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Research Paper

Effect of cyclodextrin concentration on the oral bioavailability of danazol and cinnarizine in rats

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ABSTRACT

Cyclodextrins (CDs) are frequently used as an excipient to enhance the intestinal drug absorption of compounds with a low aqueous solubility. However, there exists an intricate interplay between opposing effects that determine the optimal dosing criterion. These opposing effects are the benefits of circumventing the dissolution time required to dissolve the non-absorbable drug particles in the intestine *versus* the disadvantage of decreasing the concentration of the drug available to permeate the intestinal membrane if excessive CD concentrations are used. This study investigated whether there is a potential risk of overdosing CDs in aqueous formulations resulting in suboptimal bioavailability. This was done by measuring the *in vivo* pharmacokinetics of danazol, which has a high affinity for hydroxypropyl-βCD, and cinnarizine, which has a pH-dependent low to medium affinity. Pharmacokinetic studies of danazol in rats showed a significant longer T_{max} and decreased C_{max} resulting in decreased bioavailability when the CD concentration was increased. No significant difference was seen for any of the pharmacokinetic parameters for cinnarizine as a function of CD dose. The present study thus demonstrates that surplus CD concentrations can have a major effect on the pharmacokinetic profile of one compound and a minor effect on the pharmacokinetic profile of another. This suggests that there are some compounds where the CD excipient should be used with care and others where it can be used without major concerns.

1. Introduction

Cyclodextrins (CDs) are cyclic macromolecules capable of including a large variety of smaller molecules into their cavity region by non-covalent interactions with the interior of the CD. In the pharmaceutical field, this phenomenon is utilized to enhance the proportion of dissolved drug [1], which may lead to an increased bioavailability for compounds with a low aqueous solubility [2].

Oral delivery is the most frequent route of drug administration, mainly due to its ease of use. The absorption kinetics of a CD complexed drug taken by oral administration involves a number of processes and parameters and is therefore a relatively complicated process. In addition to simple complex formation, a variety of lipophilic compounds originating from ingested meals and gastrointestinal secretions may have the propensity to displace the drug molecule from the CD cavity [3,4]. Bile salts are of particular interest in connection with the displacement of drugs from CD complexes, as they have high affinity for β- and γCD [5,6] and are secreted in large amounts into the duodenum. Thus, bile salt displacement of drug molecules may take place in the small intestine, where large amounts of bile salt are present [7]. Only the free form of the drug, which is in equilibrium with the molecular complex, is available for absorption [8] and hence for providing the pharmacological effect. Consequently, the bioavailability of drug compounds with high CD stability constants and low aqueous solubility [4] may be reduced when high amounts of CDs are co-administered, simply because the proportion of free drug remains low when un-complexed CD is abundant. This has been demonstrated both *in vitro* [9] and *in vivo* in rats [10]. Westerberg and Wiklund [10] administered small amounts of benzo[a]pyrene with different amounts of natural βCD and observed a very significant decline in bioavailability after administration of relative small quantities

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of βCD to rats. In a recent study, however, Olesen et al. [11] investigated the potential differences between a digestible CD (γCD) and the more commonly used hydroxypropyl-βCD (HPβCD) in rats with a protocol similar to the one used by Westerberg and Wiklund [10]. Olesen and coworkers [11] reported a very limited decrease in the absorption of benzo[a]pyrene; hence, the *in vivo* potential for overdosing with CD is still an unclear risk, which needs further investigations to be clarified further by additional studies using other compounds.

The purpose of the present study was therefore to investigate the absorption of two compounds with different stability constants and complexation efficiencies toward HPβCD in rats, one with high stability constant (danazol) and one with medium (cinnarizine), both administered in solutions with increasing amounts of HPβCD to provide an estimate of the risk of overdosing the excipient and thereby potentially reducing the bioavailability of the compound.

2. Materials and methods

2.1. Materials

Danazol was purchased from Unikem A/S (Copenhagen, Denmark) and the internal standard, citalopram, obtained from H. Lundbeck A/S (Valby, Denmark). HPβCD was from Roquette (Lestrem, France) with a degree of substitution of 4.46 [5]. Cinnarizine and the internal standard, flunarizine, were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanol and acetonitrile were of HPLC grade and were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). Purified water was obtained from a Millipore Milli-Q Ultrapure Water purification system (Billeria, MA, USA). All other chemicals were of analytical grade.

2.2. Preparation of formulations

The oral formulations contained danazol or cinnarizine solubilized in CD solutions. These were prepared by weighing appropriate amounts of CD, dried in vacuum at 45 °C for 48 h before weighing. Water was then added and the solution stirred until all CD was dissolved and finally corrected to the right volume. Danazol or cinnarizine was weighed and added to the appropriate amounts of the different CD solutions. For danazol these contained 8.5%, 10%, 20% and 30% (w/v) of HPβCD. For cinnarizine the concentrations dosed were 3%, 5.2%, 13%, 20%, 25%, 30% and 50% (w/v) of HPβCD, where the lowest concentration for the two compounds was the minimum amount of HPβCD to solubilize 1.4 mg/mL danazol or 1.0 cinnarizine, at approximately pH 4.5 and 6.5, respectively. All formulations were dosed at 10 mL/kg to the animals.

The intravenous formulations contained 1.4 mg/mL danazol or 2 mg/mL cinnarizine in a solution containing 10% (w/v) HPβCD and 4.4% (w/v) glucose monohydrate. The CD solution was prepared and the compounds were solubilized by stirring. After all of the compound was solubilized the formulation was filtered through a sterile 0.22 μm filter before use. The two intravenous formulations were dosed at 5 mL/kg to the animals.

2.3. In vivo studies

The protocol used for the *in vivo* study in rats was approved by the institutional animal ethics committee in accordance with Danish law regulating experiments on animals and in compliance with EC directive 2010/63/EU, and the NIH guidelines on animal welfare. Male Sprague Dawley rats, weighing 276–322 g on the day of administration, obtained from Charles River (Sulzfeld, Germany), were used for the pharmacokinetic studies ($n = 6$ per group). The animals were acclimated for a minimum of 5 days in groups of 2 on wooden bedding (Tapvei, Kortteinen, Finland) in plastic cages, 595 × 380 × 200 mm, with a stainless steel lid (Scanbur, Sollentuna, Sweden) in humidity- and temperature-controlled ventilation cupboards (Scantainers, Scanbur Technology, Karlslunde, Denmark), relative humidity 40–60%, temperature 20 ± 1 °C, and light from 06:00 to 18:00 h. The animals had free access to a standard rodent diet (Altromin 1325, Brogaarden, Denmark) and water *ad libitum* during the study.

The animals were randomly assigned their group ($n = 6$ per group) receiving 14 mg/kg of danazol or 10 mg/kg of cinnarizine by oral gavage in different cyclodextrin formulations. Two other groups of rats were administered 7 mg/kg of danazol or 10 mg/kg of cinnarizine by injection into the tail vein. Blood samples of 0.2 mL were collected into plasma collection tubes containing dipotassium EDTA from Sarstedt (Nümbrecht, Germany). Samples were collected at 5, 15 and 30 min as well as 1, 2, 3, 4, 6, 8, 24 h for the animals dosed with danazol and at 0.5, 1, 2, 4, 6, 8, and 24 h for animals dosed with cinnarizine, plus 5 and 15 min for the animals dosed intravenously with cinnarizine. The plasma was harvested immediately by centrifugation for 10 min at 3600*g* and stored at −80 °C until further analysis. At the end of the experiment the animals were euthanized.

2.4. Quantitative determination of danazol in plasma

The concentration of danazol in plasma was determined by a turboflow chromatographic system with MS/MS detection as previously described [12]. Plasma samples were spiked 1:1 with internal standard in 10% methanol (200 ng/mL citalopram). The samples were centrifuged at 6000*g* for 20 min at 5 °C. A turboflow TLX-2 system with a Cyclone turboflow column (0.5 mm × 50 mm, 60 μm) from Cohesive Technologies (Franklin, MA, USA) and a C8 XTerra MS analytical column (2.1 mm × 20 mm, 3.5 μm) from Waters Corp. (Milford, MA, USA) were used for the analyses. 10 μL was injected to the turboflow column in which danazol and the internal standard were retained while matrix components from plasma were eluted. Danazol and the internal standard were then eluted from the turboflow column and transferred to the analytical column. The eluents; 0.1% NH_3 in water and 0.1% NH_3 in methanol were used in different ratios.

Mass spectrometry detection was done with a Sciex API 3000 mass spectrometer from MDS Sciex (Concord, ON, Canada). Positive ionization was achieved by electrospray with a single reaction monitoring (SRM) of m/z 338.2 → 148.2. Standards were prepared by spiking plasma obtained from non-dosed rats with danazol and internal standard. Standards ranged from 1 to 5000 ng danazol per mL plasma.

2.5. Quantitative determination of cinnarizine in plasma

Cinnarizine was extracted from plasma by adding 20 μL of plasma to 140 μL of acetonitrile containing 35 ng/mL flunarizine as internal standard as previously described [13]. The samples were vortexed thoroughly and then centrifuged at 20,000*g* for 10 min. The supernatant was removed and 25 μL was injected into the HPLC.

A reversed phase HPLC method was utilized for all analyses. This included the use of a C18 Waters Xbridge™ column (3.5 μm, 150 × 4.6 mm), an L-7300 oven at 45 °C, an L-7200 autosampler, an L-748 fluorescence detector, D-7000 interface and an L-7100 pump; all from Merck (Darmstadt, Germany). Data from the HPLC system were transferred to a computer and analyzed using Merck-Hitachi model D-7000 chromatography data station software, version 4.1. Flow was set at 1 mL/min, and the mobile phase consisted of 20 mM ammonium phosphate buffer pH 2.3 and acetonitrile (50:50, v/v). An excitation wavelength of 249 nm and an emission