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ORIGINAL ARTICLE

A formulation comparison between micro- and nanosuspensions: the importance of particle size for absorption of a model compound, following repeated oral administration to rats during early development

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Abstract

Aim: The aim of this study was to maximize the exposure of a model compound (MC) for forthcoming high-dose toxicological studies with the physical form of the original compound unaffected. *Method*: The two evaluated formulation approaches for the present poorly water-soluble compound were micro- and nanosuspensions. *Results*: The particle size was about 280 nm for the nanosuspensions and about 4 µm for the microsuspensions. The crystallinity and the crystalline form of the ground samples were conserved. The physical and the chemical stabilities of the two kinds of suspensions were unaffected during the investigated time period. The in vivo results of the study showed that the pharmacokinetic parameters investigated were comparable at the low-dose level (6 µmol/kg) for both formulations after single administration. However, at the two higher doses (60 and 300 µmol/kg), a significant difference in exposure was observed between the two suspensions with an improved exposure for smaller particles. After Day 7 of repeated administration, a significant difference in exposure was observed at all dose levels. The overall exposures were higher on Day 7, compared to the exposures on Day 1 (most significant for nanoparticles), due to an accumulation of compound in the body. *Conclusions*: The nanoparticles have a larger surface, resulting in faster in vivo dissolution rate, faster absorption, and increased bioavailability, compared to microparticles. The differences in systemic exposure of model compound, following oral administration of nano- or microparticles of the drug substance, are probably caused by differences in the in vivo dissolution rate and possibly further enhanced by saturation of the systemic elimination.

Key words: Absorption, in vivo exposure, nanosuspension, pharmacokinetic, poorly soluble, rat, saturated elimination, suspensions

Introduction

Conventional formulations of poorly water-soluble compounds are often associated with low and variable bioavailability[1,2]. Hence, it is desired to improve the solubility of the compound during preformulation and early formulation development. The approaches to increase the solubility of a drug mainly focus on either modifying the media in which the compound is dissolved or destabilizing (or modifying) the crystalline form of the compound. To overcome the former, there are many common pharmaceutical formulation approaches such as dissolution in aqueous mixtures with an organic solvent and formation of complexes, for example, using β-cyclodextrins[3–6]. Other attempts to increase the solubility are emulsions[7–9], solid dispersions[10,11], or the exploiting effects of pH[12].

The modification of the physical properties of the compound (the later approach mentioned above) can be achieved by forming a salt[12], an amorphous form of

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the compound[13–16], or a less stable crystalline form[17,18]. In all these approaches, one utilizes an apparent higher solubility, or supersaturation, before a more thermodynamic stable form is obtained or regained.

In the last decade, a third drug delivery approach has been developed to overcome the low and variable bioavailability often associated with poor aqueous solubility of drug substances. By reducing the particle size of the drugs, one could often improve the in vivo performance of poorly soluble drugs because smaller particles lead to an increased saturation solubility, enlarged surface area, and an increased dissolution rate[19–25]. A higher dissolution rate and the resulting higher concentration gradient between the gastrointestinal tract and blood increase the absorption and, consequently, increase the oral bioavailability. The process of milling is one of the most commonly used operations in pharmaceutical manufacturing, producing particles in the micrometer size or even in the nanometer range. One obvious advantage with producing nanoparticles or submicron particles is that the same formulation approach, that is, nanosuspensions, can be used for all intended administration routes. Examples where nanosuspensions have been administered are oral[20,26], parenteral[20,23,27], ocular[28], and pulmonary delivery[29]. Besides, nanosuspensions can be used for targeted delivery using the right set of stabilizers[21].

The model compound (MC) is a moderately lipophilic drug with a log *P* of 2.4, a pK_a below 1, and a molecule weight of 294 g/mol. The compound is neutral at all physiological conditions and the solubility is independent of pH of the solutions. The intrinsic solubility is 4 μM (1 μg/mL) at room temperature. Because of the very low pK_a of the compound, only a few counter ions were amenable to salt formation. Of the ions tested, only the bromide salt was possible to isolate. This salt was, however, found to be too unstable for further development. Neither was it possible to form amorphous material. Besides, only one crystal modification has been identified so far. The permeability of MC in the in vitro Caco-2 cell model was classified as high. Hence, according to the Biopharmaceutical Classification System (BCS), the compound is a BCS II substance, that is, high-permeability and low-solubility compound[30]. This suggested that the oral absorption of MC would most likely be limited by solubility and dissolution, not by intestinal permeability. Hence, a nanosuspension formulation of MC for oral administration could improve the kinetic solubility and the dissolution velocity of the drug and should therefore lead to increased systemic exposure compared to a microsuspension formulation. The aim of this study was to investigate whether the particle size of the MC would affect the rate and extent of the systemic exposure after oral administration, at three different dose levels and for 7 consecutive days, to rats.

Materials and methods

The model compound

The MC was synthesized by AstraZeneca (Mölndal, Sweden).

Chemicals

Polyethylene glycol (PEG)400 was bought from BASF (Ludwigshafen, Germany). HPMC (hydroxypropyl methylcellulose, 15000 cP) was bought from Shin-Etsu Chemicals (Tokyo, Japan). Polyvinylpyrrolidone K30 (PVP) is a nonionic polymer, which was bought from BASF (Gothenburg, Sweden). PVP is a stabilizer and is expected to cover the surface of the pure drug when dispersed in water. The sodium dodecyl sulfate (SDS) from Sigma (Steinheim, Germany) is a surface-active agent with function similar to PVP. Glycerol was from Sigma and was added as a cryoprotectant to the nanosuspensions to avoid freezing damage due to ice formation and to reduce particle size growth during freezing.

Preparation of microsuspensions

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

Preparation of crystalline nanosuspensions

Typically, 55 mg of substance was weighed into a 4-mL sample vial and 495 mg aqueous stabilizer solution containing 1.0% (w/w) PVP and 0.2% (w/w) SDS in deionized, distilled water was added giving a slurry holding 10% (w/w) drug, that is, 100 mg/mL. The crude suspension was stirred and treated with ultrasonic for 20 minutes, which gave a well-dispersed slurry. A 0.5-mL slurry was placed in a 1.2-mL milling vessel and 2 g of washed beads (zirconium oxide milling beads, diameter 0.6–0.8 mm, obtained from Glen Creston Ltd., Stanmore, Middlesex, UK) was added. The vessel was sealed and the slurry milled at 700 rpm for four cycles of 30 minutes with intermediate pauses of 15 minutes (to avoid high temperature in the vessel, <50°C), using the Fritsch Planetary Micromill P7 (Fritsch, Idar-Oberstein, Germany). The milled suspension was collected combined with rinsing of the beads and vessel with 2.6% (v/v) glycerol (aq) solution. The formulation was sonicated before administration. The volume-averaged particle size (diameter) of the crystalline suspensions was measured by laser diffraction (Malvern Mastersizer 2000). The zeta potential was measured by a Zetasizer (nano ZS) from the same company.

Formulation analysis

The assay was determined using a reversed-phase liquid chromatography gradient system with UV detection

(Waters, Milford, MA, USA). The mobile phase was a mixture of acetonitrile, water, and 0.03% (v/v) trifluoroacetic acid, and the column used was Waters XBridge 50 × 3.0 mm, 3.5 µm (Waters, En Yvelines Cedex, France). Detection wavelength was 280 nm. When investigating the presence of organic impurities (and possible degradation products), a Waters XBridge 150 × 3.0 mm, 3.5 µm column (from the same supplier as above) was used. Detection wavelengths were 220 and 280 nm.

Study design

Animal handling

Twenty-four male, healthy, Wistar Hannover rats (aged 11–12 weeks, body weight 261–344 g at dosing) were supplied by Charles River Limited (Edinburgh, UK). Four rats in each dose and formulation group were dosed oral. The rats were acclimatized over a period of 5 days prior to dose administration. During this acclimatization period, the animals were carefully observed to ensure that they were in good health and suitable for inclusion in the study. Holding and study areas had automatic control of light cycles and temperature. Light hours were 07.00–19.00. Ranges of temperature and humidity measured during the study were 19–21°C and 48–51%, respectively. A standard laboratory diet of known formulation (SDS Rat and Mouse Maintenance Diet No. 1, Special Diets Services, 1 Stepfield, Witham, Essex, UK) and domestic mains tap water were available ad libitum. The diet (ssniff ®R/M-H V 534) was removed from the animals at least 12 hours before administration and replaced 6 hours after dosing. During on-study periods, rats were housed singly in polypropylene and stainless steel cages. The animals were uniquely identified by tail and/or paws marking. The rats were checked following dosing and any clinical signs observed were recorded. After the last sampling time, the rats were killed by cardiac puncture under anesthesia.

Study design

Four male Wistar rats in each dose and formulation group were dosed orally by gastric gavage once daily for Day 7. Whole blood samples were collected at intervals up to 24 hours post-dose on Days 1 and 7.

Doses administered were 6, 60, and 300 µmol/kg. The dose volume was 5 mL/kg body weight. The exact start time of dosing and the exact dose volume were noted.

Blood sampling

Blood samples (~0.5 mL) for determination of MC in plasma were collected from the tail vein by venipuncture and put into tubes containing spray-dried ethylenediaminetetraacetic acid (EDTA). The samples were collected on Days 1 and 7 of dosing at 30 minutes and 2, 4, 8, and 24 hours post-dose administration. The exact dosing and sampling times were recorded. The blood samples were immediately placed on ice and centrifuged within 30 minutes at 4°C for 10 minutes at 1500 *g* to obtain plasma. The plasma was transferred to Nunc Cryotubes® (InterMed A/S, Kamstrup, Denmark). The plasma samples were stored frozen at or below −70°C until bioanalysis.

Bioanalytical methods

Quantitative analysis of the total plasma concentration was performed by liquid–liquid extraction followed by reversed-phase liquid chromatography and atmospheric pressure chemical ionization tandem mass spectrometry. The method is described in an internally documented method. The lower limit of quantification (LLOQ) in plasma was 1.0 nmol/L. The mean accuracy, reported as the percentage difference from the nominal value, was within ±15%. The precisions, expressed as the relative standard deviations, were in the range 2–10%.

Pharmacokinetic evaluation

Data on exposure of animals were obtained by means of noncompartmental analysis in WinNonlin version 4.0 (Pharsight Corporation, Mountain View, CA, USA). The pharmacokinetic calculations were based on nonrounded individual total plasma concentration–time data. The maximum concentration, C_{max}, and the time of maximum concentration, t_{max}, were direct observations from the plasma concentration versus time data. $AUC_{(0\text{-}t)}$ was calculated by means of the linear up–log down method. If the plasma concentration was below LLOQ at 24 hours, the $AUC_{(0\text{-}t)}$ was extrapolated to $AUC_{(0\text{–}24)}$ by adding the ratio C_{obs} (observed plasma concentration at t_{last}) and λ_z. The terminal slope factor, λ_z. and subsequently the terminal half-life, $t½_{\lambda z}$. were estimated in WinNonlin by log-linear regression of the last two to four data points of the plasma concentration–time curve.

Nominal time points were used in the noncompartmental analysis. Observations below the LLOQ were treated as missing values in the analysis.

Statistical analysis

In the graphical illustrations and in the calculations of the mean (±SD) values, plasma concentrations below the LLOQ were treated as missing and excluded from calculations. The pharmacokinetic results are expressed as an average of four individuals (±SD). Analyses were performed of $AUC_{(0\text{–}24)}$ and C_{max} for nanoparticles versus microparticles using the *t*-test. The differences were considered significant at $P < 0.05$.

Results

Pharmaceutical characterization of solutions

According to experimental data, the solubility of crystalline MC in water is very low, 4 µM (pH 6.5, 22°C), and pH independent (see Introduction). The solubility in different cosolvent mixtures and complexing agents was very low and no acceptable solution formulation with sufficient solubility for most in vivo testing was found. The best

formulation of the compound in an acceptable physiological solution reached a solubility of 1–2 mM (in about 80% polyethylene glycol) at room temperature.

Initial pharmaceutical characterization of suspensions

The suspensions in this work showed volume-weighted means of 280 nm (>90%, <360 nm) and 4 µm (>90%, <8 µm) for nanosuspensions and microsuspensions, respectively (Figure 1). Results from X-ray powder diffractions (XRPD) showed that there were no significant difference between the particles in the nanosuspension and in the microsuspension compared to bulk particles (data not shown). This is in accordance with polymorph screen performed without finding any more crystal modification (AstraZeneca, data on file). The suspensions can, therefore, be considered to be crystalline and of the same crystalline modification as the bulk substance.

Nanosuspension formulations between 0.03 and 80 mM were chemically stable for at least 4 weeks at 5°C, 22°C, and 40°C. There was no change in volume-weighted mean (or crystalline modification) during the same storage conditions and time period, with 2.6% (v/v) glycerol present as cryoprotectant (Table 1). There was no sign of aggregation, sedimentation, or Ostwald ripening[20] of the nanosuspensions when stored at 22°C or at 4–8°C. After thawing (of frozen suspension), some aggregation was observed, but the original volume-weighted mean was obtained after 10 seconds sonication.

The zeta potential of nanosuspensions of MC diluted to 0.5 mM with 2.6% (v/v) glycerol was −23 mV after 1 hour (a relevant, or prolonged, gastric residence time in fasted rats). The zeta potential is a measure of particle surface charge, indicative of the colloidal stability of the nanosuspension. An increase in the absolute value of zeta potential is correlated with a lesser tendency to aggregate or flocculate. For a nanosuspension that is both electrostatically and sterically stabilized, an absolute zeta potential of 20 mV is sufficient[31]. The volume-weighted mean was followed in a similar sample at pH 3 (for rats, a pH in the interval 3–5 is realistic for both fed and fasted animals in the gastric tract[32–34]). No aggregation or precipitation was observed after 1 hour.

The microsuspensions were chemically and physically (volume-weighted mean and no phase transition) stable for at least 1 week at room temperature. However, sedimentation occurred during the week, but after shaking (and/or stirring) the formulation was resuspended with similar volume-weighted mean (from now referred to as 'particle size') as originally obtained.

When adding nano- and microsuspensions to an aqueous solution (22°C or 37°C), it was obvious that the former dissolved faster.

Table 1. Particle size (nm) of nanosuspensions at different times after preparation and stored at different conditions.

Conditions	Day 0	2 weeks	4 weeks
5°C	278	287	270
22°C	278	289	268
40°C	278	252	242

Pharmacokinetics in rat

Six different formulations were administered orally to rats, containing 6, 60, or 300 µmol/kg of MC. All animals dosed with MC were systemically exposed to MC. A plot of mean plasma concentrations versus time is given in Figure 2 for Day 1 and in Figure 3 for Day 7 (after Day 7 of administration). Pharmacokinetic parameters are presented in Table 2. No difference in systemic exposure to MC between nano- and microsuspension was observed after one single dose of 6 µmol/kg. However, at both 60 and 300 µmol/kg, C_{max} and $AUC_{(0-24)}$ were at least twice higher using nanosuspension compared with microsuspension. After Day 7 of dosing, C_{max} and $AUC_{(0-24)}$ were two to three times higher at all dose levels (including the 6 µmol/kg dose) following an administration of nanosuspension compared to microsuspension. The statistical analyses of $AUC_{(0-24)}$ and C_{max} were performed for submicron particles versus microparticles. The values were significantly higher when administering nanoparticles compared with the values after dosing microparticles. The only exception was the low-dose Day 1. AUC was not

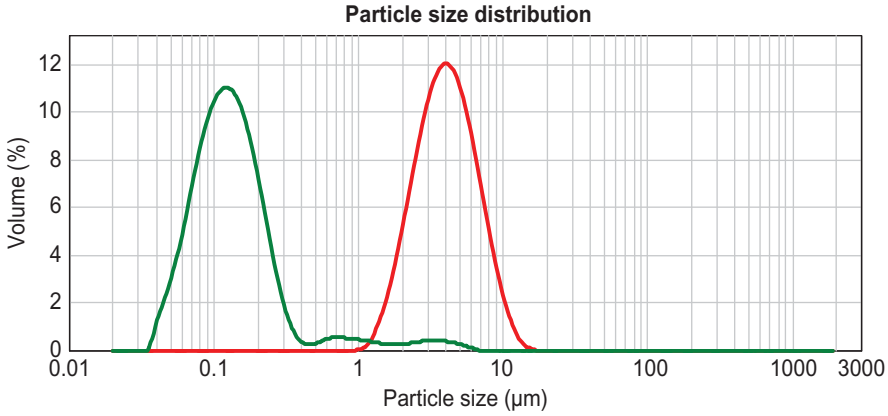

Figure 1. Particle size distribution curves for nano- and microsuspensions of MC.

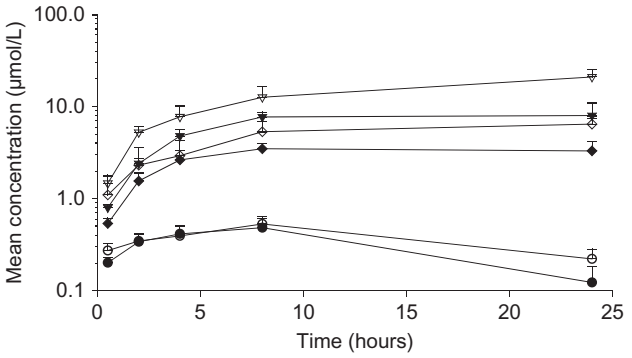

Figure 2. The mean plasma concentration (±SD) of MC versus time after Day 1 of oral administration of MC as a microsuspension and as a crystalline nanosuspension to rats at 6 (circles), 60 (diamonds), and 300 (down triangles), µmol/kg using nanosuspension (open symbols), and microsuspension (filled symbols). n = 4 for each formulation.

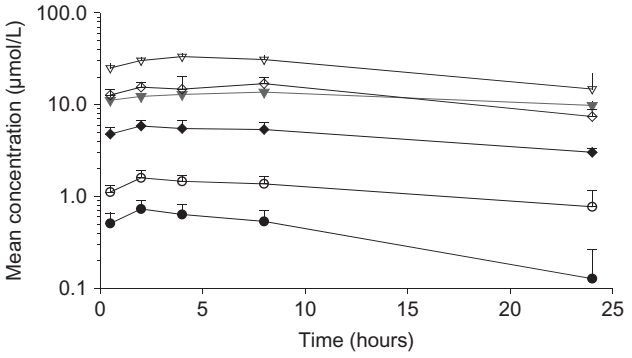

Figure 3. The mean plasma concentration (±SD) of MC versus time after Day 7 of oral administration of MC as a microsuspension and as a crystalline nanosuspension to rats at 6 (circles), 60 (diamonds), and 300 (down triangles) µmol/kg using nanosuspension (open symbols) and microsuspension (filled symbols). n = 4 for each formulation.

used in the evaluation due to the large extrapolated area and to the late t_{max} (see below). $AUC_{(0-24)}$ was used instead of AUC. Neither was $t_{1/2}$ calculated.

After administration of nanosuspension, t_{max} usually occurred later compared to microsuspension (Table 2). For both suspensions t_{max} for Day 1 occurred between 8 and 24 hours or later. After administration for Day 7, t_{max} occurred earlier compared with Day 1.

After administration of both formulations, the exposure of MC (both single dose and after Day 7 of dosing) in terms of C_{max} and $AUC_{(0-24)}$ increased in proportion to the dose of MC in the dose range 6–60 µmol/kg, and less in proportion to the dose in the dose range 60–300 µmol/kg of MC.

After Day 7 of dosing concentration–time profile was somewhat different between the two formulations (Figures 2 and 3) compared with Day 1. The initial increase in plasma exposure was not observed on Day 7. Besides, the administration of nanosuspension especially at the doses 60 and 300 µmol/kg showed a difference in plasma profile, whereas after administration of microsuspension the profiles were more similar. After administration of microsuspension, the exposure did not differ (or just a small increase) over the duration of treatment.

Discussion

The need to get sufficiently high systemic drug exposures in early efficacy and toxicology models is the major driving force for improved possibilities to formulate and administer a poorly soluble compound orally. Nanosuspensions are such an early step and are useful dosage forms for poorly soluble drugs. In this article, a comparison was made between a crystalline nanosuspension and a crystalline microsuspension of the MC administered orally to rats. The compound has high permeability and low solubility in the gastrointestinal tract, thus fulfilling the criteria for a BCS II compound. A reduction in particle size for this BCS class of compounds is supposed to increase the dissolution rate and the following systemic exposure.

Table 2. Mean (±SD) pharmacokinetic parameters following oral administration of MC in different formulations as a single dose (6, 60, and 300 µmol/kg) and after Day 7 administration (n = 4).

Dose (µmol/kg)	Formulation	Study period	t_{max} (hours)[a]	C_{max} (µmol/L)	±SD	$AUC_{(0-24)}$ (µmol·h/L)	±SD
6	Nanosuspension	Day 1	8	0.532	0.11	7.59	3.5
6	Microsuspension	Day 1	8	0.504	0.10	7.16	1.8
60	Nanosuspension	Day 1	24	6.73	0.80	119	23
60	Microsuspension	Day 1	16	3.86	0.23	72.2	4.9
300	Nanosuspension	Day 1	24	21.0	4.5	328	59
300	Microsuspension	Day 1	16	9.26	1.7	160	22
6	Nanosuspension	Day 7	3	1.65	0.31	27.8	6.3
6	Microsuspension	Day 7	2	0.731	0.18	9.01	4.2
60	Nanosuspension	Day 7	6	17.6	2.6	306	48
60	Microsuspension	Day 7	2	5.87	0.94	108	15
300	Nanosuspension	Day 7	4	33.4	2.5	585	63
300	Microsuspension	Day 7	8	13.8	1.3	287	22

[a]Median.

At the first day of administration of the study formulations, the lowest dose (6 μmol/kg) of nanosuspension did not increase either C_{max} or the extent (AUC) of absorption compared to the microsuspension at the same dose. For the two higher dose levels (60 and 300 μmol/kg), however, both the rate and extent of absorption increased significantly following administration of the nanosuspension compared to the microsuspension. To better understand the possible mechanism behind these results, the equation of Noyes–Whitney was used[35] to describe the in vivo dissolution velocity:

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

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. From this equation, it is obvious that an increase in surface area leads to an increase of the dissolution rate. In addition, smaller particles of a compound also increase the saturation solubility[19–21], which further increase the dissolution rate. One can also imagine that the thickness of the dissolution layer will decrease with smaller particles[19–22], causing an increased concentration gradient. At the lowest dose level (6 μmol/kg), the concentration of the MC in the gastrointestinal fluids was probably low in relation to the saturation solubility, that is, the term '$(C_s – C)$' was high, and therefore the dissolution rate could be high regardless of the differences in particle sizes (i.e., surface area) between the two formulations. Under these conditions, the rate-limiting mechanisms for the overall absorption is probably a combination of gastric emptying rate and dissolution rate, and the surface area will be less important for the systemic exposure.

The two higher doses of the nanosuspension (60 and 300 μmol/kg) at Day 1 of administration both resulted in significantly higher systemic exposures of MC compared to the microsuspension at the same dose levels. Thus, at these higher doses, the increase in surface area available for dissolution (i.e., smaller particle size) resulted in a faster in vivo dissolution rate from the nanosuspension and, therefore, also an increased rate and extent of systemic exposure of MC, compared to oral administration of the microsuspension. Furthermore, the less than dose-proportional increase in systemic exposure at the highest dose (see Figure 2), both for nano- and microsuspension, suggests that the intestinal saturation solubility is limiting the in vivo dissolution rate at the highest dose. Under such conditions, the in vivo dissolution rate could be so slow that the time needed to dissolve the whole dose in vivo is longer than the gastrointestinal transit time, and both the C_{max} and the AUC will therefore increase less than dose-proportional. Furthermore, the mean plasma concentrations of MC after administration of the two highest doses (both nano- and microsuspension) at Day 1 did not decline after the 8 hours sampling point, in opposite to what was the case for the lowest dose of both nano- and microsuspensions. Instead, the plasma concentrations increased slightly at the last sampling time 24 hours, or remained at almost the same level as was observed after 8 hours (see Figure 2). One plausible explanation for this observation is that the in vivo absorption is ongoing for 24 hours after oral administration because of a slow in vivo dissolution rate, as discussed earlier. However, this explanation implies that the gastrointestinal transit in the rat is more than 24 hours. Instead, the transit time in rat has been reported to generally be much faster[33,36,37].

Another possible explanation, for the increase/plateau of plasma concentration up to 24 hours for the highest doses, is a saturation of one or more systemic elimination mechanism(s) at sufficiently high plasma concentrations of MC. This would result in a slower rate of elimination and, therefore, less decline of the plasma concentration–time curve. To further investigate this we administered two different doses (0.13 and 3.0 μmol/kg) of MC intravenously to rats (n = 3, data not shown). The resulting plasma concentrations of MC were in the same magnitude as following the oral administration of the two highest doses in this study, that is, around 1 μM. The total clearance was found to be dependent on the dose, with a decrease in clearance from about 2 L/h/kg at the low dose to about 0.4 L/h/kg at the high dose (AstraZeneca, personal communication). This shows that the elimination of MC indeed can be saturated at plasma concentrations around 1 μM in the rat. Thus, the most plausible mechanism for the 'prolonged absorption phase' observed following administration of the two highest doses of both nano- and microsuspensions is a combination of slow dissolution rate in the intestine and saturated systemic elimination of MC.

At Day 7 of consecutive once-daily oral administration of the two study formulations, the nanosuspension resulted in significantly higher systemic exposure of MC at all three doses compared to the microsuspension. Besides, at the lowest dose (6 μmol/kg), the nanosuspension did result in a significantly increased systemic exposure compared to the microsuspension at Day 7 of administration, but not at Day 1 of administration. This is probably not caused by differences in gastrointestinal conditions affecting the dissolution rate, or mechanism, between Days 1 and 7. Instead, a more plausible explanation is the previously discussed saturation of the systemic elimination of MC. At Day 7 there has been an accumulation of the compound in the body up to a plateau, that is, steady state has been reached, and the plasma concentration that saturates the systemic

elimination of MC is reached already at the lowest dose. Visual inspection of the plasma concentration–time curves in Figures 2 and 3 suggests that the elimination becomes saturated at plasma concentrations roughly about 1 μM. This has also been confirmed from the intravenous administrations of MC in the rat, as previously discussed. A small difference in dissolution rate, and subsequently absorption rate, between the two formulations in the gastrointestinal tract will become a large difference in plasma concentrations if the systemic elimination is saturated.

It is well known that a physical transformation can alter the physicochemical properties of drugs[17,18]. Moreover, the impact of changes in the physical form may affect dissolution rates, apparent solubility, and also the following bioavailability of the compound. Changes in the physical form may be polymorphic transitions, changes in solvates, or variation in the degree of crystallinity. Milling is a procedure that may cause a physical transformation of the original, neutral crystalline form of MC. However, X-ray data showed that the nanoparticles of MC were not affected, indicating that the crystalline state of the compound appeared to be unaffected following the milling operation (data not shown, AstraZeneca). That is, the increase in exposure observed after dosing 60 and 300 μmol/kg of nanosuspensions of MC, compared with the microsuspensions, was not the result of the formation of amorphous material or another less thermodynamically stable form. Moreover, the suspensions were both chemically and physically (particle size and no phase transition) stable during the period of the study. There was no risk for further precipitation due to pH shift in the gastrointestinal tract because MC is neutral around the physiological pH with a solubility that is not affected by changes in pH in the physiological pH range. Thus, the differences seen in the systemic exposure of MC following the oral administration of two suspensions, containing either nanoparticles or microparticles of the drug substance, are probably caused by differences in the in vivo dissolution rate and possibly further enhanced by saturation of the systemic elimination.

Conclusion

The in vivo results in this study demonstrated a clear correlation between particle size and in vivo exposures. The nanosuspensions provided higher exposure at two high doses (60 and 300 μmol/kg) compared with the microsuspensions after single administration and at all administered doses after repeated dosing. The differences seen in the systemic exposure of MC following the oral administration of two suspensions, containing either nanoparticles or microparticles of the drug substance, are probably caused by differences in the in vivo dissolution rate and possibly further enhanced by saturation of the systemic elimination.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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