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Does the Digestibility of Cyclodextrins Influence the *In Vivo* Absorption of Benzo[a]pyrene in Rats?

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ABSTRACT

An important excipient used to overcome poor solubility is cyclodextrin. However, data in the literature suggest that excessive overdosing of cyclodextrins can decrease the absorption of compounds administered with cyclodextrins, due to their lack of release from the complex. γ-Cyclodextrin is digestible in contrast to β-cyclodextrins. This could potentially limit the sensitivity toward overdose, which was evaluated using benzo[a]pyrene in this study, in which rats were administered benzo[a]pyrene and different doses of the 2 cyclodextrins. Both cyclodextrins lowered the area under the curve and therefore the absorption of benzo[a]pyrene by up to a factor of 2 when dosed in high concentrations, thus indicating that overdosing of cyclodextrins may limit the oral absorption of a compound. This limitation may be artificial because the molar ratio of benzo[a]pyrene:cyclodextrin was >1:50,000 at the concentration where a significant decrease in the absorption was observed. No difference was observed between the 2 cyclodextrins, so digestibility seemed less important. More interesting was that the decrease in absorption was relatively small when compared with literature values, suggesting that the effect of overdosing a compound with cyclodextrins was lower than anticipated.

Introduction

A frequently encountered problem in drug discovery and development is the low solubility of drug candidates and a large proportion of drugs in development therefore belong to class II or IV according to the Biopharmaceutical Classification System.[1] For compounds with a low aqueous solubility, dissolution in the intestinal fluid may be rate limiting and could potentially limit their bioavailability. In clinical use, the poor bioavailability of a drug might result in limited therapeutic potential leading to insufficient clinical outcomes; hence to facilitate the development of these difficult-to-formulate compounds, various technological approaches may be applied to enhance the absorption of poorly water-soluble drugs. These approaches include changing the chemical structure (e.g., developing a prodrug) or formulation strategies (e.g., physical modifications such as particle size reduction, lipid-based formulations, amorphous systems, and complexation with cyclodextrins).[1-3]

Cyclodextrins are useful functional excipients, which are used widely both in development and in several marketed pharmaceutical products.[4-7] The basis for this popularity is the ability of cyclodextrins to interact with poorly water-soluble drugs forming noncovalent dynamic inclusion complexes. On account of this, an increase in the apparent water solubility of the compounds is observed leading to an enhanced bioavailability when administered orally.[6,8] Commonly used cyclodextrins consist of 6, 7, or 8 glycosidic bonded glucose molecules, denoted α-, β-, and γ-cyclodextrins, respectively.[9] The α- and β-cyclodextrins are digested and absorbed to a very limited extent.[8,10] The drug compound needs to be displaced from the complex with cyclodextrin complex before it can be absorbed into the epithelia and from there enter the systemic blood circulation.[11] Westerberg and Wiklund[12] have reported that oral coadministration of excess β-cyclodextrin significantly decreases the oral bioavailability of benzo[a]pyrene in rats. Benzo [a]pyrene is a poorly water-soluble compound that can be solubilized by cyclodextrins and Westerberg and Wiklund[12] suggested that this phenomenon was a consequence of the strong stability constant between the cyclodextrin and benzo[a]pyrene.[12] Cyclodextrins cannot be absorbed by the gastrointestinal mucosa and

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only the free form of benzo[a]pyrene can permeate the intestinal membrane. The extent of absorption thus depends on the dissociation equilibrium, which is shifted toward the complex if the stability constant is high.[13] In addition to this effect, the dissociation equilibrium may be influenced by the presence of other hydrophobic compounds present in the intestinal chyme that can compete for the cyclodextrin cavity, thereby displacing the compound. Bile salts are the most extensively studied competitor, and are present in high concentrations in the intestine. Furthermore, bile-depleted animals have shown a lower absorption of compounds from cyclodextrin complex solutions than naive animals.[11,14] A possible explanation for the decreased absorption, when high cyclodextrin amounts were dosed could therefore be due to the strong complexation affinity between cyclodextrins and the drug compound, so that bile salts cannot compete when extensive amounts of cyclodextrin are administered.

Lumholdt et al.[10] have reported an additional mechanism for drug dissociation from cyclodextrin complexes. These authors showed that γ-cyclodextrin was a substrate for enzymatic degradation by α-amylases, while β-cyclodextrin and hydroxypropyl-β-cyclodextrin (HP-β-cyclodextrin) were degraded to a very limited extent. The ability of γ-cyclodextrin to be digested could limit the risk of reduced absorption, by releasing the complexed compound even at high cyclodextrin doses. This positive effect would only be expected if it is assumed that the degradation of the cyclodextrin does not lead to extensive precipitation of the compound. The fact that γ-cyclodextrin is degradable in the intestinal lumen as well as its high aqueous solubility and larger cavity size makes it a potentially excellent drug formulation candidate, but this could also lead to a different biopharmaceutical profile.[10]

This study investigated the effect of HP-β-cyclodextrin and γ-cyclodextrin on the oral absorption of benzo[a]pyrene; γ-cyclodextrin was of interest mainly due to its degradability explained above, while 2-HP-β-cyclodextrin was chosen as a reference. Benzo[a]pyrene was selected as the model because it enables a straightforward comparison with the results presented by Westerberg and Wiklund.[12] The purpose of this study was (1) to explore how the oral administration of escalating doses of cyclodextrins affect the absorption of benzo[a]pyrene and (2) to investigate how the different degradation profiles of β-cyclodextrin and γ-cyclodextrin influence the extent of benzo[a]pyrene absorption.

Materials and Methods

Materials

2-HP-β-cyclodextrin (degree of substitution 4.55, for characterization of the batch see Holm et al.[15]) was purchased from Roquette (Lestrem, France). γ-Cyclodextrin was purchased from Wacker Chemie (München, Germany) and both cyclodextrins were of pharmaceutical grade. [^{3}H]benzo[a]pyrene for the *in vivo* study was obtained in toluene from Biotrend with a specific radioactivity of 50 Ci/mmol (1.85 TBq/mmol). OptiPhase SuperMix® liquid scintillator was from PerkinElmer (Walthan, MA). The water used in the experiments was obtained from a Millipore purification system. All other chemicals used were of analytical grade.

Formulations for In Vivo *Study*

An ethanol solution of [^{3}H]benzo[a]pyrene was prepared by evaporation of the toluene in which it was delivered at room temperature under a stream of nitrogen. After all toluene was evaporated the solid matter left in the tube was dissolved in 96% ethanol.

The animals received a dose of a placebo cyclodextrin solution by oral gavage the day before and immediately before administration of benzo[a]pyrene. This was prepared by weighing appropriate amounts of dried cyclodextrin (dried at vacuum for 48 h before weighing). Water was then added and the solution stirred until all cyclodextrin had dissolved and finally adjusted to the correct volume. This solution was dosed at 10 mL/kg and the animals received 0, 3, 30, 400, 500, 1000, 1500, or 2000 mg/kg of either cyclodextrins. In addition, 2500, 3000, and 5000 mg/kg were dosed for 2-HP-β-cyclodextrin and 2300 mg/kg for γ-cyclodextrin, a difference based on the solubility of the 2 cyclodextrins. After the cyclodextrin solution was dosed, an ethanol solution of [^{3}H]-benzo[a]pyrene was administered by oral gavage at a dose of 1050 ng/kg (250 μCi/kg) in a volume of 0.3 mL/kg.

Experimental Design of the In Vivo *Study*

The protocol used for the rat *in vivo* studies was approved by the institutional ethics committee in accordance with Danish law regulating experiments on animals and in compliance with EU directive 2010/63/EU, and the NIH guidelines on animal welfare. Male Sprague-Dawley rats were purchased from Charles River Deutschland (Sulzfeld, Germany). The animals were acclimatized and maintained on standard feed and carrots with free access to water for a minimum of 5 days prior to the experiments. At initiation of the experiments the rats had an average weight of 286-324 g. The study used a protocol similar to the one described by Westerberg and Wiklund,[12] with the exception that the cyclodextrin was dosed in 10 mL/kg instead of the 20 mL/kg.

All groups consisted of 6 randomly assigned male rats and each received a placebo cyclodextrin solution with either HP-β-cyclodextrin or γ-cyclodextrin 24 h in advance and again immediately before a single oral dose of [^{3}H]benzo[a]pyrene (1050 ng/kg equal to 250 μCi/kg). All rats were fasted overnight prior to [^{3}H]-benzo[a]pyrene dosing and *ad libitum* access to food was restored 8 h after dosing and for the rest of the experiment. Blood samples were collected following oral administration of [^{3}H]-benzo[a]pyrene and the animals were euthanized after the last blood sample was taken. The blood samples (200 μL) were withdrawn from the tail vein at 0, 0.5, 1, 2, 4, 8, 24, 32, 48, 72, 120, and 168 h after dosing. Plasma was immediately separated by centrifugation at 3600 × *g* for 10 min and stored in polypropylene tubes at −80°C until analyzed.

Bioanalysis

Plasma samples were thawed and were subsequently mixed with the scintillation cocktail and counted directly using a Perkin Elmer (TriCarb 2900TR). Quench correction was based on external radioactivity standards. The samples were counted for 3 min. Blank counts were concurrently measured and used for background correction. The blank values originated from plasma withdrawn before dosing.

Pharmacokinetic Analysis

The pharmacokinetic parameters characterizing oral administration of benzo[a]pyrene were obtained by noncompartmental analysis using WinNonlin Professional version 5.2 (Pharsight Corporation, Mountain View, CA). The area under the curve for benzo [a]pyrene after oral administration ($AUC_{0\text{-last}}$) was calculated using the linear trapezoidal rule from time zero to the last measured plasma concentration postdose.