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Published in:
Journal of Pharmaceutical Sciences

DOI:
10.1016/j.xphs.2015.10.027

Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Olesen, N. E., Vana, V., & Holm, R. (2016). Does the Digestibility of Cyclodextrins Influence the In Vivo Absorption of Benzo[a] pyrene in Rats? *Journal of Pharmaceutical Sciences*, *105*(9), 2698-2702 . https://doi.org/10.1016/j.xphs.2015.10.027

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Download date: 15. Jul. 2025

Journal of Pharmaceutical Sciences 105 (2016) 2698–2702

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

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

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ARTICLE INFO

Article history:
Available online 14 January 2016

Keywords:
cyclodextrins
benzo[a]pyrene
stability constants
γ-cyclodextrins
rats
bile
hydroxypropyl-β-cyclodextrin

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.

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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

This article contains supplementary material available from the authors by request or via the Internet at http://dx.doi.org/10.1016/j.xphs.2015.10.027.

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http://dx.doi.org/10.1016/j.xphs.2015.10.027
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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 (Waltham, 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.

Statistical Analysis

SigmaPlot for Windows version 11.2.0.5 (Sysstat Software Inc., San Jose, CA) was used for the statistical calculations. The statistical difference between the groups was compared by one-way analysis of variance followed by comparison using the Dunn's method. p Values below 5% ($p < 0.05$) were considered statistically significant.

Results and Discussion

Coadministration of γ-Cyclodextrin

The average plasma concentration as a function of time for benzo[a]pyrene after coadministration with γ-cyclodextrin is shown in Figure 1 and the associated pharmacokinetic parameters are given in Table 1. Individual curves for each cyclodextrin concentration are in the supporting information.

No statistical difference between the groups was observed for t_{max}. The C_{max} for the control group (0 mg/kg) was significantly higher than the groups coadministered with 400 mg/kg or more of γ-cyclodextrin. The lower C_{max} at higher γ-cyclodextrin concentrations was probably due to the lower bioavailability of benzo[a]pyrene and the need for a longer absorption time, as less compound was available in the free form due to complexation with cyclodextrin. Before the compound could be absorbed it would need to be expelled from the cyclodextrins, which in accordance with Le Châtelier's principle occurs both through dilution, by a competitive replacement by intestinal bile salts[13] and potentially through digestion of the γ-cyclodextrin.[10] The decrease in C_{max} observed in this study started at much higher concentrations than reported by Westerberg and Wiklund,[12] who coadministered benzo[a]pyrene with natural β-cyclodextrin, and the C_{max} decrease (up to approximately a factor of 1.5) was relatively smaller than the decrease reported (up to a factor of 5.5) by Westerberg and Wiklund,[12] indicating that γ-cyclodextrins could induce a different biopharmaceutical processing than the nondigestible β-cyclodextrin.

The AUC was also significantly higher in the control group than in the animals coadministered with 400 mg/kg or more γ-cyclodextrin, in accordance with the findings for C_{max}. Also the animals dosed with 3 and 30 mg/kg had a significantly higher absorption than some of the groups dosed with higher cyclodextrin concentrations and a clear trend for different AUC values was observed for the animals coadministered 0, 3, and 30 mg/kg versus 400 mg/kg and above, although not significantly for all groups. There was no difference in the elimination rate constant and no difference was observed between the groups (data not shown), that is, the observed differences were a reflection of different amounts of benzo[a]pyrene absorbed and not a reflection of different elimination kinetics. The relative decrease in AUC was up by a factor of 1.7 in this study, whereas Westerberg and Wiklund[12] reported a difference of more than a factor of 15 for the highest cyclodextrin dose.

Table 1
Pharmacokinetic Parameters Obtained after Oral Administration of [^{3}H]benzo[a]pyrene and Various Doses of γ-Cyclodextrin via Noncompartmental Analysis

Dose γ-Cyclodextrin (mg/kg)	T_{max} (h)	C_{max} (pg-eq/mL)	$AUC_{0\text{-}168\ h}$ (pg-eq h/mL)
0	5.2 ± 1.9	647 ± 42[a]	32,594 ± 1584[a]
3	5.1 ± 1.0	612 ± 64	29,543 ± 1629[b]
30	5.5 ± 1.1	575 ± 58	28,308 ± 1881[c]
400	3.9 ± 0.9	496 ± 66	19,090 ± 985
500	3.4 ± 0.9	559 ± 66	22,609 ± 712
1000	5.8 ± 3.3	427 ± 47	20,286 ± 1039
1500	8.4 ± 3.3	523 ± 85	24,729 ± 1664
2000	7.3 ± 0.7	543 ± 26	23,773 ± 1314
2300	6.0 ± 0.9	561 ± 48	24,310 ± 872

Data presented as mean ± standard error ($n = 6$; $n = 11$ for 0 mg/kg).
[a] Statistically significantly different from the animals receiving 400, 500, 1000, 1500, 2000, and 2300 mg/kg γ-cyclodextrin.
[b] Statistically significantly different from the animals receiving 400, 500, and 1000 mg/kg γ-cyclodextrin.
[c] Statistically significantly different from the animals receiving 400 and 1000 mg/kg γ-cyclodextrin.

The use of benzo[a]pyrene in this study was selected based on the study published by Westerberg and Wiklund,[12] where an extensive decrease in absorption was reported when coadministered with natural β-cyclodextrin. As benzo[a]pyrene has been reported to have nonlinear pharmacokinetics,[16-18] particularly at concentrations above 200 μM,[19] it was decided to deploy a dose regime similar to that of Westerberg and Wiklund,[12] that is, a low dose of radioactive compound was selected. Foth et al.,[17] after oral dosing of radiolabeled benzo[a]pyrene, have demonstrated that only 30% of the total radioactivity corresponds to the unmetabolized compound at the peak concentration after oral dosing of radiolabelled benzo[a]pyrene. In this study, only the total drug-related radioactivity was measured, hence the study did not reveal any differences in metabolic pattern that may occur through coadministration of different cyclodextrins or amounts of cyclodextrins. The total radioactivity counted was still a reflection of the total fraction absorbed despite the subsequent metabolic fate of the compound and could therefore be used in the evaluation of influence on the total bioavailability.

Besides differences between γ-cyclodextrin and β-cyclodextrins in their potential intestinal digestibility,[10] other differences in the release mechanism could potentially occur. The release mechanism of a compound from a cyclodextrin complex upon oral administration has been suggested to be a combination of dilution, which must be assumed to be constant across groups, and competitive displacement by bile salts present within the intestinal tract,[20,21] that is, differences in stability constants between cyclodextrins of both compound and bile salts may in theory influence the release and thereby the absorption. To the best of our knowledge, the stability constant between β-cyclodextrin and benzo[a]pyrene has not been determined, but the apparent stability constants measured by phase solubility studies has been reported for

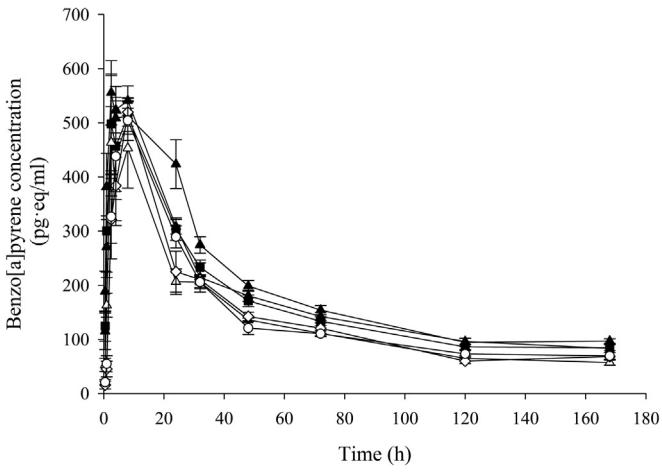

Figure 1. Total radioactivity plasma concentration time profile of [^{3}H]benzo[a]pyrene following single oral administration of 1050 ng/kg (250 mCi/kg) to rats with coadministration of (▲) 0, (▼) 3, (■) 30, (△) 500, (◇) 2000, and (○) 2300 mg/kg of γ-cyclodextrin (shown in the legend). Data are presented as mean ± standard error ($n = 6$, 11 for 0 mg/kg)

2-HP-β-cyclodextrin to be 5344 M^{-1} and 25,900 M^{-1},[22,23] the later value measured in the presence of 15 mM sodium taurocholate, that is, with competitive interaction. For γ-cyclodextrin, we have found an apparent stability constant of 37,050 M^{-1} at 37°C in a phase solubility study (data not shown); the relative affinity of benzo[a]pyrene toward the 2 different classes of cyclodextrins was in the same range. In this study, benzo[a]pyrene was administered solubilized in ethanol and 100 μL was dosed to each rat, theoretically affecting the stability constants. However, the gastric volumes in rats of ~300 g have been reported to be 2.4 mL by Takashima et al,[24] which provide a dilution of the administered ethanol to <5% vol/vol, which must be assumed to have limited influence for the compound's relative affinity toward the cyclodextrin. Also when looking at the affinity of the bile salts, Holm et al.[15,25,26] have reported similar affinities between both HP-β-cyclodextrins and γ-cyclodextrins and bile salts relevant for rats; a difference in the performance of the 2 types of cyclodextrins would therefore probably be a reflection of differences in biopharmaceutical performance rather than differences in the physical chemical interactions with key components. This study therefore indicates that γ-cyclodextrins performed better than β-cyclodextrins, when compared with the data of Westerberg and Wiklund.[12]

Table 2
Pharmacokinetic Parameters Obtained After Oral Administration of [^{3}H]benzo[a]pyrene and Various Doses of 2-HP-β-cyclodextrin via Noncompartmental Analysis

Dose HP-β-Cyclodextrin (mg/kg)	T_{max} (h)	C_{max} (pg-eq/mL)	$AUC_{0\text{-}168\ h}$ (pg-eq h/mL)
0	5.2 ± 1.9	647 ± 42[a]	32,594 ± 1585[d]
3	4.8 ± 0.9	612 ± 66[b]	32,215 ± 2232[c]
30	2.8 ± 0.3	586 ± 41[c]	28,674 ± 1248[c]
400	8.7 ± 3.2	365 ± 26	19,089 ± 962
500	3.7 ± 0.9	524 ± 78	21,376 ± 2015
1000	6.4 ± 1.0	407 ± 62	20,278 ± 1736
1500	10.8 ± 4.3	420 ± 53	20,288 ± 1320
2000	5.8 ± 1.0	395 ± 34	20,273 ± 866
2500	8.0 ± 0.0	347 ± 50	20,160 ± 2040
3000	6.2 ± 1.2	406 ± 40	19,899 ± 1210
5000	7.3 ± 0.7	276 ± 21	16,046 ± 850

Data presented as mean ± standard error ($n = 6$, $n = 11$ for 0 mg/kg).
[a] Statistically significantly different from the animals receiving 400, 1000, 1500, 2000, 2500, 3000, and 5000 mg/kg HP-β-cyclodextrin.
[b] Statistically significantly different from the animals receiving 2500 and 5000 mg/kg HP-β-cyclodextrin.
[c] Statistically significantly different from the animals receiving 5000 mg/kg HP-β-cyclodextrin.
[d] Statistically significantly different from the animals receiving 400, 2500, and 5000 mg/kg HP-β-cyclodextrin.

Coadministration of 2-Hydroxypropyl-β-Cyclodextrin

Due to the risk of interstudy comparison and as substituted β-cyclodextrins are more used than natural β-cyclodextrin, due to their higher solubility and lower toxicity, this study also included an investigation of how HP-β-cyclodextrin performed when coadministrated with benzo[a]pyrene. The average plasma concentration as a function of time for benzo[a]pyrene after coadministration with HP-β-cyclodextrin is shown in Figure 2 and the associated pharmacokinetic parameters are given in Table 2. Individual curves for each cyclodextrin concentration can be found in the supporting information.

No significant difference was found between any of the treatments with respect to t_{max}, whereas differences were observed for both C_{max} and AUC, where those groups coadministered with the lower amounts of 2-HP-β-cyclodextrin had higher C_{max} and AUC values than the animals dosed with higher amounts of 2-HP-β-cyclodextrin. Extensive coadministration of HP-β-cyclodextrin could therefore limit the bioavailability of a compound as reported by Westerberg and Wiklund for β-cyclodextrin.[12] When compared with the data obtained when benzo[a]pyrene was coadministered with γ-cyclodextrin, it is noticeable that the decrease was approximately in the same range, that is, less than a factor 2. This study therefore suggests that very high doses of both 2-HP-β-cyclodextrin and γ-cyclodextrin have a negative impact on the bioavailability of benzo[a]pyrene, but there was no difference between the 2 types of cyclodextrins. The amount of benzo[a]pyrene dosed in this study was as low as 4.2 nmol/kg. Significant decreases in the absorption of benzo[a]pyrene were not seen until 400 mg/kg of one of the 2 cyclodextrins was dosed (equal to approximately 0.2 mmol/kg). Thus, the molar difference was >50,000 before the oral bioavailability of benzo[a]pyrene was significantly reduced. Overdose of cyclodextrin in these quantities would not be relevant in most oral applications, and based on the present data the danger of oral cyclodextrin overdosing should be considered only in extreme cases. The presence of high concentrations of cyclodextrin will lower the fraction of free and uncomplexed compound,[27] but a proportion of the dose will be present in the free form and available for oral absorption, which seems sufficient to drive absorption.

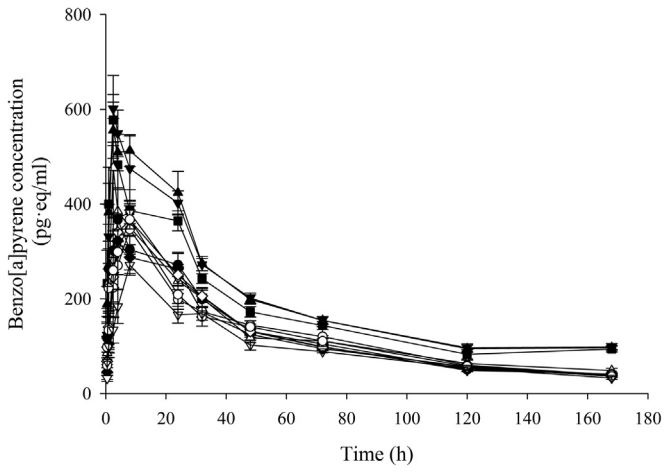

Figure 2. Total radioactivity plasma concentration time profile of [^{3}H]benzo[a]pyrene following single oral administration of 1050 ng/kg (250 mCi/kg) to rats with coadministration of (▲) 0, (▼) 3, (■) 30, (△) 500, (◇) 2000, (○) 3000, AND (▽) 5000 mg/kg of HP-β-cyclodextrin (shown in the legend). Data are presented as mean ± standard error ($n = 6$, 11 for 0 mg/kg).

The dose of benzo[a]pyrene in this study was chosen in order to compare the results with those reported by Westerberg and Wiklund,[12] as discussed above. Furthermore, the doses of cyclodextrins were selected to be in the range over which Westerberg and Wiklund[12] observed a decline in absorption, which was as low as 5 mg β-cyclodextrin/kg. In this study, a significant reduction was not observed until 400 mg/kg cyclodextrin. The reason for this difference was not clear, but a possible explanation could be the differences in degree of dilution of the doses administered. In this study, the cyclodextrin was dosed in a volume of 10 mL/kg, while Westerberg and Wiklund[12] used 20 mL/kg. This change was induced due to ethical reasons as described in good practices for studies in rodents.[28] The coadministration of a higher volume will lead to another dilution factor of the cyclodextrin and potentially to a different intestinal transit time affecting the overall absorption of benzo[a]pyrene. Another potential explanation could be that benzo[a]pyrene forms an insoluble complex with β-cyclodextrin, but not with the more soluble 2-HP-β-cyclodextrin or γ-cyclodextrin, although

in vitro data suggest that this is not the case.[22] This hypothesis may also explain why there are no stability constants between benzo[a]pyrene and natural β-cyclodextrin in the literature and why we observed very low concentrations of benzo[a]pyrene when the phase solubility study was conducted (data not shown). Another potential reason for the difference could be a different diffusion of the cyclodextrins through the unstirred water layer.[29,30] The 2 highly soluble cyclodextrins, HP-β-cyclodextrin and γ-cyclodextrin, may have an easier passage through the unstirred water layer than the poorly soluble β-cyclodextrin, resulting in a lower bioavailability for benzo[a]pyrene when coadministered with these cyclodextrins.

This study suggests that there is a small limited biopharmaceutical difference between a digestible and a nondigestible cyclodextrin. Lumholdt et al.[10] demonstrated that the digestion rate of γ-cyclodextrin *in vitro* was reduced when benzo[a]pyrene was present, which could reflect that the chemical equilibrium was faster than the enzymatic process and that enzymatic degradation was not possible when a compound was complexed within the cyclodextrin cavity. This mechanism could reduce the biopharmaceutical advantage, as observed in this study; however, these findings must be confirmed with other compounds, to ensure that it was not a benzo[a]pyrene-specific effect. More interestingly, this study also suggested that the risk of overdosing cyclodextrins may be lower than originally thought based on the study published by Westerberg and Wiklund.[12] This suggests that cyclodextrins can be used in excess without losing too much bioavailability for orally administered compounds, at least for highly permeable compounds such as benzo[a]pyrene. A better theoretical understanding of the system is still desirable as well as validation with other compounds at doses more relevant for most drug compounds.

Conclusions

In conclusion, this study has investigated the influence of oral coadministration of either 2-HP-β-cyclodextrin or γ-cyclodextrin in escalating doses on the absorption of benzo[a]pyrene to see if the digestibility of the cyclodextrin had an influence of the absorption of benzo[a]pyrene. The *in vivo* studies demonstrated a decreased absorption of low doses of benzo[a]pyrene when >400 mg/kg cyclodextrin was coadministered with the compound. The maximum decrease in the absorption was less than a factor 2. The mechanism leading to this phenomenon was thought to be a result of a strong interaction between the cyclodextrins and benzo[a]pyrene in combination with the limited amount of bile salts in the intestine ensuring displacement from the cyclodextrin cavity. No differences were observed between the 2 investigated cyclodextrins, that is, potential digestibility did not seem to be beneficial from a biopharmaceutical perspective.

The coadministration of 2-HP-β-cyclodextrin and γ-cyclodextrin led to significant lower reduction in the bioavailability when compared with data in the literature. This was suggested to be a reflection of formation of a poorly soluble complex between benzo[a]pyrene and natural β-cyclodextrin, which precipitated thereby leading to the low absorption. This was not observed with the more aqueous soluble cyclodextrins used in this study. Caution should still be taken when defining the cyclodextrin in a new formulation; though these findings suggest that the risk of overdosing with cyclodextrins may be lower than initially suggested.

Acknowledgments

The personnel in animal facilities at Lundbeck are gratefully acknowledged for their skillful conductance of the animal experiment. David John Simpson is acknowledged for his linguistic support.

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