).

#### Fabrication of Formulations

**Spray-Dried SLH.** A previously established method was followed to prepare the spray-dried SLH containing IBU (Tan et al., 2014). Soybean lecithin (emulsifier) (6% w/w) and Capmul MCG were weighed into a glass vial and sonicated for 10 minutes. IBU was subsequently added to the lipid at 75% $S_{eq}$ ($S_{eq}$ = 211 mg/ml) and sonicated until dissolved. Milli-Q water was added to the lipid to form a coarse suspension that was homogenized at 1000 bar for five cycles with an Avantium EmulsiFlex-C5 Homogenizer (ATA Scientific, Taren Point, Australia) to form a submicron oil-in-water emulsion. A 5% silica in water suspension (Aerosil Pharma 300) was prepared by sonication overnight. The 5% silica suspension was added to the emulsion to yield a final lipid:silica ratio of 2:1 w/w and was magnetically stirred overnight. The emulsion was then spray-dried using a Buchi Mini Spray Dryer B-290 apparatus (Postfach, Switzerland) to remove the water phase and obtain a dry SLH powder. The following spray drying conditions were maintained: an inlet temperature of 160°C, an outlet temperature of 65°C, aspirator setting of 100, pump set to 20%, and a product flow rate of 6 ml/min.

**Super-SLH.** A previously established method was used to prepare the super-SLH (Schultz et al., 2018). IBU and lipid were weighed into glass vials and heated to 60–70°C in an oven for approximately 10 minutes with intermittent shaking to dissolve the IBU. Porous silica microparticles (Parteck SLC 500) were added directly weighed into the vials containing hot lipid and were immediately physically mixed to obtain a white dry powder. As displayed in Table 1, the target composition of super-SLH A, B, and C were 10%, 20%, and 30% w/w IBU, respectively, with a constant 1:1 w/w ratio of lipid to silica.

**Drug Load Determination.** Solvent extraction followed by HPLC analysis was performed to extract the IBU from the spray-dried SLH, super-SLH, and crushed Nurofen tablets. Approximately 10 mg of the formulation was weighed into a glass vial to which 10 ml of methanol was added. The contents of the vial were sonicated for 30 minutes to extract the IBU. A 500 µl sample was removed from the vial and centrifuged for 20 minutes at 7270 g to separate any undissolved material. The supernatants containing the extracted IBU in methanol were diluted with mobile phase prior to HPLC analysis to determine the drug load of the IBU formulations. This was performed in triplicate.

**Surface Morphology Characterization.** A scanning electron microscope (SEM) (Carl Zeiss, Germany) operating at 1.0–2.0 kV at a working distance of 5–10 mm was used to observe the surface morphology of the spray-dried SLH and super-SLH formulations. The formulations were held in place with double-sided adhesive carbon tape and sputter coated with gold (10 nm) before imaging.
In Vitro Dissolution Study. Dissolution studies were performed using a VanKel USP Type II Paddle Apparatus (Agilent Technologies, Santa Clara, CA). Formulation samples containing 20 mg of IBU were dissolved in 450 ml of pH 2.1 HCl media. The media was stirred at 50 RPM and maintained at 37 ± 0.5°C. Five milliliters aliquots were removed at fixed time points and replaced with fresh media. The aliquots were immediately filtered using a 0.45 μm syringe filter to remove any undissolved material. The filtered samples were diluted with mobile phase prior to HPLC analysis for IBU content. This was performed in triplicate.

High-Performance Liquid Chromatography. IBU was analyzed using a Shimadzu high-performance liquid chromatograph (HPLC) (Kyoto, Japan) and a Pheno Sphere Next 3 μm C18 column (150 × 4.6 mm) (Torrance, CA) equipped with a column guard. The system was maintained at 40°C, used an injection volume of 50 μl and eluted the mobile phase at a flow rate of 0.5 ml/min. The mobile phase contained 90% methanol and 20% water adjusted to pH 2.1 with glacial acetic acid. Each sample was analyzed over 13.5 minutes and at an ultraviolet wavelength of 223 nm. The retention time of the IBU and ISTD (used in plasma samples only) were determined to be 9.1 and 11.5 minutes, respectively. The concentration of the samples were determined by using calibration curves produced by a set of IBU standards in mobile phase (9–900 μg/ml) or plasma (0.15–15 μg/ml containing a fixed concentration of ISTD).

In Vivo Pharmacokinetic Study. All animal experiments were approved by the South Australian Animal Ethics Committee under approval number U07-17. An oral PK study was performed on five groups of male Sprague-Dawley rats aged 6–8 weeks (273–463 g) sourced from the Animal Resources Centre (Canning Vale, Australia). Each group was administered one of the five formulations via the oral route: crushed Nurofen tablets, 7.1% w/w spray-dried SLH, 9.5% w/w super-SLH, 19.3% w/w super-SLH, and 29.1% w/w super-SLH. All treatments were suspended in Milli-Q water by sonicating for 5 minutes immediately before administration to the rats at an IBU dose of 10 mg/kg via gavage. The rats were caged in groups of three and fasted overnight prior to dosing to remove their gut of residual food (Vermeulen et al., 1997), and feeding was recommenced after the 2-hour time point. Water was provided ad libitum throughout the study period. Eight blood samples (0.2 ml) were taken from the saphenous vein at 12 hours post-dose and 0.5, 1, 1.5, 2, 4, 10, and 24 hour postdose. A needle was used to puncture the veins, and blood was collected in preheparinized 1.5 ml centrifuge tubes to prevent blood clotting. Plasma (approximately 70 μl) was harvested immediately by centrifugation at 7270 g (5 minutes at room temperature) and stored at −80°C until further analysis.

Plasma Sample Preparation. Fifty microliters of the plasma sample was combined with 90 μl of methanol and 10 μl of ISTD (0.3 mg/ml in methanol) in a 1.5 ml centrifuge tube to precipitate the plasma proteins. The sample was vortex mixed for 60 seconds and subsequently centrifuged at room temperature for 10 minutes at 7270 g to separate the proteins from the plasma. 50 μl of the supernatant was transferred into a fresh tube with 50 μl of mobile phase and vortex mixed for 30 seconds prior to HPLC analysis. The plasma sample dilution factor was six, and the resulting ISTD concentration was 10 μg/ml.

Blank rat plasma was used to produce a calibration curve of spiked plasma standards (0.15–15 μg/ml). The procedure followed the same protocol as the plasma sample preparation, however the 50 μl of plasma sample was replaced with 50 μl of spiked plasma standard (i.e., 45 μl blank plasma vortex mixed with 5 μl IBU standard for 30 seconds). After analysis with HPLC, the calibration curves were prepared using the ratio of HPLC response between the IBU analyte and the ISTD (R² > 0.998) and were used to determine the unknown IBU concentrations in the plasma samples.

Pharmacokinetic Data Analysis. The maximum observed plasma concentrations (Cmax) of IBU and the time for their occurrence (tmax) were noted directly from the individual plasma concentration versus time profiles. The area under the plasma concentration versus time profiles (AUC0–24 hours) were calculated using the linear trapezoidal method (Gabrielsson and Weiner, 2012) using GraphPad Prism, Version 7.03 (La Jolla, CA). Due to the IBU plasma concentrations at 10 and 24 hours being below the limit of quantification of the assay after plasma sample preparation (150 ng/ml), accurate determination of AUC0–24 hours was not possible. The relative bioavailability was calculated with respect to Nurofen, i.e., formulation AUC0–10 hours/Nurofen AUC0–10 hours.

Statistical and Correlation Analyses. All statistical and correlation analyses were performed using GraphPad Prism Version 7.03. The areas under plasma concentration–time curves (in vivo AUC0–10 hours) were plotted against the areas under the solubilization–time curves (in vitro AUC0–1 hours) (values provided in Supplemental Table 1). The Pearson correlation coefficient was calculated to determine if IVIVC existed between the in vivo and in vitro performances of the IBU formulations. Statistically significant differences in AUC, Cmax, and tmax were determined by one-way ANOVA followed by Tukey’s post-test for multiple comparisons. Differences were considered statistically significantly when P < 0.05.

Results

Formulation Characterization. The super-SLH and spray-dried SLH were successfully fabricated as described in Table 1. Super-SLH A, B, and C contained 9.5, 19.3, and 29.1% w/w IBU, respectively, and possessed S.D. ≤0.4% w/w and loading efficiencies ≥95%. The Seq of IBU in lipid (Capmul PG8) is 211 mg/g (Schultz et al., 2018). The drug loads of the formulations were also reported in terms of % of the Seq to compare the degree of supersaturation of the formulations. Super-SLH A, B, and C contained IBU at 99.5%, 227%, and 389% of the Seq, respectively. The spray-dried SLH was found
to possess a drug load of 7.1% w/w (unsaturated), and the crushed Nurofen tablet was found to contain 44.7% w/w IBU.

The SEM images, displayed in Fig. 1, illustrate the differences in the surface morphologies of the spray-dried SLH and super-SLH. The spray-dried SLH appeared consistent with previous reports (Bremell et al., 2013; Rao et al., 2015; Dening et al., 2016b), displaying spherical porous microparticles composed of many silica nanoparticles. In contrast, the super-SLH formulations displayed larger, irregular-shaped, silica microparticles with no evidence of incomplete lipid or drug loading as seen in previous super-SLH studies (Schultz et al., 2018). As super-SLH A, B, and C all appeared similar under SEM, only the super-SLH A SEM image is displayed.

**In Vitro Dissolution Study.** Figure 2A illustrates the in vitro dissolution profiles of the IBU formulations in pH 2.1 media. The super-SLH A, B, and C and Nurofen data produced by Schultz et al. (2018) were included with permission, and the spray-dried SLH data were collected in the current study. It is clear that the spray-dried SLH possesses rapid dissolution kinetics compared with the other formulations. The spray-dried SLH released 90% of the IBU load within 10 minutes and plateaued at ~92% by 15 minutes. Conversely, the other formulations demonstrated more sustained release, i.e., 28%–37% of IBU released in 10 minutes and were expected to continue releasing beyond 60 minutes. The differences in the dissolution profiles of super-SLH A, B, and C were previously described (Schultz et al., 2018). Briefly, the dissolution decreased with an increase in supersaturated drug load, which was associated with an increase in crystalline IBU content.

Figure 2B displays the areas under the solubilization-time curves (in vitro AUC0–1 hour) of each formulation relative to Nurofen. The in vitro AUCs of spray-dried SLH and super-SLH A and B were significantly greater than Nurofen (P < 0.0001). Spray-dried SLH achieved the greatest in vitro AUC, approximately twofold greater than Nurofen. Super-SLH A and B achieved similar relative in vitro AUCs (P = 0.5653), less than spray-dried SLH but approximately 1.3-fold greater than Nurofen. Conversely, super-SLH C achieved a relative in vitro AUC of 0.85, significantly less than Nurofen (P = 0.0078) and super-SLH A and B (P < 0.0001).

**In Vivo Pharmacokinetic Study.** The oral PK profiles of the IBU formulations dosed to fasted male Sprague-Dawley rats are displayed in Fig. 3A, the areas under the plasma concentration-time curves (in vivo AUC0–10 hours) (relative bioavailability) of each formulation relative to Nurofen is depicted in Fig. 3B, and the corresponding PK parameters are displayed in Table 2.

Nurofen was used to compare the formulations performances to a current commercial product, while spray-dried SLH was used to compare the formulations performances to a well-established LBF that has been used clinically. Nurofen achieved a Cmax of 7 µg/ml at a tmax of 0.9 hours, and an AUC of 21.1 µg/ml·h. The performance of the spray-dried SLH was superior to the Nurofen, exhibiting the greatest Cmax of 17.6 µg/ml at a tmax of 1.0 hour and in vivo AUC of 48.0 µg/ml·h.

The super-SLH A and B were comparable, reaching statistically equivalent Cmax of 14.1 and 13.7 µg/ml, respectively (P = 0.9985). Their Cmax were approximately 100% greater than Nurofen and 20% less than the spray-dried SLH. The AUC for super-SLH A and B, respectively, were 47.1 and 47.0 µg/ml·h, which led to relative bioavailabilities of 2.23 and 2.22 in comparison with Nurofen; these relative bioavailabilities were equivalent to that of spray-dried SLH, 2.27 (P = 0.9985 and P = 0.9976). Conversely, the in vivo performance of super-SLH C was poor compared with super-SLH A and B, attaining a Cmax of 8.2 µg/ml and AUC of 19.3 µg/ml·h. The in vivo AUC was 60% less compared with the super-SLH A and B and spray-dried SLH. Despite exhibiting a higher Cmax, the super-SLH C possessed a relative bioavailability of 0.91 when compared with Nurofen.

The tmax of the different formulations ranged from 0.5 to 1.4 hour with S.D. ranging from 0 to 0.8 hour. The different formulations did not cause statistically significant differences in IBU tmax (all P ≥ 0.9668).

**In Vitro-In Vivo Correlation.** In an attempt to explore the IVIVC between the in vitro dissolution and in vivo PK data, the areas under plasma concentration-time curves (in vivo AUC0–10 hours) were plotted against the area under the
solubilization-time curves (in vitro AUC\(_0^{–1}\) hour) and displayed in Fig. 4. The Pearson correlation coefficient indicated a strong positive correlation between the measured in vitro AUC and measured in vivo AUC data (\(r = 0.9137, P = 0.0109\)). Additionally, the relative AUCs for the in vitro dissolution and in vivo PK data were compared in Fig. 5. Greater relative AUCs were observed in vivo than in vitro. Furthermore, for each formulation, the difference between the in vitro and in vivo relative AUC was statistically significantly different for super-SLH A and B, however not for Nurofen, spray-dried SLH, or super-SLH C.

**Discussion**

Super-SLH is a strategy to improve the drug loading of solid-state LBF through drug supersaturation of the lipid followed by encapsulation in the mesopores of silica microparticles. While the super-SLH drug loads are greater than spray-dried SLH, the supersaturated levels of drug may influence the in vitro and in vivo performance of the formulation. Hence, super-SLH containing three IBU supersaturation levels (99.5%, 227%, and 389% \(S_{eq}\)) and spray-dried SLH were fabricated and directly compared in vitro and in vivo. Nurofen was also investigated to determine whether super-SLH outperforms the current commercial IBU product.

**Formulation Drug Loading and Crystalline Content.** Super-SLH with target drug loads of 10%, 20%, and 30% w/w (super-SLH A, B, and C) were fabricated using the reported method (Schultz et al., 2018). They contained slightly greater drug loads, 9.5%, 19.3%, and 29.1% w/w, than previously reported due to higher drug loading efficiencies. Super-SLH are known to possess different proportions of crystalline IBU as previously reported, with super-SLH A containing no crystalline IBU and super-SLH B and C containing small amounts of crystalline IBU (C containing more than B) (Schultz et al., 2018). This difference in crystalline drug content between the formulations is owing to their drug loads, where the greater the extent above the \(S_{eq}\), the greater the crystalline drug content of the super-SLH (Table 2). Spray-dried SLH was fabricated using an established method (Tan et al., 2014); however, the lipid Capmul PG8 was used rather than a mixture of lipids (Captex 300, Capmul MCM, and soybean oil) for direct comparisons to super-SLH, leading to a greater drug loading of 7.1% w/w compared with 5.6% w/w as reported previously. As spray-dried SLH was fabricated containing an IBU concentration below its \(S_{eq}\), it was expected that the IBU was molecularly dispersed and no crystalline drug was present. The Nurofen tablets contained crystalline IBU, which were ground into a fine powder prior to use.

**In Vitro Dissolution Performance.** After in vitro dissolution for 60 minutes at pH 2.1, the rankings of the formulations in respect to AUC were spray-dried SLH > super-SLH A > super-SLH B > Nurofen > super-SLH C, as shown in Fig. 2B. Two formulation parameters were identified as major influencers of dissolution: 1) crystalline IBU content and 2) particle morphology. The crystalline drug content within the formulation had an inverse effect on dissolution performance. Crystalline drug, as opposed to noncrystalline (molecularly dispersed) drug, requires greater energy to overcome the intermolecular forces within the crystal lattice to undergo dissolution. Spray-dried SLH containing molecularly dispersed IBU exhibited excellent in vitro performance, whereas Nurofen containing crystalline IBU exhibited poor performance. The super-SLH exhibited a decrease in dissolution with an increase in crystalline content. However, despite super-SLH A containing molecularly dispersed IBU and super-SLH B containing a small proportion of crystalline IBU, the difference between super-SLH A and B was not significant, suggesting a small portion of crystalline drug will not substantially influence the overall dissolution of a formulation. Interestingly, the super-SLH C, which...
contained portions of crystalline and molecularly dispersed IBU, performed poorer than Nurofen containing crystalline IBU. This may be contributable to Nurofen containing additional solubilizing excipients, such as the surfactant sodium lauryl sulfate, to assist in dissolution (Medicines and Healthcare Products Regulatory Agency, 2017) and a potentially smaller crystal size due to the grinding of the tablets (Meola et al., 2018). The large difference in the rate and extent of dissolution between the spray-dried SLH and the super-SLH can be explained through the differences in the particle morphology. The super-SLH is composed of mesoporous microparticles loaded with lipid and drug, resulting in slow partitioning of the drug from the lipid, located inside the pores, into the dissolution media. Conversely, the spray-dried SLH is composed of porous nanoparticles, comprised of a matrix of nanoparticles and submicron lipid droplets containing drug, which when placed in dissolution media, allow rapid drug release due to their large surface area (Tan et al., 2009).

While the dissolution conditions employed were not a biorelevant representation of the environment within the gastrointestinal tract, they were adequately discriminatory for the IBU formulations and enabled differentiation between the IBU formulations’ dissolution kinetics. The conditions did not take into consideration the different pH conditions present throughout the gastrointestinal tract, the digestion of the lipid that occurs by gastrointestinal lipases, or the dynamic absorption of the drug from the gut driving further IBU release from the formulation (Porter and Charman, 2001). In vitro lipolysis studies were considered to obtain a more biorelevant representation of the solubilization capacity of the super-SLH formulations under digestive conditions (Fatouros and Mullertz, 2008); however, due to ionization and high solubility of IBU in the fasted state lipolysis medium (pH 7.5)—(~7.7 mg/ml), discriminatory non-sink conditions were not possible without dosing an excessive amount of IBU formulation (Dening et al., 2018). Therefore, the next logical step was to conduct oral PK studies in rats to compare the performance of super-SLH, spray-dried SLH, and Nurofen in vivo.

**In Vivo Oral PK Performance.** After orally administering the formulation suspensions to rats in the in vivo PK study, the rankings of the formulations in respect to AUC (bioavailability) were spray-dried SLH > super-SLH A > super-SLH B > Nurofen > super-SLH C, as shown in

![Fig. 3. In vivo PK data for IBU formulations orally administered to Sprague-Dawley rats at a dose of 10 mg/kg. (A) The PK profiles of the IBU formulations over 10 hours (24-hour data are supplied in Supplemental Fig. 1) [mean ± S.E., n = 4 or 5 (Nurofen)]. (B) The area under the plasma concentration-time curves (in vivo AUC0–10 h) of each formulation relative to Nurofen (relative bioavailability) [mean ± S.E., n = 4 or 5 (Nurofen)]. **** indicates significantly greater than Nurofen where P < 0.0001 and ns indicates not significantly greater than Nurofen, P = 0.9746.](image)

**TABLE 2**

PK parameters for IBU formulations following oral administration at 10 mg/kg to fasted male Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Cmax (µg/ml)</th>
<th>tmax (h)</th>
<th>AUC&lt;sub&gt;0–10&lt;/sub&gt; (µg/ml·h)</th>
<th>Relative bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nurofen</td>
<td>7.0 ± 3.0</td>
<td>0.9 ± 0.4</td>
<td>21.1 ± 2.5</td>
<td>1.00</td>
</tr>
<tr>
<td>Spray-dried SLH</td>
<td>17.6 ± 2.9*</td>
<td>1.0 ± 0.4</td>
<td>48.0 ± 5.1*</td>
<td>2.27</td>
</tr>
<tr>
<td>Super-SLH A</td>
<td>14.1 ± 3.7*</td>
<td>0.5 ± 0.0</td>
<td>47.1 ± 7.1*</td>
<td>2.23</td>
</tr>
<tr>
<td>Super-SLH B</td>
<td>13.7 ± 4.5*</td>
<td>1.4 ± 0.8</td>
<td>47.0 ± 5.3*</td>
<td>2.22</td>
</tr>
<tr>
<td>Super-SLH C</td>
<td>8.2 ± 1.9</td>
<td>0.5 ± 0.0</td>
<td>19.3 ± 2.3</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*P < 0.0001 statistically significant different compared with Nurofen.
The data points represent the following formulations: spray-dried SLH (blue square), super-SLH A (green circle), super-SLH B (gold triangle), super-SLH C (purple upside down triangle), and Nurofen (red diamond).

Fig. 4. In vitro—in vivo correlation plotted as area under the IBU plasma concentration–time curve following oral administration to fasted male Sprague-Dawley rats [mean ± S.E., n = 4 or 5 (Nurofen)] vs. the area under the IBU dissolution concentration–time curve (mean ± S.E., n = 3).

The bioavailabilities of super-SLH A and B and spray-dried SLH were comparable, achieving an approximate 2.2-fold increase in bioavailability compared with that of Nurofen. This suggests that super-SLH A and B retain the beneficial properties of a spray-dried formulation, despite the differences in particle morphology, drug load, and crystalline drug content. This is promising as this may translate to other super-SLH benefits previously demonstrated by spray-dried SLH, including improved bioavailability for a range of BCS class II drugs in in vivo rat and dog models as well as in humans (Tan et al., 2011, 2014; Nguyen et al., 2013; Rao et al., 2014). Super-SLH A and B and spray-dried SLH were expected to achieve bioavailabilities greater than that of Nurofen due to the beneficial properties of LBFs and containing molecularly dispersed drug, rather than crystalline IBU, as described in literature (Balakrishnan et al., 2009; Kim et al., 2012). However, this was not the case for super-SLH C, which did not perform better than the Nurofen. As mentioned above, this was due to super-SLH C containing a high proportion of crystalline IBU, limiting dissolution and hence absorption. This demonstrates that super-SLH is an effective solid-state LBF strategy to enhance the bioavailability of IBU and outperforms the commercial product Nurofen, which suggests the formulation has commercial potential. However, the degree of crystallinity has demonstrated to be an important factor in in vitro and in vivo performance and must be taken into consideration.

The formulations that achieved the highest bioavailability enhancement, i.e., spray-dried SLH and super-SLH A and B, had different drug loads, and hence different formulation doses were administered to achieve the same IBU dose. To achieve the same 2.2-fold improvement in bioavailability, 140.8 mg of spray-dried SLH (7.1% w/w IBU), 105.2 mg of super-SLH A (9.5% w/w IBU), and 52.4 mg of super-SLH B (19.3% w/w) were dosed to rats. Super-SLH B required 50% less formulation than super-SLH A and 63% less formulation than spray-dried SLH. Of the formulations investigated, super-SLH B was considered the superior formulation, achieving the highest bioavailability and drug load, translating to considerably smaller formulation doses (less tablets/capsules) in a clinical setting, leading to better patient compliance and quality of life (Williams et al., 2005; Ingersoll and Cohen, 2008). It also translates to benefits for the pharmaceutical industry, including a more efficient medication and requirement for lower quantities of active ingredient and excipients. Large-scale manufacture is expected to be achievable utilizing commonly employed industrial processes and fabrication approaches, involving simple solution preparation and heating and mixing with excipients.

IVIVC. The Pearson correlation coefficient confirmed a strong IVIVC (r = 0.9137, P = 0.0109) between the IBU formulations’ in vitro dissolution and in vivo PK performances. The correlation suggests that the in vitro dissolution study in pH 2.1 media may offer a simple initial test to predict the performance of these formulations in vivo. This was despite the significant difference between the in vitro and in vivo performance of super-SLH A and B that resulted in a change in the performance (AUC) ranking of the formulations. All IBU formulations exhibited greater improvements in relative AUC in vivo than in vitro. This is owing to the advantages of the LBF exhibited in vivo, i.e., enhanced drug solubilization due to lipid digestion products, inhibition of P-glycoprotein-mediated drug efflux, promotion of lymphatic transport, and increased gastrointestinal membrane permeability (Hauss, 2007). Super-SLH A and B performed 78% and 85% greater in vivo than in vitro, respectively, a significant improvement (P < 0.0001), as their full potential was not demonstrated in vitro, due to slow drug partitioning from the lipid within the silica pores into the dissolution medium. This is a slow process due to the relatively larger lipid droplets with low surface area contained within the pores, compared with
the submicron lipid droplets distributed throughout the spray-dried SLH microparticles with high surface area (Joyce et al., 2018b). In vivo, this process of drug partitioning played a far smaller role, as lipolysis drove the release of IBU. An insignificant increase in AUC was observed by super-SLH C in vivo compared with in vitro, as it contained a large proportion of crystalline IBU that must undergo dissolution, limiting the advantageous effects that can be achieved by the LBF.

Conclusion

The in vivo performance of IBU was significantly enhanced by the super-SLH formulation, achieving equivalent bioavailability to spray-dried SLH and 2.2-fold greater bioavailability than Nurofen. A super-SLH formulation containing a drug load of 19.3% w/w IBU (supersaturated at 227% of the SOD), 2.7-fold greater than spray-dried SLH, allowed a 63% reduction in administered formulation. Supersaturated drug loading influenced the in vivo performance of super-SLH, as a decrease in bioavailability was observed by the super-SLH C due to its larger content of crystalline drug. Strong positive IVIVC was observed, suggesting that the simple in vitro dissolution study in pH 2.1 media approximates the in vivo bioavailability of super-SLH in rats. Super-SLH has potential to be applied to other BCS class II drugs to form solid LBF that enhance bioavailability and reduce the pill burden on patients due to its improved drug loading.

Acknowledgments

We acknowledge that this work was performed in part at the University of South Australia node of the Australian National Fabrication Facility, a company established under the National Collaborative Research Infrastructure Strategy to provide nanomaterials and micro-fabrication facilities for Australia’s researchers.

Authorship Contributions

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Conducted experiments: Schultz, Kovalainen, Peressin.
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References


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