Drug Deliv. and Transl. Res. (2014) 4:212–221
DOI 10.1007/s13346-013-0172-9

CLINICAL TRIAL

First in man bioavailability and tolerability studies of a silica–lipid hybrid (Lipoceramic) formulation: a Phase I study with ibuprofen

Angel Tan · Nasrin Ghouchi Eskandar · Shasha Rao · Clive A. Prestidge

Published online: 4 September 2013

Abstract Clinical trials addressing the viability of lipid and nanoparticle-based solid dosage forms for the oral delivery of poorly water-soluble drugs are limited to date. This Phase I study aimed to assess the comparative tolerability and oral pharmacokinetics of a novel silica nanoparticle–lipid hybrid formulation encapsulating ibuprofen (i.e., Lipoceramic-IBU) with reference to a commercial tablet (i.e., Nurofen®). The test (Lipoceramic-IBU) and reference (Nurofen®) ibuprofen formulations were characterised for physicochemical properties and in vitro solubilisation performance prior to the clinical study. A randomised, double-blinded, one-period single oral dose (20 mg ibuprofen) study was performed in 16 healthy male subjects under fasting conditions. Encapsulation of ibuprofen in a molecularly dispersed form in the Lipoceramic nanostructured silica–lipid matrices was shown to produce superior drug solubilisation in comparison to Nurofen® and the pure drug during a two-step dissolution (or solubilisation) study in aqueous buffers of pH 1.2 followed by pH 6.5. Pharmacokinetic profiles revealed an approximately 1.95-fold increased bioavailability (p=0.02) and a 1.5-fold higher maximum plasma concentration (p=0.14) for Lipoceramic-IBU with reference to Nurofen®. Review of the safety assessments, including physical examinations, clinical laboratory tests and reports of adverse events, confirmed negligible acute side effects related to the administration of blank and ibuprofen-loaded Lipoceramic formulations. This first in man study of a dry lipid and nanoparticle-based formulation successfully demonstrated the safe use and effectiveness of the nanostructured Lipoceramic microparticles in mimicking the food effects for optimising the oral absorption of poorly water-soluble compounds.

Keywords Lipid-based formulations · Silica nanoparticles · Poorly water-soluble drugs · Human clinical trial · Bioavailability · Tolerability

Introduction

There has been substantial pre-clinical and clinical evidence that lipid and surfactant-based colloidal carriers are effective in mimicking the post-prandial solubilising effects of dietary fats for enhancing the oral bioavailability of drugs with limited aqueous solubility (i.e., dose/solubility ratio of >250 ml or water solubility of <100 μg/ml) [1–3]. At present, commercialised oral lipid-based formulations are mostly formulated based on liquid or semisolid lipid excipients. To facilitate unit dosing, these products are typically administered in the form of solutions (e.g., Natopherol® vitamin E and Advil Liqui-Gels® ibuprofen) or self-emulsifying pre-concentrates (e.g., Neoral® cyclosporine A and Norvir® ritonavir) encapsulated in soft or hard gelatin capsules [4, 5]. The technology of capsule liquid-filling is known to be associated with a number of practical and stability challenges, particularly high-cost and stringent process control, as well as possible leakage and partitioning of fill materials into the shell component [6]. As a more attractive strategy to confer manufacturing convenience and less demanding handling processes, transformation of liquid lipid precursors into solid forms (i.e., powders, granules, pellets) has recently formed a new frontier in the research area of lipid-based formulations [7, 8].

The conversion of liquid to solid forms often involves the use of a chemically inert solid carrier for adsorbing or encapsulating the liquid or semisolid lipid matrices prior to a drying process. Porous, silica-based adsorbents, such as Aerosil®

A. Tan (✉) · N. G. Eskandar · S. Rao · C. A. Prestidge (✉)
Ian Wark Research Institute, University of South Australia, Mawson Lakes Campus, Mawson Lakes, South Australia 5095, Australia
e-mail: angel.tan@unisa.edu.au
e-mail: clive.prestidge@unisa.edu.au

fumed silica, Syloid® micronised silica gels and Neusilin® magnesium aluminometasilicate, are among the most commonly used solid carriers for lipid colloids [9–13]. By virtue of their relatively high specific surface area (i.e., typically 100–400 m^2/g), these silica-based carriers potentially enable extremely high adsorption capacity of liquid lipids at more than twice the weight of the corresponding carriers. Advances in various solidification techniques has led to the emergence of various free-flowing, powdery lipid-based formulations suitable for hard capsule filling or tablet compression using conventional equipment [7, 14]. Although full mechanisms have yet to be elucidated in the aspect of controlling the adsorption/desorption of lipids loaded onto solid carriers, promising bioavailability enhancement assessed using animal models have been reported for a wide range of transformed solid-state lipid-based formulations. Some key examples are dry emulsions [15, 16], solid self-(micro/nano)emulsifying drug delivery systems [17, 18], and silica–lipid hybrid (Lipoceramic™) microparticles [9, 19]. Despite the fact that most of the solid carriers employed, including colloidal silica, are generally regarded as orally biocompatible with relatively lenient daily intake limits [20], the clinical acceptability of combined lipid and nanoparticle-based formulations remains to be evaluated.

To the best of our knowledge, the current work represents the first in human Phase I study to assess the oral tolerability and pharmacokinetics of a silica nanoparticle–lipid hybrid (Lipoceramic™) formulation encapsulating ibuprofen (IBU) as a model Biopharmaceutics Classification Scheme (BCS) Class II drug (i.e., poor solubility, high permeability). IBU, a propionic acid derivative (log $P \approx 4$, $pK_a \approx 4.4$), is a well-known non-steroidal anti-inflammatory drug (NSAID) that exhibits pH-dependent solubility in water [21]. The equilibrium solubility of IBU racemate in water (at 25 °C) has been reported to be ~46 μg/ml at pH 1.5, and this increases to >300 μg/ml above pH 7 [22]. For this property, IBU has been employed as an excellent model drug in lipid or polymer-based formulation research studies aiming for enhanced immediate release and solubilisation, as well as for enteric-targeted release or sustained absorption/efficacy. Examples of formulation approaches that have been evaluated in vivo include a multiple-unit polystyrene-based microparticle formulation [23], pegylated lipid-based nanocapsules [24], cubic phase nanoparticles [25] and a myriad of other in vitro investigations [26–28].

As a continuation to explore the bench-top to clinic translation of the Lipoceramic™ formulation technology, this pilot study was performed in 16 healthy volunteers with a primary aim of determining any toxicology concerns associated with the test Lipoceramic formulation, and secondarily to compare the oral pharmacokinetics of IBU when formulated as Lipoceramic-IBU microparticles in comparison with a reference commercial tablet (i.e., Nurofen®, Reckitt Benckiser). The powdery Lipoceramic microparticles have previously been well characterised for physicochemical and biopharmaceutical properties through a number of proof-of-concept studies using both acidic (e.g., indomethacin [29, 30]) and basic model drugs (e.g., celecoxib [19, 31, 32], albendazole [33]). A series of systematic oral bioavailability and efficacy studies in rats and dogs have revealed significantly increased oral drug bioavailability (by 2- to 6-fold) from the Lipoceramic formulations in comparison with pure drugs and simple lipid solutions under fasted and/or fed conditions. These Lipoceramic microparticle prototypes, featured by their highly porous and nanostructured internal matrices (with pore widths of 3–20 nm), effectively facilitate greater drug encapsulation efficiencies via solubilisation in lipids in conjunction with adsorption onto the amorphous silica matrices [33]. It has been shown feasible to enhance and control digestibility of lipid droplets embedded in the Lipoceramic microparticles by engineering them with different internal nanostructures based on silica nanoparticles of different porosity and sizes [34]. The Lipoceramic powder is different from conventional liquid/semisolid lipid-based formulations that the microparticles exhibited acceptable compressibility for tabletisation when further enhanced for their flowability and mechanical strength in the presence of silica nanoparticle–mannitol–lipid mixtures [35]. In this investigation, insights gained from the established fabrication and characterisation techniques were extrapolated towards the development of a clinical batch of Lipoceramic-IBU microparticle formulation for undertaking the Phase I study.

Materials and methods

Materials

Ibuprofen (USP grade) and soybean oil were purchased from Sigma-Aldrich (Australia). Captex 300 (glyceryl tricaprylate/tricaprate) and Capmul MCM (glyceryl caprylate/caprate) were generous gifts obtained from Abitech Corporation (USA). Soybean lecithin (>94 % phosphatidylcholine and <2 % triglycerides) was supplied by BDH Merck (Australia). Aerosil 380 fumed silica (with a primary average diameter of 7 nm and a specific surface area of 380 $m^2\ g^{-1}$) was obtained from Evonik Degussa (Germany). All other chemicals and reagents were of analytical grade and used as received. High-purity (Milli-Q) water was used throughout the study.

Preparation of Lipoceramic-IBU microparticles

The Lipoceramic-IBU microparticle formulation was prepared from submicron lipid emulsions consisting of medium chain triglycerides (MCT, Captex 300), medium chain mono/diglycerides (MG/DG mixture, Capmul MCM), and long chain triglycerides (LCT, soybean oil), using soybean lecithin

as an emulsifier. IBU was pre-dissolved in the lipid mixtures according to the equilibrium solubility pre-determined using the conventional shake-flask method (Table 1). Adopting the previously established method [31], the oil-in-water emulsions were first homogenised (Avestin EmulsiFlex-C5) under a pressure of 1,000 bar for five volume cycles, followed by stabilisation with dispersed silica nanoparticles for 12 h. The silica nanoparticle-stabilised emulsions were then spray-dried using a BÜCHI Mini Spray Dryer B-290 to produce powdery Lipoceramic-IBU microparticles under the following conditions: emulsion flow rate 6 ml/min, aspirator setting 100 %, air flow rate 0.6 m^3/min, inlet and outlet temperature 160 °C and 85 °C, respectively. Blank Lipoceramic microparticles were prepared in the same way without the addition of drug.

Physicochemical characterisation of Lipoceramic-IBU formulations

Solid-state imaging

The solid-state surface morphology of the Lipoceramic-IBU microparticles was examined by high resolution analytical scanning electron microscopy (SEM, CamScan CS44FE). A microparticle sample was mounted on double-faced adhesive tape and sputter-coated with gold/palladium (60 %:40 %) prior to imaging at an accelerating voltage of 10 kV, spot size 10 μm, and an aperture of 50 μm.

Redispersed properties

Particle sizes of the precursor lipid emulsions and the spray-dried Lipoceramic-IBU microparticles were characterised using dynamic light scattering (Malvern Zetasizer Nano ZS) and laser diffraction (Malvern Mastersizer 2000), respectively. Water (refractive index=1.33) was used as the dispersant. The particle refractive index was pre-set at 1.45 for lipids and silica particles. Zeta potentials were measured using phase analysis light scattering (Malvern Zetasizer Nano ZS) for the emulsions and microparticles redispersed in phosphate buffer (0.05 M, pH 7.2). The samples were diluted 100-fold with water prior to measurement at 25°C.

Table 1 Equilibrium solubility of ibuprofen in different oils at 25 °C

Types of lipids	IBU solubility (% w/w)
Medium chain MG/DG mixture	15.1
MCT	6.6
MCT + lecithin (1:0.05)	7.3
LCT	5.9

Lipid content

Lipid content of the Lipoceramic-IBU microparticles was determined by thermogravimetric analysis (Hi-Res Modulated TGA 2950, TA Instruments). Approximately 10 mg sample was heated from 25 °C to 600 °C at a scanning rate of 10 °C/min under nitrogen gas purging. The lipid content was evaporated in the range of 140–450 °C while the silica component remained thermally stable. After subtraction for the water content of spray-dried silica (determined to be 1.5±0.0 %) and the encapsulated drug content, the subtracted weight loss computed by using the TA Universal Analysis software corresponds to the lipid content of the microparticles.

Drug content

IBU content of the spray-dried Lipoceramic-IBU formulation and Nurofen® tablets (six tablets crushed with mortar and pestle) was determined by using solvent extraction and high-performance liquid chromatography (HPLC) analysis (Shimadzu UFLC-XR Prominence System). The encapsulated IBU was extracted by dissolving 10–15 mg of the formulation powder in 10 ml methanol (which has been shown to give >98 % extraction efficiencies), followed by centrifugation at 9,400×g for 10 min to remove the undissolved excipients. The supernatant was taken and diluted suitably with the mobile phase solution prior to HPLC quantification for IBU content. An isocratic elution method employing UV detection at 225 nm and a mobile phase consisting of 80:20 (v/v) methanol/water (containing 0.2 % glacial acetic acid) was performed at a flow rate of 1 ml/min using an Alltima™ reverse-phase C-18 analytical column (5 μm, 4.6 mm ID × 250 mm). Linear calibration curves (R^2=0.99) were plotted for chromatographic peak areas against concentrations of IBU over the range of 1–100 μg/ml with a retention time of approximately 5.9 min. The intra- and inter-day assay precision and accuracy were acceptable based on the relative standard deviations (i.e., <1 %) and percentage bias (i.e., <5 %), respectively.

The state of crystallinity of the IBU content was monitored by using a differential scanning calorimeter (TA Instruments Q100 DSC) calibrated based on an indium standard. Approximately 5 mg of each sample was heated in a hermetically sealed aluminium pan at a rate of 5 °C/min over a temperature range of 25–200 °C, under a flow of dry nitrogen gas at 70 ml/min.

Dissolution/solubilisation study

A two-step dissolution test (for pure IBU and the Nurofen® tablet materials) or dispersion/solubilisation study (for the Lipoceramic-IBU formulation) was performed in 0.1 N HCl (pH 1.2, 500 ml) for 1.5 h followed by adjustment with phosphate buffer to pH 6.5 (900 ml), using USP 23 type II

apparatus operating at 50 rpm. Each sample equivalent to 20 mg IBU was added into the dissolution medium maintained at 37±0.5 °C. Aliquots of 4 ml were drawn at fixed time points and replaced with an equal volume of blank dissolution medium. The drawn samples were centrifuged at 9,400×*g* for 15 min to remove undissolved materials and the supernatant was diluted with the mobile-phase solution prior to HPLC analysis as described previously.

Clinical study

Treatment protocol and procedure

This Phase I clinical trial was approved by the Bellberry Human Research Ethics Committee and was conducted at one clinical research facility (Clinical Medical and Analytical eXellence, Adelaide, Australia) in accordance with the Therapeutic Goods Administration (TGA) Note for Guidance on Good Clinical Practice, the ethical rules contained in the National Statement on Ethical Conduct in Human Research, and the Declaration of Helsinki (1964).

A randomised, double-blinded, one-period single oral dose study was performed in 16 clinically healthy men aged from 18 to 52 years under fasting conditions. The participants were selected based on the study inclusion/exclusion criteria and randomised into four treatment groups, each receiving one of the following formulations filled into a size AA hard gelatin capsule: (1) Lipoceramic-IBU equivalent to 20 mg IBU (n=6); (2) blank Lipoceramic microparticles (n=6); (3) Nurofen® tablet ground to fine powder equivalent to 20 mg IBU (n=2); and (4) blank placebo without any active ingredient and silica/lipid excipients (n=2). Each dose was swallowed whole with 240 ml of room temperature water following an overnight fast of at least 10 h. Each participant was instructed to abstain from fluid intake within 1 h before and 2 h after dosing; and fasting was continued for another 4 h following dosing. During the study period, each participant was kept seated upright for the first 4 h and prohibited from strenuous activity. Blood samples (8 ml) were collected via the cannulated arm vein at pre-dose (i.e., within 1 h prior to dosing), 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4 and 6 h following dose administration. The blood plasma samples were subjected to pharmacokinetic analysis using a validated HPLC assay method at TetraQ, University of Queensland (Brisbane, Australia).

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters were computed based on the standard non-compartmental model using WinNonlin version 5.2 software (Pharsight Corporation). The maximum observed plasma concentration (C_{max}) and the time at which C_{max} occurred (t_{max}) were obtained directly from the individual plasma concentration–time curves. The area under the plasma concentration versus time curve from time zero to the last measured concentration at 6 h ($AUC_{0\rightarrow 6\ h}$), calculated using the linear trapezoidal rule, was used to estimate the relative bioavailability (F_{rel}) of the test formulation with reference to that of the Nurofen® tablet materials:

$$F_{rel.} = \frac{AUC_{0\rightarrow 6\ h}(test)}{AUC_{0\rightarrow 6\ h}(reference)} \times \frac{Dose\ (reference)}{Dose\ (test)}$$

All values are expressed as the arithmetic mean ± standard deviation (SD) or alternatively, arithmetic mean ± error in the control groups comprised of duplicated data (i.e., n=2 in Nurofen® reference and blank placebo groups). Pharmacokinetic data of the test versus reference formulations were analysed statistically using a two-tailed, unpaired Student t-test. The level of statistical significance was set at $p<0.05$. The current study is limited by the low number of subjects included in the Nurofen® and placebo control groups, therefore statistical methods typically used in clinical trials or bioequivalence assessments (e.g., 90 % confidence interval approach) were not adopted.

Clinical examination

Physical examination and clinical laboratory tests, including biochemistry (e.g., sodium, potassium, glucose, urea), haematology (e.g., haemoglobin, red blood cell count, platelets, white cell count), HIV/HbsAg/HCV screening, urinalysis (e.g., pH, blood, ketone, glucose), urine drug screening and alcohol breath test, were performed during the pre-study screening phase and in the follow-up visit within 5–8 days after completion of the dosing period. During the study period, seated vital signs (i.e., blood pressure, radial pulse rate, aural temperature and respiratory rate) and electrocardiograms (ECGs) were monitored. Abnormal laboratory findings or clinically significant deviations from the baseline values were recorded as adverse events (AEs). Each participant was specifically questioned with regard to AEs. The nature, time of onset, duration and severity of all AEs reported were documented and assessed for the relationship to drug and/or formulation administration.

Results and discussion

Physicochemical properties of Lipoceramic-IBU microparticles

The Lipoceramic formulations were fabricated based on submicron lipid emulsions (282±8 nm) as the drug solubilising vehicles, and fumed silica nanoparticles (~50 nm) as an amorphous stabiliser and solid carrier. Following optimisation from several trial batches, a clinical batch of nanostructured

Lipoceramic microparticles encapsulating 5.6 % IBU and 58 % lipids (i.e., a mixture of Captex 300, Capmul MCM and soybean oil) was produced (Fig. 1). This is equivalent to approximately 80 % of the equilibrium solubility of IBU in the selected lipid mixtures and approximately 95 % of lipid encapsulation efficiency. Similar to previously reported observations [30–32], SEM examination confirmed that the spray-dried Lipoceramic-IBU microparticles were consisted of well separated, enclosed spherical structures. It is thus confirmed that the established silica/lipid mass ratio of 1:2 is effective in encapsulating lipid droplets of different acyl chain lengths, resulting in lipid load and encapsulation efficiency comparable to our previously reported values for Lipoceramic systems encapsulating only medium chain lipids (i.e., 42–62 % lipid load corresponding to 92–100 % encapsulation) [30–32]. Laser diffraction particle sizing and zeta potential measurements showed that the microparticles were readily redispersed in water to form negatively charged particles (average zeta potentials of negative 46±15 mV) with a median volumetric diameter of 15±9.6 μm. Owing to the presence of a long chain lipid (i.e., soybean oil), the clinical batch is relatively larger in the particle sizes as compared to previous prototypes prepared using medium chain lipids (typically <10 μm). While it remains challenging to establish a direct correlation between particle size and drug composition, it is generalised that the drug content of each microparticle is dependent on the drug solubility in the lipid phase, and the amount of lipid adsorbed onto the microparticles is likely to depend on the carrier porosity. The analysis of drug and lipid content for the current clinical and several trial batches have shown an acceptable range of deviations, which is within 5 % of the experimental average values. These composition analyses were performed in triplicates based on random sampling. Therefore, it can be inferred that the microparticles are homogenously distributed in the bulk sample.

Chemical stability of the Lipoceramic-IBU formulation was assessed based on its IBU content when stored at ambient conditions (i.e., in airtight glass vials at 25–30 °C). The Lipoceramic-IBU formulation was found to be well preserved with negligible changes in its composition levels over a period of 12 months (Fig. 2). DSC crystallinity analysis was performed for a control system containing the USP grade pure ibuprofen powder physically mixed (but not loaded) with silica particles to achieve 6 % content (equivalent to the Lipoceramic-IBU formulation), Nurofen® tablet materials and the Lipoceramic-IBU powder. The DSC thermograms show that the IBU–silica physical mixture as well as the reference Nurofen® tablet materials exhibited a clear melting peak at approximately 73 °C (Fig. 3). This endothermic peak, which corresponded to the presence of crystalline IBU, was not detected for the Lipoceramic-IBU formulation even when re-analysed at 12 months after preparation. It is thus confirmed that the drugs were retained in a molecularly dispersed state in the silica–lipid hybrid matrices, most likely attributed to the drug content below its equilibrium solubility in the selected lipids; it could also be the stabilising effect of silica nanoparticles in preserving the drug in its amorphous state via confinement in the nanostructured pores [36, 37].

The Lipoceramic microparticles could be handled as normal pharmaceutical powders to facilitate filling into hard gelatine capsules. Using a powder gun which compresses powder materials into pellets, a mass of up to 400 mg of Lipoceramic-IBU microparticles was fillable into a size AA hard gelatine capsule (volume capacity=0.94 ml, internal diameters=9.08 mm), which is commonly used in clinical trials and slightly larger than a size 00 capsule (volume capacity=0.95 ml, outer diameters=8.53 mm). This enabled feasible oral dosing of Lipoceramic-IBU formulation in the form of filled capsules for comparison with that of the pure drug and commercial tablet materials. The total mass of Lipoceramic formulation administered to the clinical study volunteers was approximately 355 mg for a 20-mg ibuprofen dose based on the current clinical batch encapsulating ~5.6 % IBU.

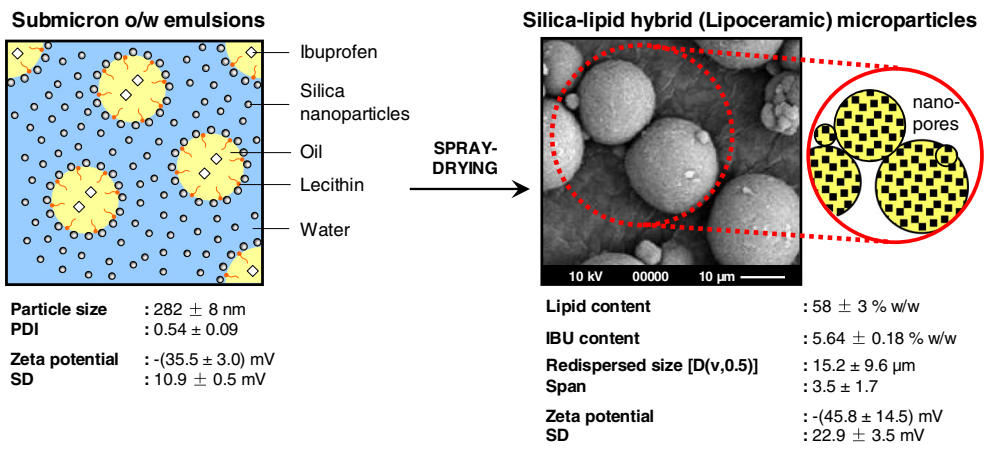

Fig. 1 Formation of silica–lipid hybrid (Lipoceramic) microparticles with internal nanoporous matrix structures from submicron o/w emulsions stabilised by dispersion of silica nanoparticles via spray-drying (n=3, mean ± SD)

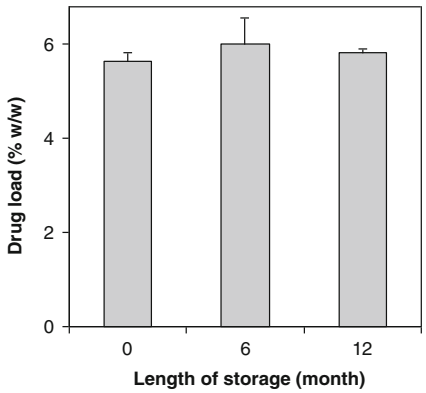

Fig. 2 Drug content of Lipoceramic-ibuprofen microparticles stored in transparent, airtight glass vials at ambient conditions (25–30 °C) for up to 12 months (n=3, mean ± SD)

Solubilisation properties of Lipoceramic-IBU microparticles

Compendial dissolution (or solubilisation) tests, as officially adopted by the Food and Drug Administration since 1970 [38], were employed as a pre-clinical in vitro tool for probing the biopharmaceutical performance of the Lipoceramic-IBU formulation in comparison with Nurofen® tablet materials and the pure drug. Initially, the test was performed at a dose of 20 mg IBU in 500 ml of the USP recommended medium, i.e., 0.05 M phosphate buffer (pH 7.2) at a paddle rate of 50 rpm. This dissolution method demonstrated comparable solubilisation profiles among the three investigated systems, including pure IBU, Nurofen® tablet materials and Lipoceramic-IBU microparticles, with >90 % drug solubilisation achieved within the first 5 min. Although meeting the immediate release criterion for IBU tablets, specifically not less than 80 % of the labelled amount of IBU dissolved in 60 min, the solubilisation outcome was poorly discriminating mainly ascribable to the high solubility of IBU at pH of 7 and above.

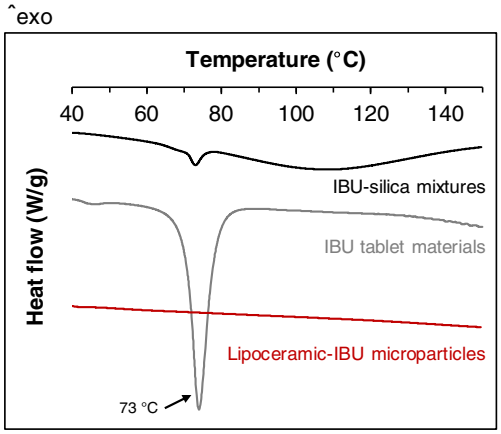

Fig. 3 Differential scanning calorimetry (*DSC*) thermograms of physical mixtures of ibuprofen (*IBU*) and silica nanoparticles, reference IBU tablet materials (Nurofen®), and Lipoceramic-IBU microparticles (drug contents are approximately 6 %, 44 %, and 6 %, respectively)

In order to better simulate the gastrointestinal transit, a stepwise dissolution (or solubilisation) test was performed commencing from acidic environment in which IBU exists in the unionised and poorly soluble form, followed by more solubilising intestinal pH conditions. This demonstrated distinguishable dissolution kinetics where the Lipoceramic-IBU microparticles produced higher rate and degree of drug solubilisation in comparison with the tablet material and pure drug, particularly within the first hour under acidic environment (Fig. 4). The major contributing factor for the superior solubilisation level is that IBU was fully pre-dissolved or molecularly dispersed within the Lipoceramic matrices as previously confirmed by the DSC analysis. Encapsulation in the solubilised form inherently eliminates the steps of wetting and solid-to-liquid dissolution transition in the stomach fluids, i.e., the biggest obstacle to the absorption of acidic drugs such as IBU. The relatively incomplete drug solubilisation or dispersion in the acidic fluids as observed for the Lipoceramic-IBU formulation is likely to result from preferential drug partitioning in the lipid cores; when the aqueous medium was adjusted to pH 6.5, immediate burst drug partition to the aqueous phase was observed mainly due to increased ionisation degree of the drug molecules (pK_a≈4.4). Correlation between the solubilisation profiles and the in vivo pharmacokinetic properties is further elucidated in the following section.

Oral pharmacokinetics of Lipoceramic-IBU microparticles

All 16 recruited subjects completed the clinical study and are included in the pharmacokinetics evaluation of IBU at a single oral dose of 20 mg in the form of filled hard capsules. The dosing regimen was chosen on the basis that 20 mg IBU (rather than the typical therapeutic range of 200–400 mg in a single oral dose) would allow adequate pharmacokinetics determination while minimising its interference with the assessment of AEs related to the formulation excipients under fasting conditions. The mean plasma concentration versus time profiles of each investigated formulation is presented in Fig. 5. The corresponding non-compartmental pharmacokinetic data are summarised in Table 2.

Overall, the relative bioavailability ($AUC_{0\rightarrow 6\ h}$) of the Lipoceramic-IBU treatment group was computed to be 1.95-fold greater than the Nurofen® reference group (p=0.02). Subjects who received the test Lipoceramic-IBU formulation demonstrated a relatively rapid, early absorption phase (t_{max}=1.2±0.9 h) with prolonged plasma concentrations of IBU in comparison to the reference group (t_{max}=2.0±1.0 h), although statistical significance is limited due to the low number of subjects included and large variation in the reference group data. Throughout the 6-h study period, Lipoceramic-IBU treatment group appeared to display higher plasma levels of IBU (C_{max}=2.79±0.77 μg/ml) as compared to the reference group (C_{max}=1.81±0.28 μg/ml) (p=0.14). It is of note that ten

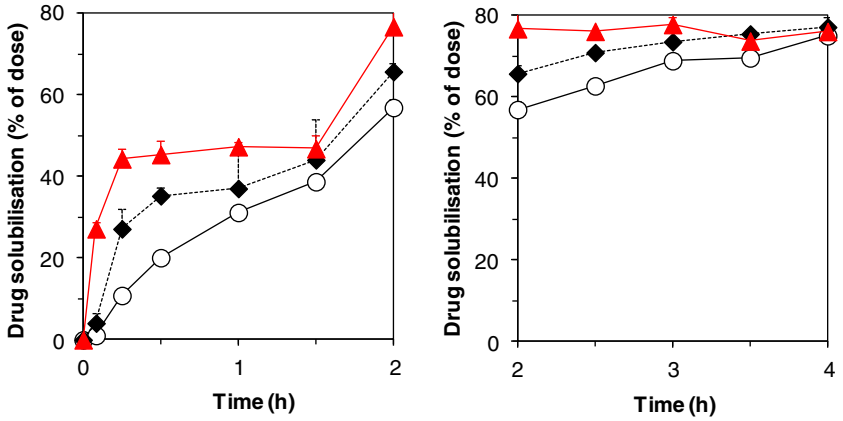

Fig. 4 Mean dissolution/solubilisation profiles of 20 mg ibuprofen in 0.1 N HCl (pH 1.2, 500 ml) for the first 1.5 h (*left*), followed by adjustment to pH 6.5 (900 ml) with phosphate buffer (*right*) at 37 °C: Pure IBU (*empty circle*), reference IBU tablet materials, Nurofen® (*filled diamond*) and Lipoceramic-IBU microparticles (*red filled triangle*) (n=3, mean ± SD)

of 16 pre-dose levels (including the placebo group) had shown detectable IBU peaks but at concentrations below the limit of quantification (i.e., <0.01 μg/ml). The detectable pre-dose 'IBU content' was likely to result from the analytical background interference or from endogenous plasma contents. The interference did not show a clear trend in any particular group. Thus, the influence of the low pre-dose IBU levels is regarded as minimal in affecting the bioavailability comparison across the treatment and reference groups.

As a further evidence to previous pre-clinical studies using rodent and dog models [19, 29–32], the current human clinical trial successfully demonstrated the feasibility of the Lipoceramic formulation in enhancing the oral pharmacokinetics of IBU, a weak acid BCS Class II drug. Weak acid compounds are predominantly unionised under gastric conditions and prone to precipitation before reaching the more solubilising duodenal environment. The major concept by which the Lipoceramic (silica–lipid hybrid) formulation works for IBU is based on preservation of the lipid–drug solubilised phases in a solid-state, nanoporous network composed of hydrophilic silica nanoparticles (Fig. 6). Such a nanoporous matrix is endowed with relatively high specific surface area which plays an important role in facilitating dispersion and release of the oil droplets and drugs into the aqueous surroundings. It is plausible that the nanoporous carriers have an effect in minimising drug re-crystallisation or precipitation based on the fact that porous silica adsorbents have been commonly used for direct loading of fully dissolved drugs via physical adsorption, and that drug re-adsorption from solution to the dispersed porous silica carriers is not impossible although the re-adsorption affinity may be quite low (i.e., less than 4 % as reported for danazol) [39]. According to the pre-determined lipid solubility data (Table 1), it is envisaged that post-digestive lipid products (particularly MG/DG) are efficient in creating a increasingly lipophilic microenvironment in the GI lumen to sustain IBU solubilisation, thereby promoting greater and prolonged absorption in comparison to the crystalline tablet product.

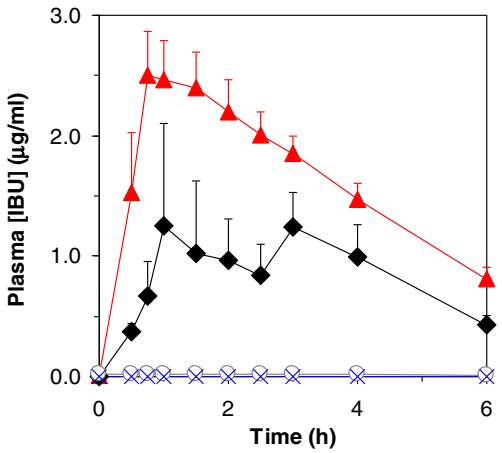

Fig. 5 Plasma concentration versus time profiles of ibuprofen in healthy, male subjects following a single oral administration of various formulations filled in hard gelatine capsules equivalent to 20 mg ibuprofen: Lipoceramic-IBU microparticles (n=6, *red filled triangle*), reference IBU tablet materials, Nurofen® (n=2, *filled diamond*), blank Lipoceramic microparticles (n=6, *empty circle*) and placebo (n=2, ×) (mean ± SD)

Table 2 Non-compartmental pharmacokinetic parameters of ibuprofen following a single oral administration of various formulations to 16 healthy, male human subjects at a dose of 20 mg ibuprofen under fasting conditions (mean ± SD)

PK parameters	IBU formulations		
	Test: Lipoceramic-IBU	Reference: IBU tablets	Placebo: Lipoceramic-blank
t_{max} (h)	1.2±0.9	2.0±1.0	NA
C_{max} (μg/ml)	2.79±0.77	1.81±0.28	0.02±0.02
$AUC_{0\rightarrow 6\ h}$ (h. μg/ml)	9.84±2.08*	5.04±0.46	0.11±0.13
F_{rel} (%)	195*	100	NA

*p<0.05 when compared to the reference group

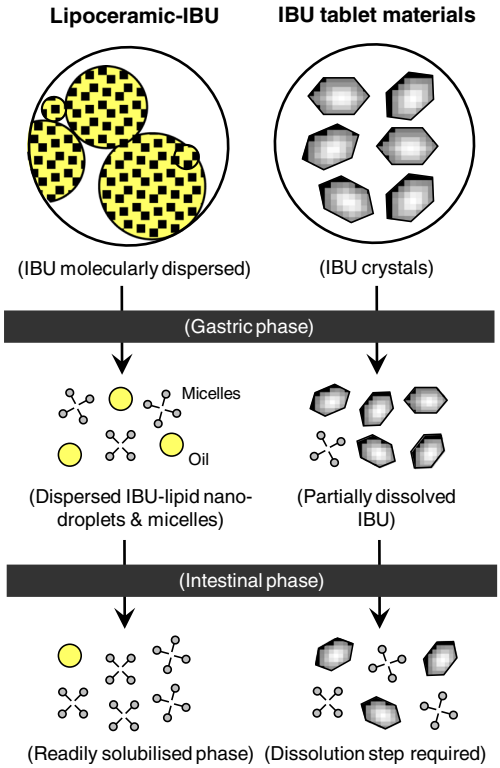

Fig. 6 Schematic representation of possible gastrointestinal fate of ibuprofen when formulated as the Lipoceramic-IBU (solubilised) microparticles and the conventional (crystalline) tablet (not drawn to scale). Formation of mixed micelles from the endogenous and exogenous lipids is critical towards adequate solubilisation of ibuprofen molecules prior to absorption

Given that the current loading content of IBU is mainly limited by its solubility in the lipid phase, the clinical applicability of the Lipoceramic formulation technology may be limited to high potency drugs that are prescribed in low doses. However, one could also infer from the pharmacokinetic results that the Lipoceramic formulation has produced an approximately 2-times higher drug bioavailability than the commercial capsules; therefore, it is possible to significantly reduce the administration dose of a drug with the Lipoceramic encapsulation technology (assuming that there is a proportional correlation between the bioavailability and efficacy).

Oral tolerability of Lipoceramic-IBU microparticles

This Phase I study essentially demonstrated the safety and tolerability of the Lipoceramic formulation for administration as a single oral dose. Each formulation component employed, including lipids (Captex 300, Capmul MCM and soybean oil), emulsifier (soybean lecithin), and colloidal silica (Aerosil silica nanoparticles), is classed as non-hazardous substance by the Occupational Safety and Health Administration (OSHA). These materials are permitted for use as additives in food processing in addition to pharmaceutical use as inactive ingredients by the Australian TGA. Each excipient was incorporated at a dose of at least 100-fold below the allowed daily quantities while colloidal silica particles are generally regarded as safe for oral administration without specific restriction on the daily oral dose [20].

Review of the frequency, severity, and relationship of the AEs documented shows no trends related to the investigational formulations. Overall, there was no report of any serious AEs. A total of three AEs were reported during the study period by two subjects randomised to the placebo group (i.e., 12.5 % of the total number of subjects) (Table 3). On the day of dosing, one subject experienced mild, transient vasovagal presyncope which was resolved in the first hour after the placebo dosing without any medical intervention. This AE was considered by the medical investigator to be unlikely related to the placebo product. During the follow-up visit performed within 5–8 days after completion of the dosing period, the same subject has reportedly contracted mild viral pharyngitis which was resolved in the next 5 days; another subject who administered the same placebo capsule had experienced moderate toothache for a day. These two AEs were evaluated to be unrelated to the placebo product. All physical examination, ECG monitoring and clinical laboratory tests

Table 3 Number and severity of reported adverse events (AEs) following a single oral administration of various formulations to 16 healthy, male human subjects at a dose of 20 mg ibuprofen under fasting conditions

Adverse events (AEs)	IBU formulations			
	Test: Lipoceramic-IBU (n=6)	Test: Lipoceramic-blank (n=6)	Reference: IBU tablets (n=2)	Placebo: Blank capsules (n=2)
Subjects with at least one AE	0	0	0	2
Nervous system disorders Vasovagal presyncope	0	0	0	1 (mild)
Infections and infestations Viral pharyngitis	0	0	0	1 (mild)
Gastrointestinal disorders Toothache	0	0	0	1 (moderate)

have shown negligible deviations from the individual baseline values. Taken together, the tolerability data have supported the safe use of Lipoceramic microparticles for the oral administration of IBU and placebo in healthy human subjects under fasting conditions, with negligible acute adverse effects or toxicology concerns. This provides an impetus for a multiple dose study which may provide further insights into the longer term safety of the Lipoceramic formulation.

Conclusion

This Phase I clinical trial provides important insight into the effectiveness and tolerability of a powderised lipid-based formulation, specifically silica nanoparticle–lipid hybrid (Lipoceramic) microparticles, for the oral delivery of poorly water-soluble drugs. Using ibuprofen (a weak acid) as a model BCS Class II drug, the Lipoceramic formulation (composed of a mixture of medium and long chain lipids) was shown to enhanced drug dispersion and solubilisation in comparison with a commercial tablet product (Nurofen®) and the pure drug powder in a sequential gastric-to-intestinal dissolution (or solubilisation) study. It was further evidenced in the randomised, single oral dose study performed under fasting conditions that the Lipoceramic formulation effectively increased the oral bioavailability of ibuprofen to 1.95-fold greater than the reference crystalline tablet product. Comprehensive review of the physical examination, clinical laboratory tests and reports of AEs essentially supported the tolerable use of the nanostructured Lipoceramic microparticles for oral drug administration in healthy human subjects. The current investigation clearly elucidated the food-mimicking solubilisation effects of the Lipoceramic microparticles in promoting more complete drug absorption with negligible acute adverse effects, and potentially fosters the likelihood of bench-to-clinic translation of a powderised lipid-based formulation.

Acknowledgments The authors gratefully acknowledge the Australian Research Council, the Australian National Health and Medical Research Council, ITEK Pty. Ltd., Bioinnovation South Australia, and the Australian Biotech Ceridia Pty. Ltd. for research funding and support. Specifically, Gregor Rossenberg, Sepehr Shakib and Mark Bruce are acknowledged for assistance and support in undertaking the human clinical trial.

Conflict of interest disclosure The authors report no conflicts of interest in this work. This study was approved by the Bellberry Human Research Ethics Committee and written informed consent was legally obtained from all subjects.

References

1. Dressman JB, Lennernas H. Oral drug absorption: Prediction and asessment. New York: Marcel Dekker; 2000.
2. Porter CJH, Trevaskis NL, Charman WN. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov. 2007;6:231–48.
3. Fatouros DG, Karpf DM, Nielsen FS, Mullertz A. Clinical studies with oral lipid based formulations of poorly soluble compounds. Ther Clin Risk Manag. 2007;3:591–604.
4. Hauss DJ. Oral lipid-based formulations: Enhancing the bioavailability of poorly water-soluble drugs. New York: Informa Healthcare USA; 2007.
5. Marchaud D, Hughes S. Solid dosage forms from self-emulsifying lipidic formulations. Pharm Tech Eur. 2008;20:46–9.
6. Cole ET, Cadé D, Benameur H. Challenges and opportunities in the encapsulation of liquid and semi-solid formulations into capsules for oral administration. Adv Drug Deliv Rev. 2008;60:747–56.
7. Jannin V, Musakhanian J, Marchaud D. Approaches for the development of solid and semi-solid lipid-based formulations. Adv Drug Deliv Rev. 2008;60:734–46.
8. Nokhodchi A, Hentzschel CM, Leopold CS. Drug release from liquisolid systems: speed it up, slow it down. Expert Opin Drug Deliv. 2011;8:191–205.
9. Simovic S, Barnes TJ, Tan A, Prestidge CA. Assembling nanoparticle coatings to improve the drug delivery performance of lipid based colloids. Nanoscale. 2012;4:1220–30.
10. Wang Z, Sun J, Wang Y, Liu X, Liu Y, Fu Q, et al. Solid self-emulsifying nitrendipine pellets: preparation and in vitro/in vivo evaluation. Int J Pharm. 2010;383:1–6.
11. Sander C, Holm P. Porous magnesium aluminometasilicate tablets as carrier of a cyclosporine self-emulsifying formulation. AAPS PharmSciTech. 2009;10:1388–95.
12. Janga KY, Jukanti R, Sunkavalli S, Velpula A, Bandari S, Kandadi P, et al. In situ absorption and relative bioavailability studies of zaleplon loaded self-nanoemulsifying powders. J Microencapsul. 2013;30:161–72.
13. Hentzschel CM, Sakmann A, Leopold CS. Suitability of various excipients as carrier and coating materials for liquisolid compacts. Drug Dev Ind Pharm. 2011;37:1200–7.
14. Shukla D, Chakraborty S, Singh S, Mishra B. Lipid-based oral multiparticulate formulations — advantages, technological advances and industrial applications. Expert Opin Drug Deliv. 2011;8:207–24.
15. Jang DJ, Jeong EJ, Lee HM, Kim BC, Lim SJ, Kim CK. Improvement of bioavailability and photostability of amlodipine using redispersible dry emulsion. Eur J Pharm Sci. 2006;28:405–11.
16. Hamoudi MC, Bourasset F, Domergue-Dupont V, Gueutin C, Nicolas V, Fattal E, et al. Formulations based on alpha cyclodextrin and soybean oil: an approach to modulate the oral release of lipophilic drugs. J Control Release. 2012;161:861–7.
17. Yi T, Wan J, Xu H, Yang X. A new solid self-microemulsifying formulation prepared by spray-drying to improve the oral bioavailability of poorly water soluble drugs. Eur J Pharm Biopharm. 2008;70:439–44.
18. Hu X, Lin C, Chen D, Zhang J, Liu Z, Wu W, et al. Sirolimus solid self-microemulsifying pellets: formulation development, characterization and bioavailability evaluation. Int J Pharm. 2012;438:123–33.
19. Nguyen T-H, Tan A, Santos L, Ngo D, Edwards GA, Porter CJH, et al. Silica–lipid hybrid (SLH) formulations enhance the oral bioavailability and efficacy of celecoxib: an in vivo evaluation. J Control Release. 2013;167:85–91.
20. Rowe R. Handbook of pharmaceutical excipients. 7th ed. London: Pharmaceutical Press; 2006.
21. Moffat AC. Clarke's analysis of drugs and poisons. 4th ed. London: Pharmaceutical Press; 2011.
22. Potthast H, Dressman JB, Junginger HE, Midha KK, Oeser H, Shah VP, et al. Biowaiver monographs for immediate release solid oral dosage forms: ibuprofen. J Pharm Sci. 2005;94:2121–31.

23. Tamilvanan S, Sa B. In vitro and in vivo evaluation of single-unit commercial conventional tablet and sustained-release capsules compared with multiple-unit polystyrene microparticle dosage forms of ibuprofen. AAPS PharmSciTech. 2006;7:E126–34.
24. Lamprecht A, Saumet J-L, Roux J, Benoit J-P. Lipid nanocarriers as drug delivery system for ibuprofen in pain treatment. Int J Pharm. 2004;278:407–14.
25. Dian L, Yang Z, Li F, Wang Z, Pan X, Peng X, et al. Cubic phase nanoparticles for sustained release of ibuprofen: formulation, characterization, and enhanced bioavailability study. Int J Nanomedicine. 2013;8:845–54.
26. Perge L, Robitzer M, Guillemot C, Devoisselle J-M, Quignard F, Legrand P. New solid lipid microparticles for controlled ibuprofen release: formulation and characterization study. Int J Pharm. 2012;422:59–67.
27. Kang MJ, Jung SY, Song WH, Park JS, Choi S-U, Oh KT, et al. Immediate release of ibuprofen from Fujicalin®-based fast-dissolving self-emulsifying tablets. Drug Dev Ind Pharm. 2011;37:1298–305.
28. Qiao M, Luo Y, Zhang L, Ma Y, Stephenson TS, Zhu J. Sustained release coating of tablets with Eudragit® RS/RL using a novel electrostatic dry powder coating process. Int J Pharm. 2010;399:37–43.
29. Simovic S, Hui H, Song Y, Davey AK, Rades T, Prestidge CA. An oral delivery system for indomethicin engineered from cationic lipid emulsions and silica nanoparticles. J Control Release. 2010;143:367–73.
30. Simovic S, Heard P, Hui H, Song Y, Peddie F, Davey AK, et al. Dry hybrid lipid–silica microcapsules engineered from submicron lipid droplets and nanoparticles as a novel delivery system for poorly soluble drugs. Mol Pharm. 2009;6:861–72.
31. Tan A, Simovic S, Davey AK, Rades T, Prestidge CA. Silica–lipid hybrid (SLH) microcapsules: a novel oral delivery system for poorly soluble drugs. J Control Release. 2009;134:62–70.
32. Tan A, Davey AK, Prestidge CA. Silica–lipid hybrid (SLH) versus non-lipid formulations for optimising the dose-dependent oral absorption of celecoxib. Pharm Res. 2011;28:2273–87.
33. Tan A, Prestidge C. Nanostructured silica–lipid hybrid microparticles: a supersaturating carrier for water- and lipid-resistant compounds. Chem Lett. 2012;41:1334–6.
34. Tan A, Martin A, Nguyen T-H, Boyd BJ, Prestidge CA. Hybrid nanomaterials that mimic the food effect: controlling enzymatic digestion for enhanced oral drug absorption. Angew Chem Int Ed. 2012;51:5475–9.
35. Bremmell KE, Tan A, Martin A, Prestidge CA. Tableting lipid-based formulations for oral drug delivery: a case study with silica nanoparticle–lipid–mannitol hybrid microparticles. J Pharm Sci. 2012;102:684–93.
36. Sanganwar GP, Gupta RB. Dissolution-rate enhancement of fenofibrate by adsorption onto silica using supercritical carbon dioxide. Int J Pharm. 2008;360:213–8.
37. Shen S-C, Ng WK, Chia L, Hu J, Tan RBH. Physical state and dissolution of ibuprofen formulated by co-spray drying with mesoporous silica: effect of pore and particle size. Int J Pharm. 2011;410:188–95.
38. Adeyeye MC, Brittain HG. Preformulation in solid dosage form development. New York: Informa Healthcare; 2008.
39. Van Speybroeck M, Williams HD, Nguyen T-H, Anby MU, Porter CJH, Augustijns P. Incomplete desorption of liquid excipients reduces the in vitro and in vivo performance of self-emulsifying drug delivery systems solidified by adsorption onto an inorganic mesoporous carrier. Mol Pharm. 2012;9:2750–60.