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Research paper

A formulation comparison, using a solution and different nanosuspensions of a poorly soluble compound

Kalle Sigfridsson [a,*], Sara Forssén [a], Paula Holländer [b], Urban Skantze [a], Jennie de Verdier [b]

[a] Pharmaceutical and Analytical R&D, AstraZeneca R&D Mölndal, Mölndal, Sweden
[b] Discovery Drug Metabolism and Pharmacokinetics & Bioanalytical Chemistry, AstraZeneca R&D Mölndal, Mölndal, Sweden

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Abstract

The pharmacokinetic parameters of AZ68 administered as a solution have been compared with those from an amorphous and a crystalline nanosuspension using rats as *in vivo* specie. All formulations were administered intravenously (i.v.) and orally. The purpose of the study was to find out if the three different formulations were comparable and safe to administer. The results indicate that AZ68 is absorbed at a lower rate for crystalline nanosuspensions compared to amorphous nanosuspensions and solutions. However, the absorbed extent of the compound is similar. The results are a consequence of the lower solubility and the slower dissolution rate for crystalline material compared to amorphous substance in the gastrointestinal tract. The dissolution process is excluded for a solution, resulting in the fastest absorption rate. No significant difference was found between pharmacokinetic parameters when comparison was made between the formulations after i.v. administration. There were no adverse events observed after i.v. administration of the nanosuspensions.

Keywords: NK receptor; Neurokinin; Nanosuspensions; Pharmacokinetic

1. Introduction

A significant proportion of drugs on the market are poorly soluble in water and it is expected that this will be even more pronounced in the future [1,2]. Formulations of poorly water-soluble compounds are a resource demanding challenge. During the discovery phase, new compounds are evaluated by both *in vitro* and *in vivo* studies, in which liquid formulations are used frequently. Poorly soluble compounds can be formulated e.g. as aqueous pH-shifted solutions, provided the molecules are ionizable, in mixtures of water and organic cosolvents, or by solubilization in cyclodextrin [3–5] or using emulsions [6–8]. With the exception of the pH-shifted aqueous solutions, significant amounts of additives are often needed to increase the solubility into the millimolar range, required for most animal studies, which may induce unwanted side effects [9,10]. It would be more desirable to have a universal formulation approach to process any poorly soluble drug. This is of particular interest for drugs being poorly soluble in aqueous media and simultaneously in organic media, thus excluding all formulation approaches involving any solvent mixture. An interesting alternative to the first category (i.e. compounds poorly soluble in water) is amorphous nanosuspensions with typical particle sizes of the order of 100–200 nm [11–13]. To obtain an amorphous nanosuspension, the drug is first dissolved in an organic water-miscible solvent and the resulting solution is then rapidly mixed with an aqueous stabilizer solution. The mechanism of particle formation by precipitation after a solvent quench has been studied in several recent papers [12–16].

* Corresponding author. AstraZeneca R&D Mölndal, Pharmaceutical and Analytical R&D, Early Development, S-431 83 Mölndal, Sweden. Tel.: +46 31 7762246; fax: +46 31 7763768.
E-mail address: carl-gustav.sigfridsson@astrazeneca.com (K. Sigfridsson).

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For crystalline compounds, which are poorly soluble in water and maybe also in organic solvents, a second approach may be needed. A classical formulation approach for such poorly soluble drugs is micronization where a coarse drug powder is milled to an ultrafine powder with a mean particle size being typically in the range of 1–10 μm [17–21]. The principle is to increase the dissolution velocity by enlarging the surface area of the drug powder. Micronization is a technology for class II drugs of the biopharmaceutical classification system (BCS), i.e. drugs having a good permeability and poor solubility [22–24]. The consequence of these properties may be low dissolution rate followed by low oral bioavailability. Nowadays, many of the new drugs exhibit so low solubility, that micronization does not lead to a sufficiently high bioavailability. Consequently, the next step was taken to move from micronization to nanometer sized particles, that means producing drug nanocrystals (typically between 200 and 500 nm) [25–27].

There are two basic disintegration technologies for drug nanocrystals: bead/ball milling [28] and high-pressure homogenization [29] with different homogenizer types/homogenization principles. Only the first technique was used in the present study.

In the milling approach, the drug macrosuspension is filled into a milling container. Milling beads from, e.g. glass, zirconiumoxide or special polymers such as hard polystyrene derivatives, are then added to the vessel. Using a planetary mill, the containers are rotated at high speed and the drug is ground to nanocrystals in between the beads.

In the present article, a comparison was made between a solution, a crystalline nanosuspension and an amorphous nanosuspension of AZ68, administered to rats. The purpose of the study was to find out if the three different formulations were comparable and safe to administer. AZ68 is a neurokinin NK receptor antagonist intended for schizophrenia treatment. The compound has high permeability and low solubility in the gastrointestinal tract, thus fulfilling the criteria for a BCS II compound and hence chosen for the present study.

2. Materials and methods

2.1. The test compound

AZ68 has a molecular mass of 380 g/mol. The substance is a crystalline compound with a melting point of about 130 °C (ΔH_m = the enthalpy of melting is 85.2 J/g, determined by Differential Scanning Calorimetry, DSC). The pK_as were measured (by CE-MS) to 3 (basic pK_a, related to an aromatic nitrogen) and 7.2 (acidic pK_a, related to an aromatic hydroxy group). Estimated log D at pH 6.8 (from $k' = 13.1$, obtained by LC-MS) is 5. The Papp value in the Caco-2 experiment was $>70 \times 10^{-6}$ cm/s in both directions at low μM concentrations. AZ68 is a typical BCS II compound, i.e. a drug having good permeability, but a low solubility, making it an attractive candidate for particle size reduction before administration.

2.2. Chemicals

N,*N*-Dimethylacetamide (DMA) was bought from Aldrich. PEG400 was bought from Hoechst (recently included in the Sanofi-Aventis group) and HP-β-cyclodextrin from Roquette. Sodium dodecyl sulphate (SDS) is an anionic surface-active agent, which was obtained from Millchem UK Ltd. Polyvinylpyrrolidone K30 (PVP) is a non-ionic polymer, which was bought from BASF. SDS and PVP (a surfactant and a polymer) are both stabilizers and are expected to cover the surface of the pure drug when dispersed in water [30,31]. The disodium salt of Aerosol OT (AOT) from Cytec Industries Inc is another surface-active agent. Miglyol 812N, used here as an Ostwald ripening inhibitor [12,13], is from Hüls (recently named Degussa-Hüls) and is a 60/40 (w/w) mixture of C8 and C10 triglycerides (Ostwald ripening is a process where the difference in (local) solubility, as a function of the particle size, leads to a transport of material from small to larger particles, with an accompanying increase in the mean particle size with time [32]). Mannitol was from Sigma and used as a tonicity modifier and as a cryoprotectant during freezing.

2.3. Determination of AZ68 solubility

The solubility of AZ68 in water, 60% PEG400, 30% HP-β-cyclodextrin and PEG400:DMA:water (1:1:1) (w/w/w) was determined by adding an excess of the crystalline drug into the solvent. The suspensions were stirred on a magnetic stirrer at 22 °C for 24 h, filtered (cut-off 0.22 μm, Millex-GV, PVDF, Millipore, Carrigtwohill, Co. Cork, Ireland) and the content of dissolved AZ68 was analyzed by HPLC.

Using the experimental data for the crystalline solubility in water, it is possible to calculate the amorphous solubility using the equation below (see [12,13] and references therein):

$$S_a^0 = S_c \exp\left\{\frac{\Delta S_m}{R} \ln\{T_m/T\}\right\}$$

where S_a^0 is the amorphous solubility of pure substance, S_c is the crystalline solubility, ΔS_m is the entropy of melting, that is obtained from $\Delta S_m = \Delta H_m/T_m$, where ΔH_m is the enthalpy of melting and T_m is the melting temperature. Finally, R is the gas constant and T is the absolute temperature.

2.4. Preparation of amorphous nanosuspensions

Amorphous nanosuspensions of AZ68 were prepared by rapidly injecting a drug solution (typically 100 mM drug dissolved in DMA) into an aqueous stabilizer solution in a vial placed on an ultrasonic bath (Elma Transsonic bath T460/H). The stabilizer solutions contained 0.2% (w/w)

PVP and 0.25 mM SDS in all cases. Ostwald ripening inhibitor used was Miglyol. The drug/inhibitor ratio was in all cases 4:1 (w/w). Typically 250 μl stock solutions of 100 mM AZ68 in DMA containing 4:1 drug:miglyol (w/w) were added to 4.75 ml stabilizer solution. The final formulation typically contained 5% DMA. For some critical *in vivo* experiments, the organic solvent can be removed by dialysis. When the nanosuspension was administered i.v., 5% (w/w) mannitol was added (if DMA were removed) in order to obtain an isotonic formulation. The average particle size of the amorphous suspensions was measured by Dynamic Light Scattering, DLS, using Fiber Optic Quasi Elastic Light Scattering (FOQELS, Brookhaven Instruments Corporation).

The formulations were examined by XRPD (Powder X-ray Diffraction, data not shown). The formulations were completely amorphous.

2.5. Preparation of crystalline nanosuspensions

Typically about 60 mg of the drug was weighed 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 ultrasonics for 10 min, which gave a well-dispersed slurry. Five-hundred and ten microliters 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 Micro mill P7. The milled suspension was collected and the milling beads were rinsed with water. The volume averaged particle size (diameter) of the crystalline suspensions was measured by laser diffraction to ensure that there was no fraction of the material that had not been properly reduced in size in the milling process (Mastersizer 2000, Malvern Instruments Ltd). The suspension was diluted to 5 mM with or without 5% mannitol. In the latter case was a 5% (w/w) mannitol solution used. Further measurements (i.e. stability studies) of the average particle size of the crystalline suspensions were carried out by DLS (Zetasizer Nano, Malvern Instruments Ltd). The two different DLS equipments (used for the two kinds of nanosuspensions) give comparable results.

XRPD were performed on the compound before and after the milling (data not shown). No changes in solid state were observed.

2.6. 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 TFA (trifluoroacetic acid).

2.7. Animal handling

The test system consisted of starved female Sprague–Dawley rats (Harlan, The Netherlands), approximately 11 weeks old on the day of arrival at AstraZeneca R&D Mölndal. The rats were housed in plastic rat cages (2 animals/cage) with aspen wood-chips (Tap-Vei, Kortteinen Kaawi, Finland) as bedding material. They were kept at 18–22 °C, and at a relative humidity of 30–60% under a 12-h light/dark cycle and had free access to food (R3, Lactamin AB, Vadstena, Sweden) and tap water. The rats were allowed to acclimatize for three weeks (weight ~250 g) after arrival at AstraZeneca R&D Mölndal.

All animals were killed, by an overdose of pentobarbitalnatrium, after the last blood sample had been collected.

2.8. Surgery

Two days prior to dosing, the rats were prepared by cannulation of the left carotid artery for blood sampling and the right jugular vein in cases for i.v. administration. 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 under isoflurane (Forene®, Abbott) anesthesia. The rats were given 0.5 ml/kg Romefen®Vet (ketoprofen 10 mg/ml, Merial) and 10 ml Rehydrex®Med (glucose 25 mg/ml, Fresenius Kabi AB) subcutaneously after surgery. They were then housed individually and had free access to food and water throughout the study. A label on the cage identified each rat. The animals were left to recover until administration of the test compounds.

2.9. Dosing

I.v. bolus injection was given via the catheter in the jugular vein (5 μmol/kg, 1 ml/kg). The oral dose was given by gavage (10 μmol/kg, 2 ml/kg).

2.10. Blood sampling

The systemic plasma concentration of AZ68 was followed up to 24 h after dosing. The samples were taken after 2, 5, 15 and 30 min and after 1, 2, 3, 5, 8 and 24 h. The 2 min sample was excluded for the oral experiments.

Blood samples of about 0.1 ml were collected from the arterial cannula. The cannula was flushed with physiological saline containing heparin (100 IE/ml) after each sample. The blood samples were collected in heparinized plastic tubes (Microvette®, Sarstedt) and kept on ice until plasma separation (5 min, 10,000 g, +4 °C). The plasma was then transferred to 96-deep well plates and stored at about −20 °C until analysis.

2.11. Bioanalytical methods

Plasma samples were analyzed by a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, which is described in an internally documented method. An Agilent 1100 LC pump (Agilent Technologies,

Waldbronn, Germany) was used with gradient elution at flow rate of 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 HyPURITY column with 5 μm particle size (Thermo Electron Corporation, Waltham, MA, USA) using a linear gradient of 5–90% B in 2 min, held at 90% for 1 min and returned to initial conditions in one step. The front was diverted to waste by using a 6-port valve (VICI AG, Schenkon, Switzerland) and after 0.5 min the effluent entered the MS without splitting. Sample storage and injection was performed with a CTC HTS Pal autosampler (CTC Analytics, Zwingen, Switzerland). 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 0.2% formic acid and internal standard. After vortexing, the plasma samples were centrifuged for 20 min at 2750 *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 reaction monitoring (MRM) using a Micromass Quattro LC triple quadropole (Waters, Manchester, UK). Instrument control, data acquisition and data evaluation were performed using Masslynx 4.0.

2.12. Pharmacokinetic evaluation

The pharmacokinetic calculations are based on individual plasma concentration–time data. Non-compartmental analysis was performed using Excel Template for Activity-Base v. The maximum plasma concentration (C_{max}) and the time at which it occurred (t_{max}) were determined. 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 area under the plasma concentration–time profile (AUC) was calculated by the linear/log trapezoidal rule up to the last data point plus the residual area up to infinity. The residual area was calculated by integration, C_p/k, where C_p is the predicted plasma concentration at the last measurable sampling point and k is the terminal slope of the ln plasma concentration-time curve. The bioavailability (F) was determined by $AUC_{oral}/AUC_{i.v.}$, corrected for the dose. Each individual per oral exposure was compared with mean i.v. AUC within each formulation group.

3. Results

3.1. Pharmaceutical characterization of solutions

The solubility of AZ68 was determined in water and in a couple of cosolvent vehicles. According to experimental data, the solubility of crystalline AZ68 in water is very low, 3 μM (pH 6.8). Using the obtained crystalline solubility, the amorphous solubility was calculated to be 58 μM. The substance is ionizable, but an aqueous pH-shifted solution did not reach the intended millimolar range necessary for the *in vivo* study. The low or high pH necessary to reach the intended concentration also discriminated a pH-shifted solution for i.v. administration. Instead mixtures of water and organic cosolvents were tested. Using 60% PEG400, a maximum solubility of 1.8 mM was obtained. In 30% HP-β-cyclodextrin, the dissolved amount was 1.2 mM. A PEG400:DMA:water (1:1:1) formulation was made and a 21 mM solution was obtained. There is always a risk for precipitation upon dilution of poorly soluble drugs administered as oral solutions when the drug gets mixed with the gastric fluid and the fluid secreted in the intestine. The precipitation may have a great influence on the absorption rate and in some cases also on the extent of drug absorbed. AZ68, being zwitterionic, has a rather broad solubility minimum in the pH range 4–6. In the stomach, however, the pH is around 1 and the solubility at this pH is expected to be, roughly, two orders of magnitude higher than at the minimum due to the basic pK_a around 3. The risk for precipitation in the stomach is therefore considered to be low. However, there may be a dilution effect when entering the intestine. In the present work, a 5 mM solution (using the PEG400:DMA:water-vehicle) was chosen, in order to reduce the risk for precipitation. Besides, the bile salts and pancreatin in the intestinal fluid are supposed to increase the solubility of lipophilic drugs and, thus, further counteract precipitation.

The risk for precipitation is evident in i.v. administration as well, since the pH in the blood is unfavourable for AZ68. However, the solubility in plasma should normally be higher than the intrinsic solubility, and the solution is quickly diluted in the blood stream.

In the development of pharmaceutical dosage forms, one of the persistent challenges is assuring acceptable stability, i.e. the storage time allowed before the content is too low or a degradation product in the dosage form achieves a sufficient level to represent a risk to the *in vivo* specie. During early stage of the project, it is important to have stability information to secure that the dose administer *in vivo* contains the desired compound and that the effect originates from the parent compound. Some initial stability studies were performed for 1.8 mM solutions of AZ68 in 60% PEG400. The formulations were kept in refrigerator (8 °C) and freezer (−20 °C) as well as at room temperature (22 °C) for one month. After one week, at room temperature, about 55% of the compound was degraded, while the corresponding figure was 80% after two weeks. In refrigerator and freezer the formulations appeared to be stable. The tendency was similar for a 5 mM solution of AZ68 in PEG400:DMA:water (1:1:1) and for a 2 mM water solution, pH 11. However, in 33% DMA (2 mM) and in 100% ethanol (2 mM), the compound was more stable. After one week at room temperature, only 3% was degraded.

Obviously, a cosolvent formulation was needed to reach the intended concentration for the *in vivo* study and to be able to administer both i.v. and orally. Moreover, AZ68

is sensitive to high pH and is affected by PEG400 and/or its impurity profile. In the *in vivo* experiments below, the solutions were freshly prepared in connection with the administration.

3.2. Pharmaceutical characterization of amorphous nanosuspensions

Some compounds have a very strong tendency to crystallize and amorphous nanosuspensions of such compounds have a very limited stability. That means that beyond particle size measurements and chemical stability, also the tendency to crystallize must be followed. No obvious chemical degradation was observed after two weeks at room temperature in a 3 mM nanosuspension. Also the particle size remained more or less unaffected under the same time period during the same storage conditions (see Table 1), i.e. no Ostwald ripening was observed (see Section 2). Results are presented as mean diameter (intensity). No crystals were observed (using planepolarized microscope and/or visual examination). In addition, two samples were frozen, with and without 5% mannitol present. Two weeks later, the particle size was 207 and 144 nm, respectively.

Notable is that no chemical degradation occurred in the nanosuspensions. This is probably due to the very low solubility and that the LC equipment cannot observe the degradation of the dissolved substance. That the solid material is stable is further supported by the fact that no chemical degradation of the crystalline powder occurred after eighteen months of storage at room temperature.

Table 1
The particle size of amorphous nanosuspensions at different times after preparation (measured with DLS)

Time	Size (nm)
0	125[a]
6 h	155
24 h	145
48 h	162
One week	151
Two weeks	151

The formulations were stored at room temperature ($n = 1$).

[a] The incorporation of a second component with low aqueous solubility leads to a difference in composition between large and small particles during the Ostwald ripening process. This difference may counterbalance the driving force for Ostwald ripening and eventually result in termination. An initial short period of growth is, thus, expected before complete inhibition of the Ostwald ripening process is observed. See also Section 2 about Ostwald ripening.

3.3. Pharmaceutical characterization of crystalline nanosuspensions

The milled nanosuspensions appeared as particles somewhat larger than for amorphous nanosuspensions described above. A freshly prepared nanosuspension contained particles around 200 nm, measured by laser diffraction (volume weighted mean 201 nm, $d(0.1) = 68$ nm, $d(0.5) = 142$ nm, $d(0.9) = 342$ nm). Stability tests were performed with DLS, and the particles were stable for at least four weeks in refrigerator (see Table 2). Results are presented as mean diameter (intensity). If the intention is to store the milled nanosuspension frozen, it is important to add mannitol to keep the particle size (see Table 2, mannitol is a commonly used cryoprotectant in freeze-drying processes [33]). Also a sonication step may be performed to separate particles which may have aggregated during the freezing step [34]. As for amorphous nanosuspensions, the formulation appeared to be chemically stable for more than four weeks in refrigerator.

3.4. In vivo studies in rats

The use of particle size reduction to increase the surface area for dissolution and thereby increase bioavailability of poorly water-soluble molecules has recently been an attractive alternative for formulation scientists. In the present article, three different formulations were administer to rats, orally and i.v., containing 5 μmol/kg (i.v.) and 10 μmol/kg (oral) AZ68. There was one cosolvent solution containing PEG400:DMA:water (1:1:1), one amorphous nanosuspension and one crystalline nanosuspension. Three (i.v.) plus three (oral) animals were used for each formulation, except for the group receiving crystalline nanosuspensions per oral. One animal was excluded from this group due to technical circumstances. There were no indications that the animals do not tolerate the formulations. The mean plasma levels obtained for the three oral formulations are presented in Fig. 1. The mean plasma levels obtained for the three i.v. formulations are presented in Fig. 2. Pharmacokinetic parameters C_{max}, t_{max} and $t_{1/2}$ are presented in Table 3 for the oral formulations. Also AUC (area under individual plasma time curve) is presented in Table 3. Absolute oral bioavailabilities of each formulation were calculated

Table 2
The particle size (nm) of crystalline nanosuspensions at different times after preparation and stored at different conditions ($n = 1$, measured with DLS)

Condition	Day 1	2 weeks, before sonication	2 weeks, after sonication	4 weeks, before sonication	4 weeks, after sonication
Fridge	300	277	238	300	283
Fridge + 5% mannitol	300	313	324	270	266
Freezer	291	>1000	380	ND	ND
Freezer + 5% mannitol	337	314	317	ND	ND

ND, not determined.

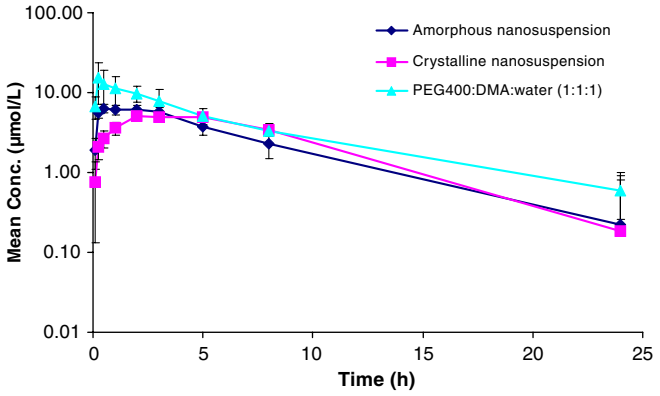

Fig. 1. The mean plasma levels of AZ68 vs. time after oral administration of AZ68 in a PEG400:DMA:water (1:1:1) solution (▲), as an amorphous nanosuspension (◆) and as a crystalline nanosuspension (■) to rats. The administered dose was 10 μmol/kg in all cases. $n = 3$ for each formulation, except for the experiments using crystalline nanosuspensions, where $n = 2$. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

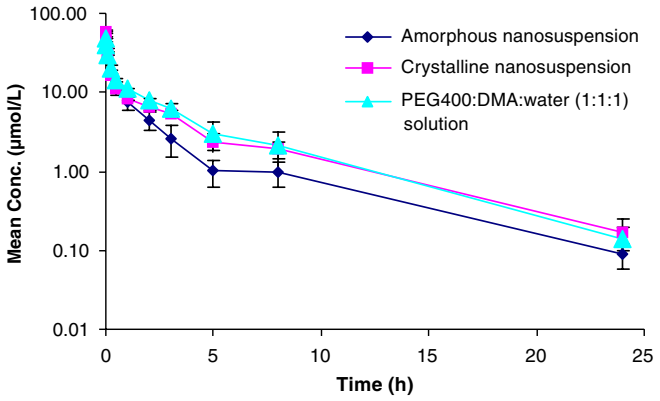

Fig. 2. The mean plasma levels of AZ68 vs. time after i.v. administration of AZ68 in a PEG400:DMA:water (1:1:1) solution (▲), as an amorphous nanosuspension (◆) and as a crystalline nanosuspension (■) to rats. The administered dose was 5 μmol/kg in all cases. $n = 3$ for each formulation. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

using the AUC values from i.v. administration (Table 4). $t_{1/2}$ are similar for the formulations, while there is a clear difference in t_{max}. The time to peak was shortest for the solution, longer for the amorphous nanosuspension and longest for the crystalline nanosuspension. The solution has higher C_{max}, while the absorbed amount of the compound was similar for the three formulations.

There have been reports in the literature regarding the use of i.v. administration of nanosuspensions in order to change the pharmacokinetic profile to more closely resemble an extended release formulation [35]. This was not evident in these trials, since no significant difference was found between pharmacokinetic parameters when comparison was made between the formulations administered i.v. The little lower AUC for amorphous nanosuspensions during the i.v. experiments was explained by a lower administered dose. There were no adverse events observed after i.v. administration of the nanosuspensions.

4. Discussion

Amorphous and crystalline nanosuspensions of drugs are attractive formulations in many cases. On a lab-scale, amorphous nanosuspensions are prepared by rapid mixing of a drug dissolved in an organic water-miscible solvent and an aqueous stabilizer solution. Crystalline nanosuspensions, on the other hand, are prepared by a milling procedure. Both formulations contain low amounts of additives and are, thus, expected to induce minimal side effects in various *in vivo* studies. In the present article, both crystalline and amorphous nanoparticles were prepared, where the amorphous particles also contained a second, water insoluble, compound to inhibit Ostwald ripening. Both kinds of nanosuspensions, and as a comparison – a solution, were administered to rats, both orally and i.v.

A very central property within the pharmaceutical world is the term shelf-life, or stability. In the present study, some initial stability investigations were performed with AZ68 in different liquid formulations. Obviously, there was some instability observed in formulations containing cosolvents and/or elevated pH. One advantage with nanosuspensions compared to solution is the better stability. The poorly soluble substance has an insignificant part in solution, which is exposed to environmental conditions. Also the particle size remained the same after storage. The good physical stability of nanosuspensions is explained by a good balance between the used stabilizers and/or the Ostwald ripening inhibitors, which inhibits the increase in particle size (see Section 2).

Absorption of a drug from a nanosuspension vehicle is considered to involve a dissolution step of the drug from the formulation into the aqueous luminal fluid followed by transport across the gastrointestinal epithelium. The dissolution rate and/or the low solubility may become the rate determining process in the bioavailability pathway. Thus, it is likely that the suspensions used in the present study provide a reservoir for the uptake of AZ68 by controlling its release in the gastrointestinal fluid, where only a small fraction can be dissolved.

After oral administration (10 μmol/kg), the two different nanoparticle suspensions were similar to the solution, with respect to exposure. However, the average plasma profiles differ. The solution resulted in the shortest t_{max} and highest C_{max} due to the fact that the compound could be absorbed without an initial, time-consuming dissolution step. The largest t_{max} was, as expected, observed in the experiments with crystalline nanosuspensions. The crystalline material has, due to the lattice energy gain, a lower free energy and, hence, a lower solubility than the amorphous material. Therefore, the dissolution rate of the crystalline material is slower. In addition, the crystalline particles are larger than the particles in the amorphous formulation, resulting in a smaller total surface exposed to the surrounding

Table 3
Mean pharmacokinetic parameters following oral administration of AZ68 in different formulations as a single dose (10 μmol/kg)

Animal, po	AUC/dose (h × kg/l)	C_{max} (μmol/l)	t_{max} (h)	$t_{1/2}$ (h)	F (%)
(A) PEG400:DMA:water (1:1:1) solution					
1	9.5	23.6	0.25	4.1	75.2
2	8.8	14.9	0.25	6.3	70.3
3	7.8	8.0	0.08	9.1	62.2
Mean	8.7 (±0.9)	15.5 (±7.8)	0.19 (±0.1)	6.5 (±2.5)	69.2 (±6.6)
(B) Amorphous nanosuspension					
1	4.6	5.0	0.5	4.9	61.7
2	6.3	9.2	0.5	5.6	85.6
3	4.4	6.6	3.0	3.4	59.1
Mean	5.1 (±1.0)	6.9 (±2.1)	1.3 (±1.4)	4.6 (±1.1)	68.8 (±14.6)
(C) Crystalline nanosuspension[a]					
1	–	–	–	–	–
2	4.5	4.9	2	4.2	40.5
3	6.1	6.1	5	3.8	54.6
Mean	5.3	5.5	3.5	4.0	47.6

The parameters presented are AUC (area under individual plasma time curve), C_{max} (peak concentration), t_{max} (time to reach peak concentration), $t_{1/2}$ (apparent terminal half-time) and F (bioavailability).
[a] No standard deviations are calculated since $n = 2$.

Table 4
Mean pharmacokinetic parameters following i.v. administration of AZ68 in different formulations as a single dose (5 μmol/kg)

Animal, i.v.	AUC/dose (h × kg/l)	$t_{1/2}$ (h)
(A) PEG400:DMA:water (1:1:1) solution		
1	15.1	4.0
2	12.8	4.7
3	9.9	4.2
Mean	12.6 (±2.6)	4.3 (±0.4)
(B) Amorphous nanosuspension		
1	6.2	6.3
2	7.8	5.4
3	8.1	4.2
Mean	7.4 (±1.0)	5.3 (±1.1)
(C) Crystalline nanosuspension		
1	11.3	5.6
2	11.7	4.0
3	10.3	5.0
Mean	11.1 (±0.7)	4.9 (±0.8)

The parameters presented are AUC (area under individual plasma time curve) and $t_{1/2}$ (apparent terminal half-time).

medium, decreasing the dissolution rate further. A combination of higher solubility, smaller particle size and higher dissolution rate from the amorphous nanoparticles explains the difference in behavior between the amorphous and the crystalline nanoparticles. However, as mentioned above, even if the above parameters differ, the total exposure and final bioavailability are comparable.

In contrast to micronized powders, the drug nanocrystals, as well as amorphous nanosuspensions, can be administered using very different administration routes, due to their small size. The nanosuspensions can be injected parenterally, e.g. i.v. The purpose with the i.v. administration was to be able to calculate the absolute bioavailability, but also to evaluate whether the nanosuspensions functioned as good and safe as the solution.

The results after i.v. administration in rats showed no significant difference among the three formulations (5 μmol/kg, see Fig. 2) by means of similar plasma profiles. Notable is that the results have very little variation between individuals. Thus, for the present compound, the nanosuspensions were as good as the solution and could be used for i.v. administration to avoid administration of solubility enhancing components such as PEG400 and DMA. Amorphous nanosuspensions have been administered frequently, mainly to gerbils, in pharmacology effect models, without any adverse events (unpublished data).

Results from the present work are encouraging. For BCS II compounds, nanosuspensions appear to be an attractive alternative to a solution. In studies where high doses are needed for oral administration, a solution may not be possible to manufacture and a microsuspension may result in too low exposure. Regarding i.v. administration, nanosuspensions may be the only alternative for a poorly soluble compound. Moreover, a nanosuspension formulation may also reduce the risk of undesired adverse effects related to the initial plasma peak, without losing the high overall exposure. In some cases, where nanosuspensions reduce the rate of absorption of the compound, but keep the overall exposure intact, the possibility of a sustained release formulation may be put forward. Nanosuspensions may give added value by allowing a reduction in either the dose or its frequency of administration.

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