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Research paper

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

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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].

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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)