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ORIGINAL ARTICLE

Particle size reduction and pharmacokinetic evaluation of a poorly soluble acid and a poorly soluble base during early development

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Abstract

Aim: The aim of the present study was to find out if nanosuspensions were a better choice compared with microsuspensions, for the present substances with water solubility in the order of 2–3 μM (pH 6.8, small intestinal pH) and no permeability limitations. The ambition was also to understand what the higher solubility in the stomach for BA99 means in terms of absorption properties of the substance. *Method*: The pharmacokinetic parameters of a poorly soluble acid (AC88) and a poorly soluble base (BA99) administered orally as nanosuspensions have been compared with those from microsuspensions using rat as in vivo species. *Results*: A significant difference was observed between the two suspensions for AC88 already at the lowest dose, 5 μmol/kg (the particle size of the nanosuspensions and the microsuspensions was about 200 nm and 14 μm, respectively). These results were further confirmed at a high dose (500 μmol/kg). However, for BA99, there were no significant differences between the two formulations at any dose investigated (the particle size of the nanosuspensions and the microsuspensions was about 280 nm and 12 μm, respectively). *Conclusions:* The study demonstrated a clear correlation between particle size and in vivo exposures for an acidic compound, the nanosuspensions providing the highest exposure. For a basic compound, on the other hand, with the present properties and doses, a microsuspension was sufficient. In the latter case, the higher solubility at gastric pH, because of the basic pK_a, limits the need for particle reduction.

Key words: Dissolution rate, nanosuspension, pharmacokinetic, poorly soluble, suspension

Introduction

The bioavailability of a drug administered by the oral route depends primarily on its ability to be absorbed by the intestinal tract[1,2]. The main absorption mechanism is a passive diffusion[3,4]. Drugs absorbed in this way must dissolve in the intestinal fluids before diffusion through the membrane. The amount of drug absorbed then depends on its solubility characteristics. Some drugs are highly soluble and it is relatively easy to obtain a good rate of dissolution with any type of formulation. The parameter limiting the absorption, in this case, is the permeability of the intestinal membrane to the compound. However, the solubility is often low that reduces the rate of dissolution. This is especially the case for newer drugs, which may have a complex structure. In these cases, the drug formulation is important because it is the main parameter dictating the rate of dissolution of active materials as absorption is determined by the amount of substance dissolved. The bioavailability of a drug is classically defined as the fraction of the administered dose that reaches the systemic circulation. In addition, the rate at which this process is done is an important parameter to be considered in the pharmacokinetic evaluation. The main, and often the only, parameter that can improve the bioavailability of a poorly soluble drug is its rate of dissolution in the intestinal

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lumen contents. Noyes and Whitney's law can be used to evaluate the rate of dissolution[5]:

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

where dC/dt is the rate of dissolution of the drug particles, D the diffusion coefficient of the drug in the gastrointestinal fluids, A the effective surface area of the drug particles in contact with the gastrointestinal fluids, h the thickness of the diffusion layer around each drug particle, C_s the saturation solubility of the drug in solution in the diffusion layer, and C 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[6–10].

Nanosizing refers to the reduction of particle size down to submicron range. Recent advances in milling technology, using crystalline material, and the understanding of colloidal systems have resulted in reproducible production of particles in 100–500 nm sizes[6,8–15]. The particles in nanosuspensions are stabilized with mixtures of surfactants and/or polymers[13,16]. By adding a suitable tonicity modifier, the formulation may be used for intravenous (i.v.) administration. The formulations could be further processed into standard dosage forms such as capsules and tablets suitable for oral administration. Nanocrystalline formulations will increase the dissolution rate, improve bioavailability, reduce variability, and eliminate food effects for orally administered drugs[16]. Five oral nanoformulations are currently on the market (Emend, Megace, Rapamune, TriCor, and Triglide[8,16,17]) with several more to come. One or more of the mentioned advantages obtained by nanosizing caused the companies to select the present formulation approach.

In this article, a comparison was made between the crystalline nano- and microsuspensions of AC88 and BA99, at two doses, administered to rats. The comparison was made to find a suitable formulation, for each substance, which was expected to give a high exposure after administering high doses in toxicological studies. Besides, there is an economic factor involved. Less amount of a compound in the formulation results in lower cost of goods. Another aim was to try to confirm that the nanosuspension approach is not necessarily the optimal one for a poorly soluble compound in general, but depends on physicochemical parameters, like acid or base properties of the specific compound. Two prerequisites for selecting the present compounds were that the particle size of the different suspensions was similar for the compounds (i.e., the particle size was similar for the nanosuspensions of the two compounds and for the microsuspensions) as well as the solubility in the intestine. The compounds have high permeability and low solubility in the gastrointestinal tract, thus fulfilling the criteria for a BCS II compound[18,19].

Material and methods

Test compounds

The acid AC88 has a molecular weight of 456 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 base BA99 has a molecular weight 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 capillary electrophoresis–mass spectrometry) to 3. There is also an acidic pK_a at 7.2. Estimated log D at pH 6.8 (from $k' = 13.1$, obtained by liquid chromatography (LC)–mass spectrometry) is 5. The solubility in a water solution is about 3 μM, at 22°C (measured from solid crystals, pH 6.8). The P_{app} values in the Caco-2 experiment were $>20 \times 10^{-6}$ cm/s at low micromolar concentrations, for both substances, with no indication of efflux. The substances are typical BCS II compounds, that is, drugs having good permeability but low solubility, making them attractive candidates for particle size reduction before administration.

Chemicals

HPMC (hydroxypropyl methylcellulose, 15000 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[17,20]. The disodium salt of Aerosol OT (AOT) from Cytec Industries Inc. (Woodland Park, NJ, USA) is a surface-active agent with functions similar to PVP. Mannitol was bought from Sigma (Steinheim, Germany) and used as a tonicity modifier and as a cryoprotectant during freezing.

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 minutes and stirred overnight. The particle size (diameter) of the suspensions was measured by laser diffraction (Malvern Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK).

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. About 10% (w/w) of crude suspension was stirred and treated with ultrasound for 10 minutes, which gave a well-dispersed slurry. About 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 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 particle size (diameter) of the crystalline suspension 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-week-old on the day of arrival at AstraZeneca R&D Mölndal. 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 per 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-hour 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 (ip), 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 using isoflurane (Forene®, Abbott, 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, Uppsala, 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 with single injection of 5 mL/kg into vena jugularis through the implanted venous cannula. The oral doses were given as single doses directly into the stomach, using gavage. The dose volumes were 5 mL/kg (low dose) and 5 mL/kg (high dose), respectively.

Blood sampling

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

Bioanalytical methods

Compound concentrations in the plasma samples were analyzed by LC–mass spectrometry. Briefly, a gradient elution on a short C_{18} column was used with acetonitrile/formic acid as the mobile-phase system. The plasma samples were protein precipitated by acetonitrile and diluted after centrifugation.

Pharmacokinetic evaluation

The pharmacokinetic calculations are based on the individual plasma concentration–time data. The calculations were made with the computer program WinNonlin™ Professional version 3.1 (Pharsight Corporation, 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 ruled 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 natural log (ln) of 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_{iv}, 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 the t-test approach.

Results

Pharmaceutical characterization of suspensions of AC88

According to the experimental data, the solubility of crystalline AC88 in water is very low of about 2 μM (pH 6.8). The substance is ionizable, with increasing

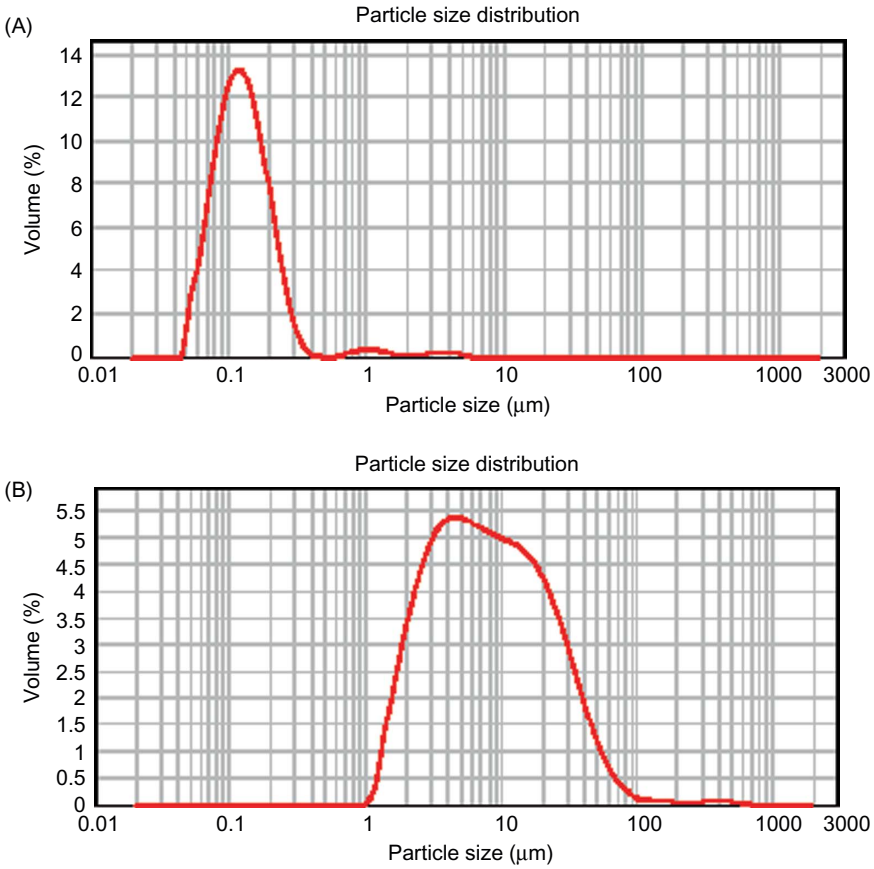

Figure 1. (A) Particle size distribution curve of AC88 as nanosuspension. (B) Particle size distribution curve of AC88 as microsuspension.

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 200 nm (>90%, <230 nm) and of about 14 μm (>90%, <25 μm) for nano- and microsuspensions, respectively (Figure 1). 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 chemically and physically stable for at least 1 month at room temperature.

Pharmaceutical characterization of suspensions of BA99

The solubility of BA99 was determined in water. According to the experimental data, the solubility of crystalline BA99 in water is very low, about 3 μM (pH 6.8). A freshly prepared nanosuspension contained particles around 280 nm (>90%, <400 nm) (Figure 2A). 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. If the intention is to store the nanosuspension frozen, it is important to add mannitol to keep the particle size[21]. Also the microsuspensions (1 and 100 mM) were chemically and physically stable (about 12 μm with >90%, <25 μm, Figure 2B) for at least 1 month at room temperature. The crystal structure was determined by X-ray diffraction to be unchanged by the dispersion and milling process (data not shown).

In vivo studies in rats using AC88

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) AC88. One nano- and one microsuspension were administered at each dose level. The mean plasma levels obtained for the formulations are presented in Figure 3. 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 3). All the substance was dissolved in the blood after i.v. administration, which could

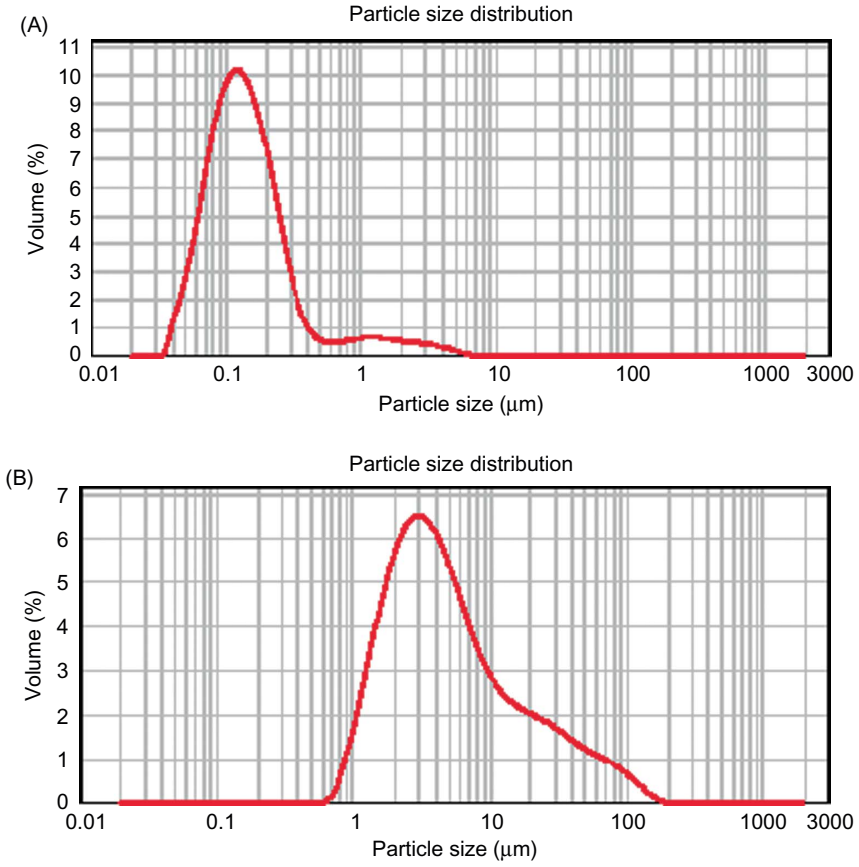

Figure 2. (A) Particle size distribution curve of BA99 as nanosuspension. (B) Particle size distribution curve of BA99 as microsuspension.

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 3). 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 1 for the oral formulations. Also AUC is presented in Table 1. The values of F 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. At the low dose, C_{max} and AUC for the animals receiving nanosuspensions were about 4 times larger compared to the dose group that received microsuspensions. This ratio between the two formulation groups was similar to the high dose. The half-life and t_{max} were not altered between the suspensions or between the dose levels. Moreover, F of AC88 using nanosuspensions (low dose: 70%; high dose: 7%) was significantly higher than when microsuspensions (low dose: 20%; high dose: 1–2%) were administered, comparing the same dose. Besides, the value of F decreased with dose within the two suspension types administered.

In vivo studies in rats using BA99

The same formulation approach was used for BA99 as for AC88 above, that is, one low (5 µmol/kg, 5 mL/kg) and one high (500 µmol/kg, 5 mL/kg) dose of nanosuspensions as well as of microsuspensions. The mean plasma levels obtained for the formulations are presented in Figure 4. Four animals were used for each formulation. 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 4). All substance was dissolved in the blood after i.v. administration (see above). There were no indications that the animals did not tolerate the formulations given orally or i.v. The main pharmacokinetic parameters are shown in Table 2.

In contrast to AC88, there were no significant differences in C_{max} or AUC for the animals receiving microsuspensions of BA99 or the nanosuspensions of the compound at any of the two doses administered. The increase in exposure was less than dose linear. The half-life and t_{max} were not altered between the two different suspensions or between the dose levels. Moreover, F of BA99 using nanosuspensions (low dose: 85%; high dose: 16%) was similar to the values obtained after the administration of microsuspensions (low dose: 76%; high dose: 11%), comparing the same dose. Besides, the value of F decreased with dose within the two suspension types administered. The results showed that the main

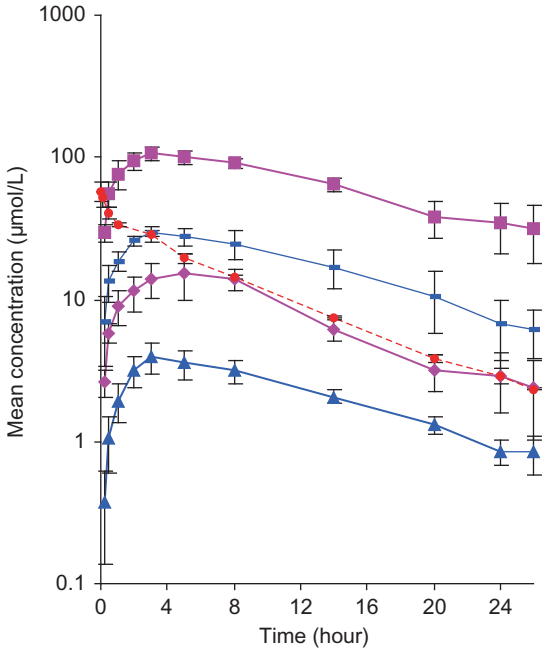

Figure 3. The mean plasma levels of AC88 versus time after oral administration (and i.v. administration, as nanosuspension 5 µmol/kg, dotted line) of AC88 as nanosuspensions (◆) and microsuspension (▲) at 5 µmol/kg to rats. At a higher dose, 500 µmol/kg, was a nanosuspension (■) and a microsuspension (–) administered. In all cases, 5 mL/kg doses were administered. $n = 4$ for each formulation, except for the i.v. administration, where $n = 2$.

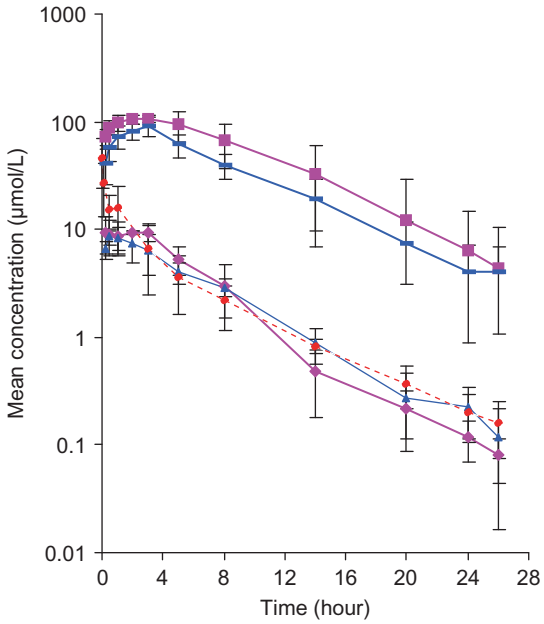

Figure 4. The mean plasma levels of BA99 versus time after oral administration (and i.v. administration, as nanosuspension 5 µmol/kg, dotted line) of BA99, as nanosuspensions (◆) and microsuspension (▲) at 5 µmol/kg to rats. At a higher dose, 500 µmol/kg, was a nanosuspension (■) and a microsuspension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

pharmacokinetic parameters obtained from BA99 in rat blood, administered as micro- and nanometer-sized particles, were not significantly different.

Discussion

For neutral drugs, like danazol[6], 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 permit i.v. administration, see discussion below). On a laboratory scale, crystalline nanosuspensions are prepared by a milling procedure[6,22,23]. The obtained formulations contain low amounts of additives and are, thus, expected to induce minimal side effects in various in vivo studies. Besides on neutral compounds, the nanosuspension approach should preferably be applied on acidic compounds[16]. 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 AC88), 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 contrary, 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

Table 1. Mean values of the pharmacokinetic parameters following oral administration of AC88 in different formulations as 5 and 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 (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

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.

*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. The differences between nano- and microsuspensions (C_{max}, AUC, and F) are significant ($P < 0.05$).

Table 2. Mean values of the pharmacokinetic parameters following oral administration of BA99 in different formulations as 5 and 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 (h × kg/L)	F (%)
Nanosuspension	500	114 ± 6	3 ± 1.2	4.4 ± 1.3	2.4 ± 0.4	16 ± 4
Microsuspension	500	92 ± 16	3 ± 0	4.5 ± 0.9	1.6 ± 0.2	11 ± 2
Nanosuspension	5	11 ± 2	1.8 ± 1.1	4.4 ± 0.8	12.8 ± 2.8	85 ± 18
Microsuspension	5	9 ± 2	1.0 ± 0.6	4.9 ± 0.9	11.5 ± 1.1	76 ± 7

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.

properties of the substance[8,14,16]. In this article, crystalline nanosuspensions of the acidic substance AC88 were prepared and compared in vivo with microsuspensions of the compound at two different doses. Besides, the basic compound, BA99, having similar properties as AC88 on the 'acidic side' but with more substance dissolved in the stomach, was investigated in parallel during similar conditions.

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[16,24]. 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 AC88. Obviously, the dissolution rate of the microparticles was significantly 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 hundred times of the higher dose, but remained about 4 : 1. This further indicates that the dissolution rate is the major rate-determining step and the ratio is similar because 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 is supposed to be mainly solubility limited). However, for the basic compound, 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 the systemic exposure of BA99. A certain amount of the substance was dissolved when it entered the small intestine (With some risk for uncontrolled precipitation when the drug and the gastric fluid were mixed with the fluid secreted in the intestine. The main part was, however, conserved as nanoparticles.) and ready for fast absorption, because of good permeability, and the acidic pK_a ensured continued dissolution and absorption (however, in the large intestine, there was less liquid and less substance will be dissolved and absorbed, resulting in less F; see below). In this case, also a significant part of the microsuspension was dissolved in the stomach, then reducing the differences in exposure between micro- and nanosized particles. Notable is the fact that both suspensions, for both substances, 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 (AC88). 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 this case, the compounds may have no or limited absorption in the colon because of instability (chemical and/or physical), low solubility, slow dissolution rate, and/or low permeability in the large intestine. The possible poor absorption in the colon suggested that the absorption of AC88 and BA99 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 substances and even the lower absorption and bioavailability of the microsuspension of AC88 compared with BA99 (for which a significant amount of the substance was already dissolved when entering the small intestine).

The i.v. administration confirmed that nanosuspensions of AC88 and BA99 could be administered without adverse events to rats (at the present dose), that is, neither the substances 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[21,25]. 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[26], 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, for example, 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 that are due to limiting physicochemical properties of the substance and formulation and not due to toxicological effects of the compound. However, the alternative is not to administer i.v. at all. Therefore, particle size 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 for an acidic compound (AC88) with low solubility, nanosuspensions providing the highest exposure at both investigated doses: 5 and 500 μmol/kg. On the contrary, for a base, with similar acidic properties as AC88, but with a basic pK_a as well (and a substantial portion of BA99 dissolved before entering the small intestine), abolished the effect of differences in particle size. The explanation for the low bioavailability of the micronized suspension of AC88 was due to insufficient drug dissolution (and solubility) achieved within the limited small intestinal transit time. Faster dissolution from the nanometer-sized particles resulted in an approved absorption in this narrow window. Besides, the high dose of both suspensions, for both substances, was too high to be able to reach the bioavailability value, which was reached at the low dose.

For BSC II compounds, nanosuspensions appear to be an attractive alternative to a microsuspension, especially for acidic compounds (and neutral compounds in the in vivo pH range). Besides, a marked increase in exposure can lead to reduced cost of goods. For basic compounds, microsuspensions may be good enough, depending mainly on pK_a, gastric solubility, and permeability. With regard to i.v. administration, nanosuspensions may be the only alternative for a poorly soluble compound. However, at least particle size, physical stability, and dose in relation to solubility in plasma are crucial parameters to evaluate before considering i.v. administration.

Declaration of interest

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

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