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ORIGINAL ARTICLE

Particle size reduction for improvement of oral absorption of the poorly soluble drug UG558 in rats during early development

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Abstract

Background: The exposure of UG558 was not good enough using traditional microsuspensions. *Aim*: The aim of this study was to find out whether nanosuspensions were a better choice compared with a microsuspension, for an acidic substance with a water solubility in the order of 2 μM (pH 6.8, small intestinal pH) and no permeability limitations. *Methods*: UG558 was ground by a planetary ball mill. The particle size was measured by laser diffraction and the stability of the particle sizes was followed. The pharmacokinetic parameters of UG558 administered as nanosuspension have been compared with those from microsuspension using rat as in vivo specie. Both formulations were administered orally. The nanosuspension was also administered intravenously. *Results*: The particle size of the nanosuspensions was about 190 nm and about 12 μm for the microsuspensions. At the administered doses, solutions were no alternative (e.g. due to limited solubility). Already at the lowest dose, 5 μmol/kg (5 ml/kg), a significant difference was observed between the two suspensions. These results were further confirmed at a high dose (500 μmol/kg, 5 mL/kg). Thus, the study demonstrated a clear correlation between particle size and in vivo exposures, where the nanosuspensions provided the highest exposure. Furthermore, no adverse events were observed for the substance nor the nanosuspension formulations (i.e., the particles) in spite of the higher exposures obtained with the nanoparticles. To make it possible to calculate the bioavailability, 5 μmol/kg doses of the nanosuspensions (5 ml/kg) were also administered intravenously. No adverse events were observed. *Conclusions*: The nanoparticles have a larger surface, resulting in faster *in vivo* dissolution rate, faster absorption, and increased bioavailability, compared to microparticles. The lower overall bioavailability observed at the high dose, compared with the low dose, was due to a combination of low dissolution rate, low solubility, and a narrow intestinal absorption window for UG558.

Key words: *Dissolution rate; nanosuspension; pharmacokinetic; poorly soluble; suspension*

Introduction

A significant proportion of drugs on the market are poorly soluble in water, and it is expected that this will be even more pronounced in the future[1,2]. Formulations of poorly water-soluble compounds are a resource demanding challenge. During the discovery phase, new compounds are evaluated by both in vitro and in vivo studies, in which liquid formulations are used frequently. Poorly soluble compounds can be formulated, for example, as aqueous pH-shifted solutions, provided the molecules are ionizable, in mixtures of water and organic cosolvents, or by solubilization in cyclodextrins[3–5] or using emulsions[6–8]. With the exception of the pH-shifted aqueous solutions, significant amounts of additives are often needed to increase the solubility into the millimolar range, required for most animal studies, which may induce unwanted side effects[9,10]. It would be more desirable to have a universal formulation approach to process any poorly soluble drug. This is of particular interest for drugs being poorly soluble in aqueous media and simultaneously in organic media,

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thus excluding all formulation approaches involving any solvent mixture. A classical formulation approach for such poorly soluble drugs is micronization, where a coarse drug powder is milled to an ultra fine powder with a mean particle size being typically in the range of 1–10 μm[11–15]. The principle is to increase the dissolution velocity by enlarging the surface area of the drug powder. Micronization is a technology mainly for class II drugs of the biopharmaceutical classification system (BCS), that is, drugs having a good permeability and poor solubility in the gastrointestinal tract[16–18]. The consequence of these properties may be low dissolution rate followed by low oral bioavailability. One example of size reduction in the micrometer scale is griseofulvin[19,20], where a reduction of particle size from about 4 to about 1 μm doubled the absorbed amount of drug in humans. Nowadays, many of the new drugs exhibit so low solubility and slow dissolution rate that micronization does not lead to a sufficiently high bioavailability. Consequently, the next step was taken to move from micronization to nanometer-sized particles, which means producing drug nanocrystals[21–23] (typically between 200 and 500 nm). This was, originally, done by wet milling using danazol, a neutral compound[21] (i.e., with no pK_as). The relative bioavailability of danazol in dogs increased more than 10 times when the particle size was reduced from 10 μm to 169 nm.

Solid drugs, for example, particles in suspensions, need to dissolve before they can be absorbed. The dissolution of drugs can be described, interpreted, and discussed in terms of the Noyes–Whitney equation[24]. This equation describes the rate of dissolution of spherical particles when the dissolution process is diffusion controlled and involves no chemical reaction.

$$\frac{dC}{dt} = \frac{DA(C_s - C)}{h},$$

where dC/dt is the rate of dissolution of the drug particles, D is the diffusion coefficient of the drug in the gastrointestinal fluids, A is the effective surface area of the drug particles in contact with the gastrointestinal fluids, h is the thickness of the diffusion layer around each drug particle, C_s is the saturation solubility of the drug in solution in the diffusion layer, and C is the concentration of the drug in the gastrointestinal fluids.

Parameters that can influence the dissolution rate can be divided into two categories: physiological and physicochemical parameters. In the first category falls properties such as pH, buffer capacity, food components, bile salts and surfactants present, viscosity in the gastrointestinal tract, motility patterns, and permeability, which all will affect some of the components in the Noyes–Whitney equation. The present substance, UG558, has no permeability limitations, which is a fundamental criteria for a class II substance in BCS. According to the Noyes–Whitney equation, a low value of C will favor more rapid dissolution of the drug by increasing the value of $(C_s - C)$. For a BCS II compound, C is normally kept very low by fast absorption (because of high permeability) of the drug.

In the second category, dealing with physicochemical properties of the substance that can influence the dissolution rate, parameters like wettability, solubility, physical form of the compound (polymorphs, neutral form or salt, crystalline material or amorphous, and solvate or not), and particle size will effect the Noyes–Whitney equation. In this article, we focus on surface area and particle size. An increase in A, which is in contact with the gastrointestinal fluids, will cause an increase in dissolution rate. Hence, smaller particle size resulting in an increased A will be followed by higher dissolution rate. Particle size reduction is thus likely to result in increased bioavailability, provided the absorption of the drug is dissolution-rate limited.

UG558 was discovered and synthesized in-house and was effective in the cardiovascular area. Despite promising early efficacy findings, the physical properties of the free form of the compound were not suited for dose escalation to the desired exposure, using simple liquid formulations. During early discovery research, different cosolvent mixtures were used for the in vivo experiments. However, the solubility in these vehicles was too low for safety assessment studies. Moreover, the amounts of cosolvents were high, which made the vehicles unattractive for long-time studies. The same conclusion was drawn for hydroxypropyl (HP)-β-cyclodextrin. Then the choice was a hydroxypropyl methylcellulose (HPMC) microsuspension, which was well tolerated and had the advantage of low vehicle contribution in the administered doses. One obvious drawback with a HPMC suspension is that a separate formulation must be developed for intravenous (i.v.) administration. Besides, with a permeability classified as high in the Caco-2 cell model and a solubility of about 2 μM in a pH 6.8 buffer, one will expect solubility and dissolution rate limitations at a certain concentration, which will cause an exposure limitation and variation. In such a case, nanosuspension is an attractive alternative, expected to increase the dissolution rate and the bioavailability.

In this article, a comparison was made between a crystalline nanosuspension and a microsuspension of UG558 at two doses administered to rats. The comparison was made to find a suitable formulation, which was expected to give a high exposure after administering high doses in toxicological studies. Besides, there was an economic aspect involved too. Less amount of the compound in the formulation results in lower cost of goods. The compound has high permeability and low solubility in the gastrointestinal tract, thus fulfilling the criteria for a BCS II compound and hence chosen for this study.

Material and methods

Test compound

UG558 has a molecule mass of 450 g/mol. The substance is a crystalline compound with a melting point of about 260°C. The pK_a was calculated to 4.7 (acidic pK_a) and log P to 5. The solubility in a water solution is about 2 μM at 25°C (measured from solid crystals, pH 6.8). The P_{app} value in the Caco-2 experiment was $>20 \times 10^{-6}$ cm/s at low μM concentrations with no indication of efflux. UG558 is a typical BCS II compound, that is, a drug having good permeability, but a low solubility, making it an attractive candidate for particle size reduction before administration.

Chemicals

HPMC (15000 cP) was bought from Shin-Etsu Chemicals (Tokyo, Japan). Polyvinylpyrrolidone K30 (PVP), is a nonionic polymer, which was bought from BASF (Ludwigshafen, Germany). It is a stabilizer and is expected to cover the surface of the pure drug when dispersed in water[25,26]. The disodium salt of Aerosol OT from Cytec Industries Inc (Woodland Park, NJ, USA). is a surface-active agent with similar function as PVP. Mannitol was bought from Sigma and used as a tonicity modifier.

Preparation of microsuspensions

Drug substance was weighed into a sample vial and a stabilizer solution of 0.5% (w/w) HPMC was added. The slurry obtained was treated with ultrasound for 10 minutes and stirred overnight. The volume-averaged particle size (diameter) of the suspensions was measured by laser diffraction (Malvern Mastersizer 2000).

Preparation of crystalline nanosuspensions

Typically, about 60 mg of the drug was weighed and brought into a 4-mL vial together with 510 μL stabilizer solution of 1.33% PVP/0.066% Aerosol OT in water. The 10% (w/w)-crude suspension was stirred and treated with ultrasonic for 10 minutes, which gave a well-dispersed slurry. The slurry (510 μL) was added to a milling vessel (1.2 mL) together with 2.4 g milling beads (0.6–0.8 mm) of zirconium oxide. The vessel was sealed and the slurry milled at 700 rpm, 4×30 minutes with intermediate pauses of 15 minutes, using the Fritsch Planetary Micromill P7. The milled suspension was collected, and the milling beads were rinsed with water. The volume-averaged particle size (diameter) of the crystalline suspensions was measured by laser diffraction (Malvern Mastersizer 2000). The suspension was diluted with or without 5% mannitol.

Formulation analysis

An HPLC gradient method was used for LC purity. This method used a reverse phase amide column and a water/acetonitrile mobile phase with trifluoroacetic acid.

Animal handling

The test system consisted of female Sprague–Dawley rats (Harlan, the Netherlands), approximately 11 weeks old on the day of arrival at AstraZeneca R&D Mölndal. After arrival, the rats were allowed to acclimatize for at least 1 week before surgery. The rats were housed in plastic rat cages (two animals/cage during acclimatization) with aspen wood chips (Tap-Vei, Kortteinen Kaawi, Finland) as bedding material. They were kept at room temperature, 18–22°C, and at a relative humidity of 30–60% during a 12-hour light/dark cycle and had free access to food (R3; Lactamin AB, Vadstena, Sweden) and tap water. The weight of the rats was 200–240 g. All animals were killed, by an overdose of pentobarbital sodium, after the last blood sample had been collected.

Surgery

Two days prior to dosing, the rats were prepared by cannulation of the left carotid artery for blood sampling. The jugular vein was cannulated for i.v. dosing. The cannulas were filled with heparin (100 IU/mL) and were exteriorized at the nape of the neck and sealed. The surgery (for implantation of the cannulas) was performed during isoflurane (Forene®; Abbott, Scandinavia, Solna, Sweden) anesthesia. The rats were given 0.5 mL/kg Romefen®Vet (ketoprofen 10 mg/mL; Merial, Lyon, France) subcutaneously before surgery and 10 mL Rehydrex®Med (glucose 25 mg/mL; Fresenius Kabi AB, Uppsak, Sweden) subcutaneously after surgery.

Postsurgery

The animals were housed individually and left to recover until administration of the test compound. Food was replaced with drinkable Rehydrex®Med (glucose 25 mg/mL; Fresenius Kabi AB) 16 hours before dose administration until 4 hours after dosing.

Administration

The i.v. dose was given as bolus single injection of 5 mL/kg into vena jugularis via the implanted venous cannula. The oral doses were given as single doses directly into the stomach, using gavage. The dose volume was 5 mL/kg for both the low and high doses.

Blood sampling

The blood samples were taken after oral administration at 0, 15, and 30 minutes and after 1, 2, 3, 5, 8, 14, 20, 24, and 26 hours. After i.v. administration, blood samples were taken after 0, 2, 10, and 30 minutes and after 1,3, 5, 8, 14, 20, 24, and 26 hours. Blood samples of about 0.12 mL were collected from the aortic bow via the arterial cannula. The cannula was kept open and clean with flushing with physiological saline containing heparin (20 IE/mL) between blood sampling. The blood samples were collected into heparinized plastic tubes (Microvette®; Sarstedt) and kept cold until plasma separation (5 minutes, 10,000 g, +4°C). The plasma (50 µL) was transferred to 96 deep well plates and stored at about –20°C until analysis.

Bioanalytical methods

Plasma samples were analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS), with a method described in a documented internal method. An Agilent 1200 SL LC pump was used with gradient elution using a flow rate at 0.6 mL/min. The mobile phase consisted of (A) 2% acetonitrile and 0.2% formic acid in water and (B) 0.2% formic acid in acetonitrile. Separation was performed on a 30 × 2.1 mm C18 Zorbax Eclipse Plus column with 1.8 µm particle size (Agilent Technologies Inc., Wilmington, DE, USA) using a linear gradient of 5–95% B in 1.8 minutes held at 90% for 0.7 minute and returned to initial conditions in one step. The front, containing salt and highly polar compounds, was diverted to waste, and after 0.5 minute the effluent entered the MS without splitting. Sample storage and injection was performed with an Agilent ALS SL autosampler (Agilent Technologies Inc.). Before injection to the LC–MS, 50 µL of the plasma sample was protein precipitated in 96 deep well plates using a robot (GenMate, Tecan, Männedorf, Switzerland) by the addition of 150 µL acetonitrile containing internal standard. After vortexing, the plasma samples were centrifuged for 20 minutes at 2900 × *g* and 4°C. The supernatant (75 µL) was diluted with 75 µL of 0.2% formic acid in water. Detection was performed with positive electrospray ionization mode by multiple reactions monitoring using an Agilent 6410 triple quadropole (Agilent Technologies Inc.). Instrument control, data acquisition, and data evaluation were performed using Agilent MassHunter.

Pharmacokinetic evaluation

The pharmacokinetic calculations are based on individual plasma concentration–time data. The calculations were made with the computer program WinNonlin™ Professional version 3.1 (Pharsight Corporation, Mountain View, CA, USA). The maximum plasma concentration (C_{max}) and the time at which it occurred (t_{max}) were determined. The area under the plasma concentration–time profile (AUC) was calculated by the linear/log trapezoidal rule up to the last data point plus the residual area up to infinity. The residual area was calculated by integration, C_p/k, where C_p is the predicted plasma concentration at the last measurable sampling point and k the terminal slope of the ln plasma concentration–time curve. The apparent terminal half-life ($t_{1/2}$) was calculated by ln 2/k, where k is the apparent terminal slope calculated by linear regression of ln concentration–time data. The bioavailability (F) was determined by $AUC_{oral}/AUC_{i.v.}$, corrected for the dose. Each individual per oral exposure was compared with the AUC obtained with the i.v. dose.

Statistical analysis

Plasma concentrations of the compound and pharmacokinetic parameters in vivo are expressed as the mean ± SD. The statistical analysis for calculated pharmacokinetic parameters was made using the *t*-test. Analyses were performed for AUC, C_{max}, and F. The differences were considered significant at $P < 0.05$.

Results

Pharmaceutical characterization of suspensions

According to experimental data, the solubility of crystalline UG558 in water is very low, about 2 µM (pH 6.8). The substance is ionizable, with increasing solubility at higher pH, but an aqueous pH-shifted solution did not reach the intended millimolar range necessary for the in vivo study. Instead, suspensions with different particle sizes were used. The suspensions in this work showed volume-weighted means of about 190 nm (>90%, <230 nm) and about 12 µm (>90%, <25 µm) for nanosuspensions and microsuspensions, respectively. The crystal structure was determined by X-ray diffraction to be unchanged by the dispersion and milling process (data not shown).

Two nanosuspension formulations, 1 and 100 mM, were chemically and physically (particle size) stable for at least 1 month at room temperature. The two formulations were frozen and then thawed after 3 weeks. There were no degradation observed and no change in particle size when 5% mannitol was present. Also the microsuspensions (1 and 100 mM) were chemical and physical stable for at least 1 month at room temperature.

In vivo studies in rats

Four different formulations were administered orally to rats, containing 5 µmol/kg (low dose, 5 mL/kg) and 500 µmol/kg (high dose, 5 mL/kg) UG558. One nanosuspension and one microsuspension were administered at each dose level. The mean plasma levels obtained for the formulations are presented in Figure 1. Four animals were used for each formulation. One animal receiving the high dose of nanosuspensions was excluded after the 14-hour blood sample because of technical circumstances. To make it possible to calculate a comparison value of *F*, two animals received an i.v. dose of 5 µmol/kg (5 mL/kg) as nanosuspension (Figure 1). All substance was dissolved in the blood after i.v. administration, which could be expected from the water solubility (2 µM at pH 6.8), the higher pH in blood, and the actual C_{max} values after oral administration. The i.v. exposure was well below the exposure obtained with the high dose after oral administration (Figure 1 and Table 1). There were no indications that the animals did not tolerate the formulations given orally or i.v. Pharmacokinetic parameters C_{max} (peak concentration), t_{max} (time to reach the peak concentration), and $t_{1/2}$ (terminal half-life) are presented in Table 2 for the oral formulations. Also AUC (area under individual plasma time curves) is presented in Table 2. Absolute oral bioavailabilities of each formulation were calculated using the AUC values from i.v. administration.

Compared with the microsuspension groups, the nanosuspension groups significantly increased C_{max} and AUC at both 5 and 500 µmol/kg ($P < 0.05$). At the low dose, C_{max} and AUC for the animals receiving nanosuspensions were about four times larger compared to the dose group that received microsuspensions. This ratio between the two formulation groups was similar at the high dose. The half-life and t_{max} were not altered between the suspensions or between the dose levels. Moreover, *F* of UG558 using nanosuspensions (low dose, 70% and high dose, 7%) was significantly higher than when microsuspensions (low dose, 20% and high dose, 1–2%) were administered, comparing the same dose ($P < 0.05$). Besides, the value of *F* decreased with dose within the two suspension types administered ($P < 0.05$).

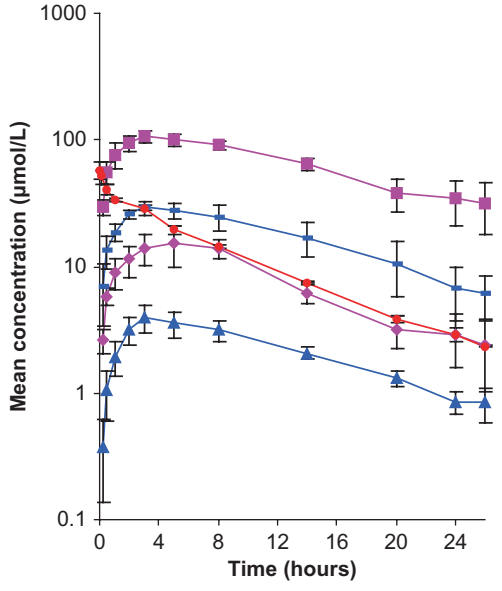

Figure 1. The mean plasma levels of UG558 versus time after oral administration (and i.v. administration, as nanosuspension 5 µmol/kg, dotted line) of UG558 as nanosuspensions (✦) and microsuspension (▲) at 5 µmol/kg to rats. At a higher dose, 500 µmol/kg, was a nanosuspension (■) and a microsuspension (-) administered. In all cases were 5 mL/kg administered. $n = 4$ for each formulation, except for the i.v. administration, where $n = 2$.

Table 1. Mean values of the pharmacokinetic parameters following i.v. administration of UG558 in nanosuspensions as a single dose (5 µmol/kg, 5 mL/kg) to rats.

Animal (i.v.)	AUC/dose (h kg/L)	$t_{1/2}$ (hours)	Cl (mL/min/kg)
1	72	7.9	0.23
2	66	6.9	0.25
Mean	69	7.4	0.24

The parameters presented are AUC (area under individual plasma time curve), $t_{1/2}$ (apparent terminal half-time), and Cl (clearance, volume of blood cleared of compound per unit time). No standard deviations are calculated as $n = 2$.

Table 2. Mean values of the pharmacokinetic parameters following oral administration of UG558 in different formulations as 5 and 500 µmoL/kg doses (5 mL/kg) to rats.

Formulation	Dose (µmol/kg)	C_{max} (µmol/L)	t_{max} (h)	$t_{1/2}$ (h)	AUC (h kg/L)	*F* (%)
Nanosuspension	500*	106 ± 8.0	3.3 ± 0	12.8 ± 4.5	4.6 ± 0.8	6.7 ± 1.2
Microsuspension	500	29.9 ± 2.0	3.5 ± 0.4	8.2 ± 1.8	1.0 ± 0.2	1.5 ± 0.3
Nanosuspension	5	16.6 ± 3.5	5.3 ± 1.7	10.0 ± 5.2	48.6 ± 8.4	71 ± 12
Microsuspension	5	4.4 ± 0.6	4.0 ± 1.0	9.9 ± 2.9	13.4 ± 1.2	20 ± 2.0

The parameters presented are AUC (area under individual plasma time curve), C_{max} (peak concentration), t_{max} (time to reach peak concentration), $t_{1/2}$ (apparent terminal half-time), and *F* (bioavailability). $n = 4$ for all experiments. The values are expressed as mean ± SD.
*One animal receiving the high dose of nanosuspensions was excluded after the 14-hour blood sample because of technical circumstances. However, the parameters of the subject are included in the calculations.

Discussion

For neutral drugs, like danazol[21], suspension is an attractive approach and maybe the only one to reach sufficient in vivo exposure and effect. To optimize the exposure for poorly soluble compounds, one can take the development one step further and reduce the particle size to the nanometer range (which has the advantage to be able to be used also for i.v. administration). On a laboratory scale, crystalline nanosuspensions are prepared by a milling procedure[21–23]. The obtained formulations contain low amounts of additives and are, thus, expected to induce minimal side effects in various in vivo studies. Besides neutral compounds, the nanosuspension approach should preferably be applied on acidic compounds. A suspension of an acidic compound, where a well-defined formulation is administered, reaches and passes the stomach as a conserved suspension (with only a minor part dissolved, in the nanomolar range for UG558) and then starts to dissolve (depending on pK_a) when it reaches the small intestine (higher pH). For a suspension of a basic compound, on the other hand, the substance may dissolve (or a part of it, depending on solubility and pK_a) already in the stomach and then it may be absorbed in the small intestine or it may precipitate (in different ways ranging from a gel to a nice, easily redissolved material), all depending on the physicochemical properties of the substance. In this article, crystalline nanosuspensions of the acidic substance UG558 were prepared and compared in vivo with microsuspensions of the compound at two different doses. The aim was to find an optimal formulation, resulting in high exposure in the forthcoming toxicological high-dose studies, using a minimum amount of drug and animals.

In the development of pharmaceutical dosage forms, one of the persistent challenges is assuring acceptable stability, that is, the storage time allowed before the content is too low or a degradation product in the dosage form achieves a sufficient level to represent a risk to the in vivo specie[27,28]. During early stage of the project, it is important to have stability information to secure that the dose administered in vivo contains the desired compound and that the effect originates from the parent compound. In this study, some initial stability investigations were performed with UG558 in the suspensions. No chemical degradation was observed at any of the investigated conditions. Also the particle size remained similar after storage.

Absorption of a drug from a suspension vehicle is considered to involve a dissolution step of the drug from the formulation into the aqueous luminal fluid followed by transport across the gastrointestinal epithelium. The dissolution rate and/or the low solubility may become the rate determining process in the bioavailability pathway[29,30]. The dissolution rate is supposed to be slower for larger particles, that is, at a specific concentration (and above it) the dissolution rate (and the solubility) is supposed to be rate limiting, resulting in a better exposure for nanoparticles compared to microparticles. Already at the lowest dose (5 μmol/kg), after oral administration, the two different suspensions differed significantly with respect to exposure and bioavailability of UG558. Obviously, the dissolution rate of the microparticles was significant slower. The appearance was similar at the higher concentration (500 μmol/kg). The ratio in plasma exposure between the two different suspensions did not significantly increase at the hundred times higher dose, but remained about four times. This further indicates that the dissolution rate is the major rate-limiting step and the ratio is similar as the particle size is conserved at the higher concentration. Besides, the exposure was not dose linear, indicating that also the low solubility in the intestinal region may be a factor to consider (at a specific dose, the exposure of a specific compound in a certain suspension will not increase with dose anymore, and the exposure was supposed to be mainly solubility limited). Notably is the fact that both suspensions resulted in lower bioavailability at the high dose compared to the low dose.

Limited solubilization capacity of the gastrointestinal tract and the slower dissolution rate for larger particles resulted in a lower bioavailability when microsuspensions were used. Moreover, in general, for drugs that are evenly absorbed over the entire intestinal tract, rate of dissolution will not influence the extent of absorption. In the present case, however, the compound may have no or limited absorption in colon because of instability (chemical and/or physical), low solubility, slow dissolution rate (both solubility and dissolution rate were further affected by the low liquid content in colon), and/or low permeability in the large intestine. The possible poor absorption in the colon suggested that the absorption of UG558 almost exclusively occurred in the upper gastrointestinal tract (supported by the good permeability achieved using Caco-2 cells, see Material and Methods). These properties explain the lower bioavailability for the 500-μmol/kg doses, compared to the 5-μmol/kg doses, of the two formulations and the even lower absorption and bioavailability of the microsuspension.

The i.v. administration confirmed that nanosuspensions of UG558 could be administered without adverse events to rats (at the present dose), that is, neither the substance nor the particles caused negative effects. The possible alternatives for projects in general, where cosolvents are used in the formulation, may cause pain and local irritation for the subjects. In fact, nanosuspensions may be the only alternative for i.v. administration of some poorly soluble compounds[31]. The simple technical approach yields a more physically stable (more comparable with solid-state stability than solution

stability) and safer product than solvent mixtures. This is also in accordance with the Chinese Pharmacopoeia (2005) for injectable emulsion[32], where it is stated that the number of particles larger than 1 μm should be less than 10%, and the maximum particle size should be below 5 μm. The smallest blood capillaries are about 5 μm in width. Particles larger than 5 μm may then cause blockade or embolism. However, in addition to the particle size, care must be taken so that the final administered concentration does not exceed the solubility of the drug in plasma too much (the water solubility can be used as a first approximation). If the injected amount of the drug exceeds a substance-specific level, the solid concentration of particles will be high, the particles may aggregate, and then the interpretation of the effect and the pharmacokinetics will be difficult. Besides, negative events for the subjects may appear, which are because of limited suitable physicochemical properties of the substance and formulation and not because of toxicological effects of the compound. However, the alternative is not to administer i.v. at all. So, particle size (and the physical stability) and dose in relation to solubility in plasma are the two main parameters to evaluate before using nanosuspensions for i.v. administration.

In conclusion, this study demonstrated a clear correlation between particle size and in vivo exposures, where nanosuspensions provided the highest exposure at both investigated doses; 5 and 500 μmol/kg. The explanation for the low bioavailability of the micronized suspension was mainly because of insufficient drug dissolution achieved within the limited small intestinal transit time. Faster dissolution from the nanometer-sized particles resulted in an improved absorption in this narrow window. Besides, the high dose of both suspensions was too high to be able to reach the bioavailability value, which was reached at the low dose.

For BCS II compounds, nanosuspensions appear to be an attractive alternative to a microsuspension. In studies where high doses are needed for oral administration, a solution may not be possible to manufacture and a microsuspension may result in too low exposure. Regarding i.v. administration, nanosuspensions may be the only alternative for a poorly soluble compound.

Declaration of interest: The authors report no conflicts of interest.

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