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RESEARCH ARTICLE

Co-administration of a nanosuspension of a poorly soluble basic compound and a solution of a proton pump inhibitor—the importance of gastrointestinal pH and solubility for the *in vivo* exposure

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Abstract

In Sigfridsson et al. (2011, *Drug Dev Ind Pharm*, 37:243–251), there was no difference in plasma concentration of BA99 when administering the drug as nanosuspension or microsuspension and analyzing the blood samples by liquid chromatography–mass spectrometry. This was related to the dissolved amount of drug in the gastric tract, which was high enough to support fast absorption when the drug reached the small intestine. One single physicochemical property (pK_a, about 3 for BA99) abolished the benefit of small particles. These results were further confirmed in the present study, where a proton pump inhibitor, AZD0865, was used to elevate the gastric pH and then drastically decreased the gastric solubility. In this way, BA99 could be considered as a model compound for a neutral substance. By increasing the gastric pH to 5–6 and 8–9, respectively, in rats, the plasma concentrations of BA99, after administering nanosuspensions, were unchanged compared with untreated (i.e. no AZD0865) animals. For microsuspensions of the test compound, on the other hand, the exposure of BA99 was 2- to 3-fold lower than for nanosuspensions at both pHs. Moreover, the blood concentrations of BA99 administered as microsuspension were also 2- to 3-fold lower compared with untreated (no AZD0865) individuals receiving both nanoparticles and microparticles of BA99. Obviously, for *neutral* compounds, with similar physicochemical properties as the present compound, size reduction will be crucial for increased plasma exposure. For *basic* compounds, with similar physicochemical properties as the present compound, the crucial step for absorption is the dissolution and solubility in the gastric tract.

Keywords: Dissolution rate, gastric pH, nanosuspension, pharmacokinetic, poorly soluble, proton pump inhibitor, suspension

Introduction

Insufficient exposure of poorly water-soluble drugs such as BCS class II (Biopharmaceutical Classification System, where class II means low-soluble–high-permeable drugs as defined by the Food and Drug Administration)[1–3] compounds after oral administration, due to a lack of or nonlinear drug absorption, is a more and more common challenge in the pharmaceutical industry today. This property often makes it difficult to evaluate drug efficacy and safety in both the preclinical and the clinical stage of development. By reducing the particle size of the drugs, one could often improve the *in vivo* performance of poorly soluble drugs, since smaller particles lead to increased saturation solubility, enlarged surface area, and an increased dissolution rate[4–7]. 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 in the nanometer range, all depending on equipment used

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and milling conditions. One obvious advantage with producing nanoparticles is that the same formulation approach, that is, nanosuspensions, can be used for all intended administration routes[6].

Our previous work described the exposure in plasma after administration of nanosuspensions and microsuspensions of a poorly soluble base and a poorly soluble acid to rats[8]. Both compounds had many similar physicochemical properties, for example, lipophilicity, solubility (about 3 μM) at intestinal pH, mass, and permeability (high). Besides, the particle sizes of the formulations for the different compounds were similar. For the acidic compound, there was an obvious effect of particle size, where smaller particles resulted in higher systemic exposure. However, for the basic compound (with a basic pK_a of 3), there was no difference in exposure (Figure 1). The explanation was that independent of particle size, the suspensions were dissolved to a large degree in the gastric tract and then rapidly absorbed when it reached the small intestine.

In the present study, we continue to work with the base, BA99, but convert its behavior to one of the neutral compounds in the physiological interval (pH 1–7) by pre-administering a proton pump inhibitor, AZD0865[9], increasing the gastric pH to 5–6 and 8–9, respectively, depending on the dose of AZD0865 administered. With this approach, the intention was to confirm the importance of small particles for absorption of a neutral compound (with similar small intestinal solubility) and also to confirm the importance of pre-dissolved compound in the gastric tract before the subsequent, optimal, absorption in the small intestine for class II basic compounds. Besides, a brief discussion around possible dissolution rate and solubility limitations affecting the subsequent plasma exposure is performed, utilizing the present data.

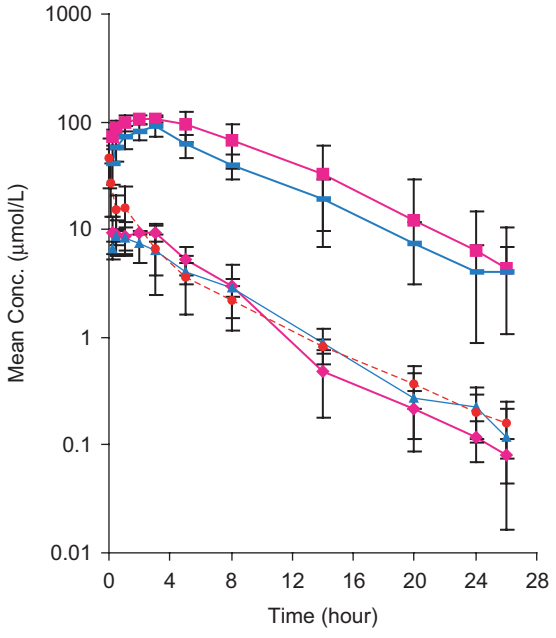

Figure 1. The mean (±SD) plasma levels of BA99 versus time after oral administration (and i.v. administration, as nanosuspension 5 μmol/kg, dotted line) of BA99, as nanosuspension (◆) and microsuspension (▲) at 5 μmol/kg to rats. At a higher dose, 500 μmol/kg, a nanosuspension (■) and a microsuspension (–) were administered. In all cases, 5 mL/kg were administered. $N=4$ for each formulation, except for the i.v. administration, where $n=2$ (taken from ref. 8).

Materials and methods

Test compounds

The base, BA99, has a molecular mass of about 380 g/mol. The substance is a crystalline compound with a melting point of about 130°C. The basic pK_a was measured (by CE-MS) to 3. There is also an acidic pK_a at 7.2. Estimated log *D* (the distribution coefficient between octanol/water) at pH 6.8 (from $k' = 13.1$, obtained by LC-MS) is 5. The solubility in a water solution is about 3 μM, at 22°C (measured from solid crystals, pH 6.8). The model drug used in this study was classified as a BCS class II because the proposed dose (>3 mg/kg) will not be dissolved in the whole gastrointestinal tract (pH 1–7) and permeability through the Caco-2 monolayer is higher than metoprolol and considered high and not limiting.

AZD0865 is an efficient acid secretion inhibitor that was discovered and developed by AstraZeneca. The compound was discontinued for toxicological reasons. An anti-secretory effect in the rat was achieved within 2 h after an oral dose of 1 μmol/kg. The maximum effect was reached after 3.5 h. The *in vivo* pH after different doses were carefully evaluated (ref. 9, and references therein).

Chemicals

Hydroxypropylmethylcellulose (HPMC; 15,000 cP) was bought from Shin-Etsu Chemicals (Tokyo, Japan). Polyvinylpyrrolidone (PVP) K30 is a nonionic polymer, which was bought from BASF (Göteborg, Sweden). PVP is a stabilizer and is expected to cover the surface of the pure drug when dispersed in water. The disodium salt of aerosol OT (AOT) from Cytec Industries Inc. is a surface-active agent with similar function as PVP. Mannitol was bought from Sigma (Stenhein, Germany) and used as a tonicity modifier.

Preparation of microsuspensions

Drug substance was weighed into a sample vial and stabilizer solution of 0.5% (w/w) HPMC was added. The slurry obtained was treated with ultrasound for 10 min and stirred overnight. The 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% AOT in water. The 10% (w/w) crude suspension was stirred and treated with ultrasound for 10 min, which gave a well-dispersed slurry.

The 510 µL of the slurry 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 min with intermediate pauses of 15 min, using the Fritsch Planetary Micromill P7. The milled suspension was collected and the milling beads were rinsed with water. The particle size (diameter) of the crystalline suspensions was measured by laser diffraction (Malvern Mastersizer 2000). The suspension was diluted with 5% mannitol.

Formulation analysis

BA99 concentrations in suspensions were evaluated using a reverse-phase liquid chromatography gradient system with UV detection. An XBridge Shield RP18 column (3.5 µm, 3.0 × 50 mm; Waters, En Yvelines Cedex, France) was used with a mobile phase of water (90% v/v)/acetonitrile (10% v/v) with 0.03% (v/v) trifluoroacetic acid. The detection wavelength was 280 nm.

Animal handling

The test system consisted of female Sprague Dawley rats (Harlan, The Netherlands), ~11 weeks old on the day of arrival at AstraZeneca R&D Mölndal (Sweden). After arrival, the rats were allowed to acclimatize for at least 5 days before surgery. The rats were housed in Macrolon III cages (two animals/cage during acclimatization) with aspen wood-chips (TapVei, Estonia) as bedding material. They were kept at room temperature, 20 ± 2°C, and at a relative humidity of 45 ± 15% during a 12-h light/dark cycle and had free access to food (R3, Lantmännen AB, Vadstena, Sweden) and tap water. The weight of the rats was 200–240 g. All animals were euthanized, by an overdose of pentobarbital sodium (i.p.), after the last blood sample had been collected.

Administration

The oral doses were given as single doses directly into the stomach, using gavage. The dose volumes were 5 mL/kg.

Blood sampling

The blood samples were taken after 0, 15, and 30 min and after 1, 2, 3, 5, 8, 20, 24, 26, and 28 h after oral administration. After i.v. administration, the blood samples were taken after 0, 2, 10, and 30 min and after 1, 3, 5, 8, 14, 20, 24, and 26 h. 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 min, 10,000 *g*, +4°C). Fifty microliters of the plasma 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) using a linear gradient of 5–95% B in 1.8 min, held at 90% for 0.7 min, and returned to initial conditions in one step. The front, containing salt and highly polar compounds, was diverted to waste and after 0.5 min the effluent entered the MS without splitting. Sample storage and injection was performed with a Agilent ALS SL autosampler (Agilent Technologies Inc.). Before injection to the LC-MS, 50 µL of plasma sample was protein-precipitated in 96-deep well plates using a robot (GenMate, Tecan, Männedorf, Switzerland) by addition of 150 µL acetonitrile containing internal standard. After vortexing, the plasma samples were centrifuged for 20 min at 2900 *g* and 4°C. Seventy-five microliters of the supernatant was diluted with 75 µL of 0.2% formic acid in water. Detection was performed with positive electrospray ionization mode by multiple reactions monitoring (MRM) using a Agilent 6410 triple quadrupole (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). The maximum plasma concentration (C_{max}) and the time at which it occurred (t_{max}) were determined. The area under the plasma concentration–time curve, AUC, was calculated by the linear/log trapezoidal rule. The AUC was extrapolated 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 is the terminal slope of the logarithmic 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 logarithmic 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

A P-value <0.05 was deemed to be statistically significant using a t-test approach.

Results

The *in vivo* oral administration study with different-sized BA99 particles (Figure 1) showed that the AUC values of plasma concentrations after the administrations of

smaller particles (280 nm) were similar to that of larger particles (12 μm)[8]. In the present study, the same bulk batch of the drug was used and the particle sizes of the formulations were similar. Figures 2 and 3 show the blood concentration–time profiles of BA99 in rats after oral administration of freshly prepared BA99 formulations (500 μmol/kg, both suspensions administered as 5 mL/kg). Before BA99 was administered, 5 mL/kg, 0.5 μmol/kg, or 1.0 μmol/kg of AZD0865 was administered per oral, 3.5 h prior to BA99 to adjust the gastric pH to 5–6 and 8–9, respectively. The relevant pharmacokinetic parameters including C_{max}, t_{max}, $t_{1/2}$, AUC, and bioavailability (F) are listed in Table 1. Oral administration of BA99 and AZD0865 resulted in an elevation of BA99 levels up to C_{max} 70–80 μmol/L and 140–160 μmol/L, using microsuspensions and nanosuspensions, respectively, as formulations. The AUC/dose values were calculated to be 0.9–1.0 h·kg/L and 2.3–2.8 h·kg/L, respectively. t_{max} and $t_{1/2}$ between the trials were not further evaluated due to broad and flat peaks. These results (and in ref. 8) indicated that the effect of particle size on oral absorption varies depending on the physiological environment. The compound, as well as the combination of BA99 and AZD0865, was well-tolerated by the animals at high doses when administering suspensions. As shown in Table 1, the bioavailabilities of BA99, for each formulation and amount AZD0865 pre-administered, were calculated to be 6–7% and 15–18% (similar as in ref. 8) for administering microsuspensions and nanosuspensions of BA99, respectively. The calculations were performed on the basis of the AUC value obtained after i.v. administration of BA99 (5 mg/kg, 5 mL/kg, as nanosuspension, Figure 1). Upon these findings, nanosuspension formulations of BA99 (acting as a substitute for a neutral compound) could provide wider therapeutic safety margins when compared with formulations with larger particle sizes.

The plasma concentrations from nanosuspensions of the drug were higher than those from the formulations of larger particles, indicating a limit of absorption from larger solid particles. The plasma exposure after administering nanosuspensions of BA99 was only 10 times

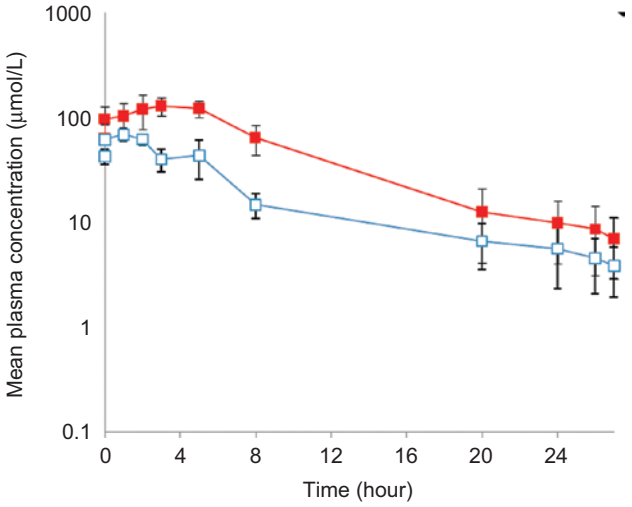

Figure 2. The mean (±SD) plasma levels of BA99 versus time after oral administration of BA99, as nanosuspension (filled symbols) and microsuspension (open symbols) at 500 μmol/kg to rats. In all cases, 5 mL/kg were administered. $N=4$ for each formulation. Rats were orally treated with 0.5 μmol/kg AZD0865 to maintain high gastric pH (5–6) before administration of BA99.

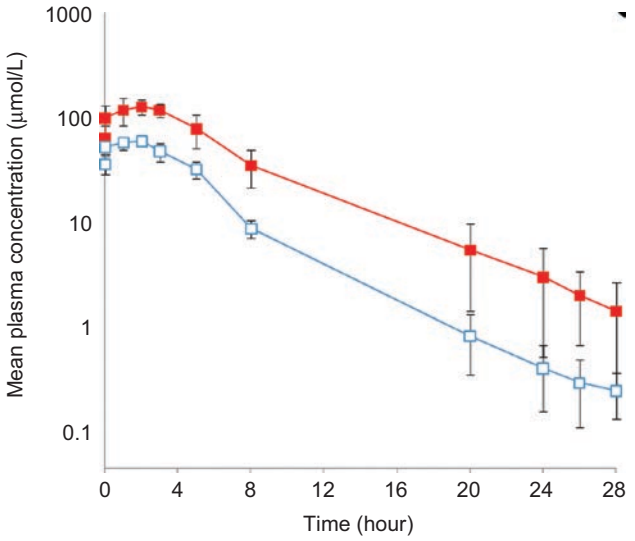

Figure 3. The mean (±SD) plasma levels of BA99 versus time after oral administration of BA99, as nanosuspension (filled symbols) and microsuspension (open symbols) at 500 μmol/kg to rats. In all cases, 5 mL/kg were administered. $N=4$ for each formulation. Rats were orally treated with 1.0 μmol/kg AZD0865 to maintain high gastric pH (8–9) before administration of BA99.

Table 1. Mean values of the pharmacokinetic parameters following oral administration of BA99 in different formulations as 500 μmol/kg doses (5 mL/kg) to rats ($n=4$, mean ± SD).

Formulation	Dose (μmol/kg)	C_{max} (μmol/L)	t_{max} (h)	$t_{1/2}$ (h)	AUC/dose (h·kg/L)	F (%)
Nanosuspension + 0.5 μmol/kg AZD0865	500	141 ± 23	4.3 ± 1.5	7.4 ± 1.4	2.8 ± 0.2	18 ± 2
Microsuspension + 0.5 μmol/kg AZD0865	500	69 ± 8	1.3 ± 0.5	4.5 ± 0.3	1.0 ± 0.1	6.9 ± 1.1
Nanosuspension + 1.0 μmol/kg AZD0865	500	158 ± 16	1.8 ± 0.9	4.7 ± 0.7	2.3 ± 0.2	15 ± 2
Microsuspension + 1.0 μmol/kg AZD0865	500	77 ± 6	1.5 ± 0.6	5.1 ± 1.0	0.9 ± 0.1	5.8 ± 0.5

The animals were pretreated, per oral (3.5 h prior BA99), with AZD0865 to obtain high gastric pH before administration of BA99. The parameters presented are AUC (area under individual plasma time curve, presented as AUC/dose), C_{max} (peak concentration), t_{max} (time to reach peak concentration), $t_{1/2}$ (apparent terminal half-time), and F (bioavailability). The statistical analysis was performed of AUC, C_{max}, and F for nanoparticles versus microparticles. The values were significantly higher when administering nanoparticles compared with the values after dosing microparticles ($P<0.05$).

greater after the 500 μmol/kg dose compared with the 5 μmol/kg dose (Figure 1), thus exhibiting dose nonlinear absorption.

Discussion

Our previous work described the exposure in plasma after administration of nanosuspensions and microsuspensions of a poorly soluble base (BA99) and a poorly soluble acid (AC88) administered to rats[8]. Both compounds had many physicochemical properties in common and similar particle sizes. For both substances, the milling approach was used to prepare the nanosuspensions. For the acidic compound, there was an obvious effect of particle size, the smaller particles resulting in higher systemic exposure. For the basic compound, on the other hand, with the present properties and doses, a nanosuspension did not increase the exposure (Figure 1). The higher solubility at gastric pH limits the need for particle size reduction. A significant part of the microsuspension was dissolved in the stomach, then reducing the differences in exposure between micronized and nanosized particles. The aim in the present report was to find out if there was a difference in plasma exposure, due to different particle sizes, when the present compound was treated as a neutral compound in the gastrointestinal tract, that is, if the compound was able to respond to the physiological environmental as a neutral compound was supposed to do. Another goal was to further confirm the advantage of dissolved drug when it reaches the small intestine to achieve maximal plasma exposure for a poorly soluble base[8]. One way to create a neutral compound is to remove the basic part of the compound and then form another chemical identity. However, the chemical structure of BA99 plays a pivotal role in its biological activity. Thus, a modification, like modulating the aromatic nitrogroup (responsible for the basic pK_a) to create a structure analog to BA99, may hamper the already optimized pharmacological efficacies. The other possibility is to change the physiological environment for the compound. In the present report, the latter approach was used. A proton pump inhibitor, AZD0865[9], developed at AstraZeneca, was administered before BA99 to adjust the gastric pH to 5–6 and 8–9, respectively. In this way, BA99 was exposed to an environment treating the compound as a "neutral-like" compound during the gastrointestinal journey.

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-limiting process in the bioavailability pathway[5,10]. The dissolution rate is supposed to be slower for larger particles, that is, at a specific concentration (and above it) the dissolution rate (and/or the solubility) is supposed to be rate-limiting, resulting in a better exposure for nanoparticles compared with microparticles (see below). For BA99, the larger surface area of the nanosuspensions in contact with the liquid environment in combination with the gastric pH favorable to dissolution was supposed to give optimal prerequisites for systemic exposure of BA99. A certain amount of the substance was dissolved when it entered the small intestine and ready for fast absorption, due to good permeability. The presence of bile salts ensured continued dissolution and absorption[11] (however, in the large intestine, there was less liquid and less substance will be dissolved and absorbed, resulting in less *F*, see below). Also, a significant part of the microsuspension was dissolved in the stomach, reducing the differences in exposure between micronized and nanosized particles. By adding AZD0865, the solubility in the gastric tract was abolished and the solubility decreased to <3 μM. By manipulating the surrounding pH for the substance, the environmental pH in the gastrointestinal tract was 5–6 and 8–9, respectively, and the basic properties were "lost." The behavior mimics the one for a neutral compound, and the particle size of the suspension become important. The larger particles do dissolve slower compared with the smaller ones, at both pHs (5–6 and 8–9). pH in the small intestine and colon is not supposed to be affected by the increase in gastric pH. When chyme (the stomach content) enters the upper part of the small intestine, it is quickly neutralized by the alkaline secretion of the Brunner`s glands and by the pancreatic bicarbonate output. In the present case, where the gastric pH was elevated, no influence on the intestinal pH was expected[12]. Then the data can be explained, discussed, and understood in terms of the classical theory of Noyes and Witney. Noyes and Witney's law can be used to evaluate the rate of dissolution[13] for preferable neutral compounds and acids:

$$dC/dt = DA(C_s - C)/h$$

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. These parameters in the equation can be considered as constant, except for A and C. Increasing A allows, to a first approximation, an improvement in the rate of dissolution. A can be increased by reducing the particle size[14–16]. Besides, the solubility is an important parameter and the relationship between these two properties, dissolution rate and solubility, will be further discussed below.

The oral absorption of neutral drugs such as danazol and naproxen largely depends on dissolution[14,17] in the intestine because neutral drugs (and acids) mainly dissolve in the intestine, not in the stomach. For compounds with the solubility in the low μM range, like danazol, naproxen, and BA99 ("neutral" during the present conditions), the presence of bile salts will

probably become necessary. The results from different simulated dissolution media clearly show that solubilization with bile salts is important in the oral absorption of poorly soluble drugs[18–20]. This is especially true *in vivo* for rats that lack a gall bladder, where the bile is stored and concentrated[12]. Instead, bile enters the small intestine continuously as it is produced. The amounts of bile solutions in rats (48 mL bile/kg body weight each day[21]) exceed the amounts in humans (2–22 mL bile/kg body weight each day[22]), the latter released only when chyme is present. Another way to increase the plasma exposure of microsuspensions of BA99 is to utilize the basic pK_a and increase the solubility of the drug in the gastric tract. That is, for a poorly soluble basic class II compound (with high permeability), the solubility in the stomach is of outmost importance for the subsequent optimal plasma levels.

Low solubility might lead to nonlinear dose-dependent oral absorption; that is, the fraction of dose absorbed decreases as dose increases. In preclinical studies, variable and nonlinear absorption from a low-solubility compound makes it difficult to evaluate the safety of a drug. Moreover, the efficacy and therapeutic window of a drug might be underestimated and, thus, the probability of successful development is diminished. Limited solubilization capacity of the gastrointestinal tract and the slower dissolution rate for larger particles resulted in a lower bioavailability when microsuspensions were used (after AZD0865 was administered). Moreover, in general, for drugs that are evenly absorbed over the entire intestinal tract, the rate of dissolution will not influence the extent of absorption. In the present case, the compound may have no or limited absorption in colon due to instability (chemical and/or physical), low solubility (due to, e.g. pH and low liquid volume), slow dissolution rate, and/or low permeability in the large intestine. The possible poor absorption in the colon suggested that the absorption of BA99 almost exclusively occurred in the upper gastrointestinal tract, supported by the good permeability achieved using Caco-2 cells (see Materials and methods). Efficient absorption in the small intestine is supported by the large surface area (about 200 m^2 in humans and 1 m^2 in rats), a result of the folds, villi and microvilli present here[4,12,23]. In contrast, the surface area in the large intestine is small (about 0.35 m^2 in humans and 0.034 m^2 in rats). Besides, during the 3–4 h residence time of the compound in the small intestine (for both humans and rats), a major fraction is supposed to be absorbed in this part of the intestine. Further support for the upper tract being the essential absorptive region is that in the micronized suspension a substantial portion of BA99 was dissolved in the gastric tract and then immediately absorbed when entering the upper part of the small intestine, thus reducing the difference in AUC between the micronized and nanosized particles. Elevating the gastric pH reduced the amount dissolved material (from a microsuspension) entering the upper intestinal tract and reduced the AUC, inducing an exposure difference between the formulations. The remaining intestine regions could not compensate for this initial "burst" of API. The above properties explain the lower bioavailability for the microparticles of BA99, compared with the nanoparticles of the drug, at both the pH intervals (pH 5–6 and 8–9).

A clear understanding of the rate-limiting process of oral absorption is crucial for improving oral absorption. Our results suggest that the rate-limiting steps on oral absorption shift between dissolution rate-limited and solubility-limited for BA99 with an increase in the administered dose. Oral absorption *in vivo* of BA99 was clearly improved by particle size reduction (after AZD0865 administration; Figures 2 and 3), suggesting that absorption of BA99 from larger particles was limited by the dissolution rate (at the present conditions). The absorption of BA99 did not show linear correlation with dose (5–500 μmol/kg; Figure 1), indicating that absorption from small particles at 500 μmol/kg was limited not only by the dissolution rate but also by solubility. In the case of solubility-limited absorption, dissolution occurs under non-sink conditions (i.e. the parameter C in the Noyes and Witney equation becomes more significant) and, thus, increases in the dissolution rate (reduction in particle size) or the administration dose do not lead to an even increase in plasma exposure. The amount of drug absorbed reaches saturation in exposure (for the specific formulation). Therefore, if the oral absorption is solubility-limited, it is difficult to improve the exposure of a drug either by increasing dose or by reducing particle size (even if a little improvement in solubility may follow further reduction in particle size[5]). Instead, increased solubility is most likely required. There are some commonly used approaches taking advantage of a time-limited supersaturation of the drug, like using salts, amorphous material, or a less thermodynamically stable polymorph of the compound[24–28]. Also, co-crystals may be a possible way forward to improve oral bioavailability[29,30].

Conclusion

BA99 (pK_a of 3, solubility of 3 μM at pH 6.8, and particle sizes of 280 nm and 12 μm) dissolves not only in the small intestine but also mainly in the stomach due to the low gastric pH, resulting in blood concentrations that is dissolution rate- and solubility-limited at the investigated doses, since exposure does not increase linearly with dose-administered[8]. When the gastric pH was elevated, BA99 dissolved in the small intestine and not so much in the stomach, confirming the importance of small particle size to the overall dissolution process. The presence of bile salts in the small intestine and the short gastric residence time contributed to the absorption of BA99 in the small intestine. Under normal conditions, the pK_a of the base and its gastric solubility is important for its plasma exposure and if a certain fraction is dissolved before reaching

the intestine the total exposure will be higher compared with if the substance was dissolved just in the intestine. It is more important for the absorption with favorable conditions in the gastric environment compared with the intestine environment. From the experiments performed with BA99 and AZD0865, one can argue for a marked improvement in pharmacokinetic behavior for nanosuspensions of a neutral compound with similar physicochemical properties as BA99, evidenced by an increase in blood concentrations after administering smaller particles. Nanosuspensions of poorly soluble neutral compounds are efficacious formulation approaches to enhance bioavailability. In addition, the physiological conditions in the gastrointestinal lumen can have an obvious effect on the dissolution of certain drugs.

Declaration of interest

The authors report no declarations of interest.

References

1. Amidon GL, Lennernäs H, Shah VP, Crison JR. (1995). A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm Res, 12:413–420.
2. Yu LX, Amidon GL, Polli JE, Zhao H, Mehta MU, Conner DP et al. (2002). Biopharmaceutics classification system: the scientific basis for biowaiver extensions. Pharm Res, 19:921–925.
3. Kasim NA, Whitehouse M, Ramachandran C, Bermejo M, Lennernäs H, Hussain AS et al. (2004). Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. Mol Pharm, 1:85–96.
4. Hörter D, Dressman JB. (2001). Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. Adv Drug Deliv Rev, 46:75–87.
5. Kesisoglou F, Panmai S, Wu Y. (2007). Nanosizing—oral formulation development and biopharmaceutical evaluation. Adv Drug Deliv Rev, 59:631–644.
6. Gao L, Zhang D, Chen M. (2008). Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. J Nanopart Res, 10:845–862.
7. Sigfridsson K, Forssén S, Holländer P, Skantze U, de Verdier J. (2007). A formulation comparison, using a solution and different nanosuspensions of a poorly soluble compound. Eur J Pharm Biopharm, 67:540–547.
8. Sigfridsson K, Lundqvist AJ, Strimfors M. (2011). Particle size reduction and pharmacokinetic evaluation of a poorly soluble acid and a poorly soluble base during early development. Drug Dev Ind Pharm, 37:243–251.
9. Andersson K, Carlsson E. (2005). Potassium-competitive acid blockade: a new therapeutic strategy in acid-related diseases. Pharmacol Ther, 108:294–307.
10. Dressman JB, Reppas C. (2000). *In vitro-in vivo* correlations for lipophilic, poorly water-soluble drugs. Eur J Pharm Sci, 11 (Suppl 2):S73–S80.
11. Sutton SC. (2004). Companion animal physiology and dosage form performance. Adv Drug Deliv Rev, 56:1383–1398.
12. DeSesso JM, Jacobson CF. (2001). Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats. Food Chem Toxicol, 39:209–228.
13. Nernst W, Brunner E. (1904). Theorie der reaktionsgeschwindigkeit in heterogenen systemen. Z Physik Chemie, 47:52–110.
14. Liversidge GG, Cundy K. (1995). Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. Int J Pharm, 125:91–97.
15. Müller RH, Jacobs C, Kayser O. (2001). Nanosuspensions as particulate drug formulations in therapy. Rationale for development and what we can expect for the future. Adv Drug Deliv Rev, 47:3–19.
16. Wu Y, Loper A, Landis E, Hettrick L, Novak L, Lynn K et al. (2004). The role of biopharmaceutics in the development of a clinical nanoparticle formulation of MK-0869: a Beagle dog model predicts improved bioavailability and diminished food effect on absorption in human. Int J Pharm, 285:135–146.
17. Liversidge GG, Conzentino P. (1995). Drug particle reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats. Int J Pharm, 125:309–313.
18. Dressman JB. (1986). Comparison of canine and human gastrointestinal physiology. Pharm Res, 3:123–131.
19. Kalantzi L, Persson E, Polentarutti B, Abrahamsson B, Goumas K, Dressman JB et al. (2006). Canine intestinal contents vs. simulated media for the assessment of solubility of two weak bases in the human small intestinal contents. Pharm Res, 23:1373–1381.
20. Jantratid E, Janssen N, Reppas C, Dressman JB. (2008). Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. Pharm Res, 25:1663–1676.
21. Kararli TT. (1989). Gastrointestinal absorption of drugs. Crit Rev Ther Drug Carrier Syst, 6:39–86.
22. Dressman JB, Yamada K. (1991). Animal models for oral drug absorption. In: Welling PG, Tse FLS, Dighe SV. (Eds.), Pharmaceutical Bioequivalence. Marcel Dekker, New York, pp. 235–266.
23. Watts PJ, Illum L. (1997). Colonic drug delivery. Drug Dev Ind Pharm, 23:893–913.
24. Van den Mooter G, Wuyts M, Blaton N, Busson R, Grobet P, Augustijns P et al. (2001). Physical stabilisation of amorphous ketoconazole in solid dispersions with polyvinylpyrrolidone K25. Eur J Pharm Sci, 12:261–269.
25. Hirasawa N, Ishise S, Miyata H, Danjo K. (2003). An attempt to stabilize nilvadipine solid dispersion by the use of ternary systems. Drug Dev Ind Pharm, 29:997–1004.
26. Morissette SL, Almarsson O, Peterson ML, Remenar JF, Read MJ, Lemmo AV et al. (2004). High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids. Adv Drug Deliv Rev, 56:275–300.
27. Pudipeddi M, Serajuddin AT. (2005). Trends in solubility of polymorphs. J Pharm Sci, 94:929–939.
28. Serajuddin AT. (2007). Salt formation to improve drug solubility. Adv Drug Deliv Rev, 59:603–616.
29. McNamara DP, Childs SL, Giordano J, Iarriccio A, Cassidy J, Shet MS et al. (2006). Use of a glutaric acid cocrystal to improve oral bioavailability of a low solubility API. Pharm Res, 23:1888–1897.
30. Schultheiss N, Newman A. (2009). Pharmaceutical cocrystals and their physicochemical properties. Cryst Growth Des, 9:2950–2967.