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Evaluation of preclinical formulations for a poorly water-soluble compound

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ABSTRACT

One central aim of the present work was to find a robust oral formulation approach for Compound A, both to achieve reliable pharmacodynamic read outs but also for long time safety assessment studies. The compound has low aqueous solubility (0.4 μM at 37 °C), is highly lipophilic and has high Caco-2 permeability, i.e. a typical BCS II compound. A nanocrystal formulation, some oil approaches and a fat diet approach were evaluated *in vivo* in rats. The two latter strategies resulted in significantly higher *in vivo* exposures after oral administration compared to the nanocrystal approach. For simplicity, and due to the project development program, a food pellet formulation was selected. In addition, tentative data from a subcutaneous study in mice using nanocrystals of the compound are presented, showing extended profiles on the cost of C_{max}. Exposure data in monkeys after administration of nanocrystals both intravenously and per oral are presented. When switched from nanocrystals to an oil formulation, the observed oral exposure behavior was similar as observed in rats.

1. Introduction

The progress of many therapeutic compounds showing promising *in vitro* activity are impeded because such activity is not observed *in vivo*. Some factors responsible for this may be low aqueous solubility, poor absorption and low bioavailability. A significant number of new compounds are neither pH-adjustable nor soluble in the strongest cosolvent vehicles accepted. The next step is to investigate the possibility for an aqueous based suspension. For a compound with intermediate solubility a microsuspension might give an adequate exposure, but the more sparingly soluble compounds might not get a fair chance to absorb. A nanosuspension with a significantly reduced particle size will dissolve and reach the solubility faster and in that way give the sparingly soluble drug a much better opportunity for absorption (Gao et al., 2012; Möschwitzer, 2013). Another possibility, if allowed by the animal model (from a safety and pharmacokinetic/pharmacodynamic (PK/PD) read out perspective), is to improve dissolution of poorly water soluble drugs due to high efficiency of the oil phase to solubilize the lipophilic drug. Oily solutions or different kinds of emulsions are common approaches for dissolution enhancement (Gursoy and Benita, 2004; Kohli et al., 2010). Lipophilic drugs are easily solubilized in oils and can be presented directly in solution, avoiding the dissolution step and thereby enhancing absorption and the subsequent *in vivo* exposure (Pouton, 1997). A further step is to transfer the formulation into a solid form for long time studies, to simplify dosing and handling (Patel and Vavia, 2007; Tang et al., 2008; Thomas et al., 2013; Inugala et al., 2015). The test compound used, Compound A, is a cyclic sulphonamide (Fig. 1). The project objective was to develop an orally active drug for effective and safe therapy of cardiovascular diseases. The solubility of the test compound at pH 7.4, 37 °C, was 0.4 μM and in FaSSIF (fasted state simulated intestinal fluid) 6.2 μM. The permeability of Compound A was high. The calculated log P was 5.5.

Advanced drug delivery provides many potential benefits including increases in efficacy, reduction in dose and decrease in toxicity. Strategies to promote advanced drug delivery remain a focus of intensive research within mainly academia. This effort has been most prominent in early preclinical studies. In contradiction to this, Big Pharma has selected another way forward for small organic molecules; simplicity that cannot fail. Today, formulation development and manufacturing for early animal studies and the

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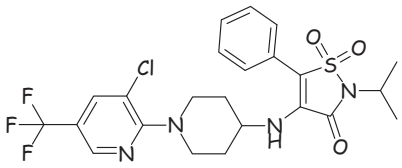

Fig. 1. The molecular structure of the test compound; Compound A.

actual in life phase are rarely conducted at the same location. Thus, the necessity for advanced liquid formulations, where for instance stability is an issue, has decreased. Today focus is on sending formulations that do not change properties whatever happens or sending a bulk compound that arrives stable and is easily dissolved in a suitable solute. In the present work we have investigated a compound on the basis of its physicochemical properties with the aim of finding a simple formulation, with optimal exposure, that could not be handled incorrectly. The formulation is supposed to be transported worldwide. A traditional approach for a poorly soluble drug is to decrease the particle size for a suspension, even down to nanosize (Gao et al., 2012; Möschwitzer, 2013). For the present drug, nanoparticles in a liquid formulation were not a way forward. Instead was the intention to use oil based, solid, robust formulations that could increase the systemic exposure compared to a traditional nanosuspension and, in addition, have the possibility to reach the lymph, i.e. a delicate balance between complexity and simplicity. Not only the formulation itself but also different routes of administration need to be explored e.g. an intravenous (i.v.) dose is needed to be able to calculated different basic pharmacokinetic properties. The subcutaneous (s.c.) route is a possible way forward for extended exposure profiles of poorly soluble compounds. In addition, initial kinetics and tolerability for different species were investigated with the described formulation approaches.

2. Material and methods

2.1. Characteristics of the test compound

Compound A was synthesized at AstraZeneca R&D Gothenburg as neutral form. The molecular structure is shown in Fig. 1. The free base of the compound was crystalline with a melting point of 160 °C and the molecular weight was calculated to 529 g/mol. The Caco-2 permeability was measured to 176 * 10^{-6} cm/s and defined as "high". The value for the calculated pK_a was 5. The solubility is pH dependent with solubility increasing at pH below pK_a. The crystalline solubility in PBS buffer, at pH 7.4 and 37 °C, was 0.4 μM. The measured solubility in FaSSIF and Miglyol was 6 μM and 25 mM, respectively, at 37 °C. The drug is a BCS class II compound.

2.2. Chemicals

Polyvinylpyrrolidone K30 (PVP) was bought from BASF (Göteborg, Sweden). The disodium salt of Aerosol OT (AOT) was purchased from Cytec Industries Inc (Woodland Park, NJ). Mannitol were purchased from Sigma (Stenhein, Germany) and used as a tonicity modifier and as a cryoprotectant during freezing of the nanocrystals. Miglyol 812 a fractioned coconut oil, was purchased from Hüls (Degussa-Hüls, Düsseldorf, Germany). The high fat diet, R638 containing 0.15% cholesterol and 21% cacao butter, was purchased from Lantmännen (Stockholm, Sweden). PEG400 was bought from Hoechst (today included in the Sanofi-Aventis group). *N,N*-dimethylacetamide (DMA) and Solutol HS15 was bought from Sigma-Aldrich and was used as a non-ionic emulsifying agent/surfactant.

2.3. Formulations

2.3.1. Preparation of crystalline nanosuspensions

The nanosuspension for oral administration was produced by water milling the test compound using the Fritsch Planetary Micromill P7. The substance was brought together with stabilizer solution PVP and AOT in water as earlier described (Sigfridsson et al., 2011a, 2014; Sigfridsson and Palmer, 2014). The particle size (diameter) and distribution of the crystalline suspensions were measured by laser diffraction (Malvern Mastersizer 2000, Worcestershire, UK). The suspension was diluted with or without 5% mannitol. Mannitol was used as cryoprotectant when stored frozen and as an uncharged tonicity modifier, not supposed to affect the stabilization of the formulations. The suspensions were thawed in a refrigerator, stirred and sonicated for a few minutes before administration. This approach was tested and verified before the start of the *in vivo* study. No significant changes in particle size was found. The particle size was measured to about 200 nm.

2.3.2. Preparation of oil formulations

The compound was dissolved in Miglyol 812 (or Miglyol 812/Solutol HS15 1/1) at room temperature. The content of the excipients in the formulations were constant. Different amounts of drug powder was added, resulting in formulations with different drug concentrations. The formulation was stirred for approximately two hours with a magnet until all substance was dissolved. The transparent formulations were stored in a refrigerator for 24 h and stirred with a magnet before administration.

2.3.3. Preparation of food pellets

The amount of R638 powder needed for accurate compound dose was weighed. One third of the diet was mixed in a pastry machine with whiskey doughs and the compound. The compound container was carefully rinsed several times with diet powder to bring out all remaining compound. The mix was mixed for five minutes. One more third of the diet was added and mixed for five more minutes. This procedure was repeated once. 10% cold (of total diet weight) tap water was added to the compound mixture, and everything was mixed for five minutes. The mixture was ran through the pellet machine. The pellets were collected and were dried at 35 °C for 24 h. The diet was stored at −20 °C, for not more than approximately four weeks. The diet's durability (compound stability) was set to one week in room temperature. This storage time was selected mainly due to microbiologic growth and not due to chemical instability of the compound. The test compound was mixed in high fat diet at three different concentrations.

2.4. In vivo *studies*

The animal experiments were performed in accordance with the present UK Animals (Scientific Procedures) Act, approved by institutional ethical review committees and conducted under the authority of Project Licences held at both facilities. In AstraZeneca Sweden the procedures were approved by the Stockholm South Animal Ethical Committee (Stockholms Södra Djurförsöksetiska Nämnd).

2.4.1. Administration of crystalline nanosuspensions via different routes to rats and monkeys

The formulation with crystalline nanosuspension was administered to both rats and monkeys. In the rat studies male rats Wistar: Han substrain Crl:WI (Glx/BRL/Han) IGSBR were used. Handling of the animals and performance of the administration were as earlier described (Sigfridsson et al., 2014). Briefly, the animals were divided into three different groups with five animals in each group. The formulation was administrated orally daily for