Dissolution/Permeation of Albendazole in the Presence of Cyclodextrin and Bile Salts: A Mechanistic In-Vitro Study into Factors Governing Oral Bioavailability

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ABSTRACT

We aimed to understand the impact of the interplay between bile salts and cyclodextrins on the dissolution-permeation of poorly soluble drug compounds with a moderate-strong binding constant to cyclodextrin. Phase diagrams were prepared on the chosen model compound albendazole in phosphate buffer, fasted state simulated intestinal fluid (FaSSIF), and a modified fed state simulated intestinal fluid (FeSSIFmod) with (2-hydroxypropyl)-beta-cyclodextrin (HP-β-CD) concentrations of up to 10 % (m/m). Then we investigated the dissolution/permeation interplay of albendazole dissolved/suspended in the different media through a biomimetic barrier on a 96-well in vitro model. The apparent solubility of albendazole was enhanced by HP-β-CD and FaSSIF/FeSSIFmod separately. However, when albendazole was dissolved in HP-β-CD and biomimetic media together, the solubility was significantly lower than the predicted additive solubility from the solubilizing effects. It is postulated that this is due to the sodium taurocholate from the biomimetic media displacing albendazole from the hydrophobic cavity of HP-β-CD. In the permeation experiments, the highest permeation was observed at cyclodextrin concentrations able to solubilize close to the total dose of albendazole without a major surplus of solubilization capacity. Furthermore, an over-proportional permeation enhancement was observed when both, cyclodextrin and biomimetic media were present. These results indicate that the interplay between bile salts and cyclodextrins can enhance the free (molecularly dissolved) fraction of drug in solution to a greater extent than could be obtained with one of the solubilizing components alone. In conclusion, at carefully selected cyclodextrin-concentrations in combination with biomimetic media, obviously, a transient supersaturation is induced, which is made responsible for the observed major permeation enhancement.

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Introduction

Cyclodextrins are α-D-glucopyranose units linked together by α-1, 4 glycosidic bonds in the shape of a truncated cone with a hydrophobic inner and a hydrophilic external surface. The hydrophobic cavity enables cyclodextrins to incorporate small hydrophobic molecules via non-covalent interactions (e.g., van der Waals interactions and hydrogen bonds), and by this increase their apparent solubility. ¹ Apparent solubility refers to the total solubility of the dissolved and all solubilized species, i.e. it includes free molecules surrounded by a solvation shell (molecularly dissolved), molecules solubilized by incorporation within micelles and molecules complexed by complex formers like cyclodextrins. Such cyclodextrin-inclusion complexes represent a well-established and frequently used formulation strategy for poorly water-soluble drugs. ² While it is obvious that the formation of such inclusion complexes yields a substantial increase in apparent solubility and/or increase in dissolution rate, there has been raised considerable doubt in recent years, whether it is the enhanced apparent solubility per se that leads to enhanced absorption. Porter ³ and Fricker ⁴ and co-workers were among the first to demonstrate that micellarly solubilized drug molecules, in contrast to molecularly dissolved drug molecules, are not susceptible to overcome biological
In 2013, Buckley et al. postulated a generalizing hypothesis that within all enabling formulations, one may expect the concentration of molecularly dissolved drug (i.e., individual molecules surrounded by their hydration shell) to govern their permeation across biological barriers. Later that year, Dahan and co-workers demonstrated that it is not the concentration of cyclodextrin-bound drug, but the molecularly dissolved (free) drug that correlated with absorption rate, i.e. the aforementioned hypothesis also applies to this type of enabling formulations. Therefore, overdosing with cyclodextrins should be avoided as it may impair drug absorption by reducing the concentration of freely dissolved molecules. Various effects have been made responsible for the frequently observed enhanced oral bioavailability of this type of enabling formulations i.e. that they may enhance drug delivery across biological barriers. Brewster and coworkers emphasized that permeation of cyclodextrin and cyclodextrin-API complexes should be disregarded. On the other hand, a negligible permeation of cyclodextrin has been reported in literature. Alternatively, enhanced absorption has been attributed to cyclodextrins stabilizing supersaturation states, inhibiting efflux transporters, or to the ability of cyclodextrins to decrease the thickness of the unstirred water layer, i.e., an aqueous film adjacent to biological barriers, where the concentration profile of the drug is purely diffusion-controlled. On the other hand, recent work has demonstrated that cyclodextrins reduce the relative diffusivity by incorporating the molecules to reduce the concentration gradient. Both effects should delay the diffusion of complexed substrates across the unstirred water layer rather than increase the diffusion rate.

Albendazole is a poorly soluble benzimidazole anthelmintic drug which needs to be orally absorbed to be therapeutically efficient against, e.g., neurocysticercosis. The molecular structure of albendazole (MW is 265 g/mol) is shown in Fig. 1; it is a basic lipophilic compound (logD pH 7 is 3.47; pKa is 4.12/10.43). The binding constant of albendazole to (2-hydroxypropyl)-beta-cyclodextrin (HP-beta-CD) has been reported to be approx. 20,000 M⁻¹ in pure water which indicates strong interaction. Furthermore, the oral bioavailability of crystalline albendazole is too low for therapeutic use due to its low solubility. Several in vivo studies have shown that oral bioavailability can be increased when albendazole is formulated together with HP-beta-CD. However, to our knowledge, only the study by Yamashita and co-workers may serve as a reference for our mechanistic study, because their formulations were the only ones, which contained no other excipients besides cyclodextrins. The solubility of albendazole was determined in the 30mM phosphate buffer pH 6.5, FaSSIF, and FeSSIF, optionally containing HP-beta-CD at concentrations of 0.1 mg/mL albendazole, i.e., overdosing the cyclodextrin. In contrast, in vitro permeation studies showed that the permeation coefficient of albendazole increased in presence of HP-beta-CD up to HP-beta-CD concentrations necessary to completely solubilize all the albendazole at a dose of 0.1 mg/mL. However, above that HP-beta-CD concentration, the permeation was decreased.

In the present study, we want to investigate whether the effect of the presence of the bile salts would affect in vitro permeation experiments. The model drug albendazole, HP-beta-CD and different concentrations of taurocholate (as in fasted state simulated intestinal fluid (FaSSIF) or a modified fed state simulated intestinal fluid (FeSSIF_mod) at pH 6.5 to eliminate effects of the pH difference between the biomimetic buffers) were used. Solubility studies of albendazole were carried out in phosphate buffer, FaSSIF, or FeSSIF with different concentrations of cyclodextrin added. A permeability model with an artificial biomimetic barrier was selected to study the permeation of albendazole in the presence of HP-beta-CD and FaSSIF/FeSSIF_mod at conditions with albendazole both in solution and suspension.

Materials and Methods

Chemicals

Albendazole and HP-beta-CD were purchased from Abcr GmbH (Karlsruhe, Germany). FaSSIF/FeSSIF/FaSSIFGF powder was purchased from Biorelevant.com Ltd (London, United Kingdom). Trifluoroacetic acid and methanol were purchased from VWR™ International A/S (Søborg, Denmark). Sodium phosphate dibasic dihydrate and Sodium phosphate monobasic monohydrate were purchased from Sigma Aldrich ApS (Brøndby, Denmark). All the water used for experimental and analytical dispersions was analytical highly purified water prepared by a Milli-Q® reference A+ water purification system from Merck KGaA (Darmstadt, Germany).

Media Preparation

30 mM phosphate buffer pH 6.5 was composed of 19.3 mmol sodium phosphate monobasic monohydrate and 10.7 mmol sodium phosphate dibasic dihydrate per L of purified water. The pH was adjusted to 6.5 by adding a 0.1M hydrochloric acid solution while measuring the pH with the 827 pH lab apparatus (Metrohm, Herisau, Switzerland). 2.24 g/L or respectively 11.2 g/L of the commercial FaSSIF/FaSSIFGF powder was dissolved in 30mM phosphate buffer, FaSSIF and FeSSIF, optionally containing HP-beta-CD at concentrations of 0.1 mg/mL albendazole, i.e., overdosing the cyclodextrin. In contrast, in vitro permeation studies showed that the permeation coefficient of albendazole increased in presence of HP-beta-CD up to HP-beta-CD concentrations necessary to completely solubilize all the albendazole at a dose of 0.1 mg/mL. However, above that HP-beta-CD concentration, the permeation was decreased.

Phase Solubility Tests

The solubility of albendazole was determined in the 30mM phosphate buffer pH 6.5, FaSSIF, and FeSSIF_mod, optionally containing HP-beta-CD at different concentrations up to 10 % (m/m). An excess of 3 mg/mL albendazole was suspended in the selected media, followed by incubation in a Julabo SW23 shaking waterbath (Buch & Holm, Selbach, Germany) at 37°C and with a shaking rate at 150 rpm. After 24h, 1 mL samples were withdrawn from the suspensions, immediately centrifuged for 20 minutes at 15557 rcf and 37°C on the Centrifuge 5804R (Eppendorf AG, Germany) and the supernatants filtered through Whatman® Anotop® syringe filters with a pore size of 0.2 μm (GE Healthcare, Buckinghamshire, UK). The albendazole concentrations in the filtrates were determined using UHPLC-UV as described in section 2.6.

Figure 1. Chemical structure of albendazole.
In order to ensure that equilibrium solubility was reached, additional samples were withdrawn from the incubated suspensions at 24 h intervals and handled by the same procedure as the previous samples. Equilibrium was assumed to be reached when the difference between two subsequent data points (means, n = 3) at 24h intervals was less than 5% or not significant (p=0.05). The albendazole/cyclodextrin binding constant was calculated as described in supplement S1; in brief: the slope derived from plot of dissolved albendazole (at equilibrium in presence of excess albendazole) versus cyclodextrin concentration was used and compared to the concentration of dissolved albendazole in absence of cyclodextrin.

Permeation Screening of Albendazole Under Static Solubilizing Conditions

Permeation experiments were carried out to screen for how the presence of HP-β-CD in FaSSIF and FeSSIFmod solutions affected albendazole's permeation. 50 μg/mL albendazole was added to donor media containing 0, 2, 5 or 10 % (m/m) HP-β-CD in 30 mM phosphate buffer, FaSSIF or FeSSIFmod the day before experiments. The donor dispersions were sonicated for 10 minutes, followed by 16-18 h of incubation in the Julabo SW23 shaking water-bath (Buch & Holm, Seelbach, Germany) at 37°C and with a shaking rate at 150 rpm. The permeation experiments were carried out using PermeaPad® 96-well plates (InnoMe GmbH, Espelkamp, Germany). PermeaPad® is an artificial biomimetic barrier designed as a sandwich with a layer of dry phospholipids enclosed inbetween two cellulose hydrate membranes. Upon contact with aqueous media, the dry phospholipids form a layer of tightly packed phospholipid vesicles and the lipid layer resembles in its structure vesicular phospholipid gels, which earlier have been described.

The bottom compartment of the PermeaPad® 96-well plate was filled with 400 μL of donor dispersion. Then the top chambers were filled with 200 μL of a 30 mM phosphate buffer pH 6.5 containing 10 % (m/m) cyclodextrin as the acceptor medium. This was done in order to ensure a strong absorptive sink in the acceptor compartment and to increase permeation rates. The plates were sealed with adhesive aluminum foil for microplates (VWR international, USA).

The plates were incubated in a PHMP-4 microplate Thermo-Shaker (Grant Instruments, Cambridge, United Kingdom) at 37°C and with orbital shaking at 300 rpm (shaking diameter 2 mm). After respectively 5 and 24 h of incubation, 50 μL samples were drawn from the top compartment (where each well was only used for one sample after either 5 or 24 h). Donor samples were taken before experiments and after 24 h and filtered through Whatman® Anotop® syringe filters with a pore size of 0.2 μm (GE Healthcare, Buckinghamshire, UK). All samples were analyzed by UHPLC-UV as described in section 2.6.

Permeation Screening of Albendazole Under Dynamic Conditions

Another set of permeation experiments was carried out. In these experiments double-concentrated solutions/suspensions of HP-β-CD and API was prepared as well as double-concentrated FaSSIF/FeSSIFmod and incubated in the water bath overnight (16-18 h) at 37°C and 150 rpm (Table 1). Here, at the start of experiments, the PermeaPad® plate bottom wells were filled with 200 μL of both albendazole/HP-β-CD dispersions and FaSSIF/FeSSIFmod solutions to obtain the same dispersion compositions as studied in section 2.4. The rest of the permeation experiment was carried out as described in section 2.4 as well. In essence, the difference between permeation experiments described under 2.4 and 2.5 is that in the latter taurocholate was first added at the beginning of the permeation experiment and thus had no opportunity to interact with the cyclodextrin/drug-complexes prior to start of the dissolution/permeation-process.

| Table 1: Donor media used for permeation screenings under dynamic conditions. |
| --- |
| Incubated API solution/suspension | Incubated FaSSIF/FeSSIFmod solution | Final donor solution after mix |
| 4 % HP-β-CD | 6 mM taurocholate | 2 % HP-β-CD |
| 100 μg/mL albendazole | 1.5 mM phospholipids | 50 μg/mL albendazole |
| 1.5 mM phospholipids | 7.5 mM phospholipids | 0.75 mM phospholipids |
| 4 % HP-β-CD | 30 mM taurocholate | 2 % HP-β-CD |
| 100 μg/mL albendazole | 7.5 mM phospholipids | 50 μg/mL albendazole |
| 15 mM taurocholate | 3.75 mM phospholipids |
| 10 % HP-β-CD | 6 mM taurocholate | 5 % HP-β-CD |
| 100 μg/mL albendazole | 1.5 mM phospholipids | 50 μg/mL albendazole |
| 3 mM taurocholate and | 0.75 mM phospholipids |
| 20 % HP-β-CD | 6 mM taurocholate | 10 % HP-β-CD |
| 100 μg/mL albendazole | 1.5 mM phospholipids | 50 μg/mL albendazole |
| 15 mM taurocholate and | 3.75 mM phospholipids |
| 20 % HP-β-CD | 30 mM taurocholate | 10 % HP-β-CD |
| 100 μg/mL albendazole | 7.5 mM phospholipids | 50 μg/mL albendazole |
| 15 mM taurocholate | 3.75 mM phospholipids |

UHPLC-UV Quantification of Albendazole

All samples were diluted 1:1 (v/v) with methanol before analyzing. The analysis was carried out using an UltiMate 3000 UHPLC system (Thermo Fisher Scientific™, Waltham, Massachusetts, USA) with a reversed-phase Kinetix® EVO C18 LC-column 150 × 2.1 mm; particle size 1.7 μm; pore size 100 Å (Phenomenex®, Værløse, Denmark) at 40°C and a mobile phase consisting of 45% (v/v) highly purified water with 0.1 % (v/v) trifluoroacetic acid at a flow rate of 0.25 mL/min. Albendazole was UV detected with a connected Diode Array detector at a wavelength of 294 nm. Chromatograms were analyzed with the Chromeleon software version 6.8, and concentrations were determined by correlation to calibration curves. No disturbance of the analytical method by FaSSIF, FeSSIFmod, or HP-β-CD was observed (data not shown).

Statistics

Two-factor ANOVA with Holm-Sidak post hoc analysis was carried out in the software Graph-Pad Prism 8.4.2. The test analyzed permeability data within each group and compared the data sets at equal cyclodextrin concentrations. For corrected p-values ≤0.05 the data were considered significantly different.

Results and Discussion

Apparent Solubilities of Albendazole in the Presence of HP-β-CD and Biomimetic Media

Albendazole reached the equilibrium solubility in all the studied media after 48 h at the latest (table 2), as confirmed by additional samples taken 24 hours later (data not shown). The apparent solubility of albendazole in the buffer was determined as 0.74 μg/mL or 2.8 μM. This value is in excellent agreement with reported intrinsic solubility and in good agreement with earlier reported values of 0.80 and 0.85 μg/mL, respectively, at the same pH but different buffer composition. Other reported values differ to a greater extent, but they were measured at 25°C in water without pH control.
The phase solubility diagram of albendazole in HP-β-CD (Fig. 2A) in the same buffer indicates a linear increase of apparent solubility of albendazole as a function of the HP-β-CD concentration. In the concentration range studied, the phase solubility behavior can be classified as an AL-type, and a 1 to 1 stoichiometry can be assumed. The calculated K1:1 binding constant of 5220 M⁻¹/C₀ is somewhat smaller than earlier reported binding constants of 19,546 M⁻¹/C₀ and 18,106 M⁻¹/C₀, respectively. These values are in reasonable accordance with the present value bearing in mind that the former had been measured in pure water, while the value reported refers to buffer of pH 6.5.

The apparent solubility of albendazole increased with increasing concentrations of phospholipids/sodium taurocholate when comparing buffer, FaSSIF, and FeSSIFmod. The solubilizing effect, however, was much less pronounced than that of HP-β-CD. This can be seen from the plot for the apparent solubility of albendazole vs. the sodium taurocholate-concentration in a phase solubility diagram (Fig. 2B). However, taking into account the fact that several colloidal structures in the biomimetic media coexist, it is not reasonable to calculate any complex constants.

The bar graph in Fig. 3 has been constructed based on the observation that FaSSIF and FeSSIFmod as well as HP-β-CD, each increased the

![Figure 2](image-url)

**Figure 2.** The measured apparent solubility of albendazole in pH 6.5 phosphate buffer with A different concentrations of HP-β-CD or B FaSSIF and FeSSIFmod at 37°C. All data are reported as mean ± SD (n = 3).

![Figure 3](image-url)

**Figure 3.** The bars represent the predicted additive apparent solubility of albendazole with the assumption that solubilization from HP-β-CD, FaSSIF, and FeSSIFmod and free drug are the averages presented in Fig. 2. The red dots represent the experimental solubility of albendazole in the same media (FaSSIF and FeSSIFmod in the presence of different concentrations of HP-β-CD at 37°C). The experimental solubility data are reported as mean ± SD (n = 3).

### Table 2
Albendazole concentrations measured in donor solutions for permeation experiments before and after experiments. Start concentrations are reported as mean ± SD (n = 3). 24 h-concentrations are reported as mean (n = 8) with no SD due to the small volume in donor wells for filtration. Experiments in 0 and 2 % HP-β-CD were in suspensions, while all the albendazole was dissolved in 5 and 10 % HP-β-CD experiments. Solubility values from Fig. 2 have been repeated for easier comparison.

| Donorstart (μg/mL) | Donor24h (μg/mL) | Solubility (μg/mL) |
|-------------------|------------------|--------------------|
| 0% HP-β-CD        | 1.7              | 0.7 ± 0.0          |
| 0% HP-β-CD + FaSSIF | N/A             | 3.1 ± 0.0          |
| 0% HP-β-CD + FeSSIFmod | 8.9        | 10.3 ± 0.1         |
| 2% HP-β-CD        | 24.3 ± 0.2       | 29.9               |
| 2% HP-β-CD + FaSSIF | 33.8           | 54.1 ± 0.4         |
| 2% HP-β-CD + FeSSIFmod | 43.2         | 44.2 ± 1.4         |
| 5% HP-β-CD        | 48.5             | 61.4 ± 0.5         |
| 5% HP-β-CD + FaSSIF | 48.7           | 143.3 ± 0.9        |
| 5% HP-β-CD + FeSSIFmod | 46.7         | 113.2 ± 1.2        |
| 10% HP-β-CD       | 50.5 ± 0.2       | 49.2               |
| 10% HP-β-CD + FaSSIF | 49.2           | 251.2 ± 1.9        |
| 10% HP-β-CD + FeSSIFmod | 45.2         | 251.1 ± 3.0        |

| 0% HP-β-CD + FaSSIF | 1.7 ± 0.0 | 0.7 ± 0.0 |
| 0% HP-β-CD + FeSSIFmod | 3.1 ± 0.0 | 10.3 ± 0.1 |
| 2% HP-β-CD + FaSSIF | 29.9 | 54.1 ± 0.4 |
| 2% HP-β-CD + FeSSIFmod | 33.8 | 44.2 ± 1.4 |
| 5% HP-β-CD + FaSSIF | 48.5 | 143.3 ± 0.9 |
| 5% HP-β-CD + FeSSIFmod | 46.7 | 113.2 ± 1.2 |
| 10% HP-β-CD + FaSSIF | 49.2 | 251.2 ± 1.9 |
| 10% HP-β-CD + FeSSIFmod | 45.2 | 251.1 ± 3.0 |

**Note:** All data are reported as mean ± SD (n = 3).
solubility of albendazole individually. The sum of apparent solubility values, derived from independent solubility experiments has been set equal 100%, where the value obtained in buffer is assumed to represent the fraction of molecularly dissolved albendazole, the solubility values in FaSSIF and FeSSIFmod represent the molecularly dissolved fraction plus the fraction of albendazole solubilized in micelles and solubility values in cyclodextrin solution represent the fraction of molecularly dissolved albendazole plus the fraction of albendazole solubilized by cyclodextrin-complexation (a calculation example in supplement S2). One may expect additive effects on the albendazole solubilization in the presence of FaSSIF or FeSSIFmod and HP-β-CD. These are depicted in the graph for FaSSIF and FeSSIFmod in the absence and presence of HP-β-CD in concentrations up to 10% (m/m). In the same graph, the dots represent the apparent solubility of albendazole in the same media. In contrast to the expectations, a lower solubilization effect was observed in all cases when HP-β-CD and FaSSIF or FeSSIFmod were present together. In fact, the overall amount of apparently dissolved amount of albendazole in cases of HP-β-CD and FeSSIFmod was even lower than in the buffered solution of HP-β-CD. Therefore, it is hypothesized that the sodium taurocholate in FaSSIF and FeSSIFmod may interact with cyclodextrin, possibly displacing albendazole from the hydrophobic cavity of HP-β-CD and thus by competition lowering its solubilization capacity for albendazole. The fact that drugs may be displaced from the cyclodextrin cavity by endogenous compounds, among which are taurocholate, has been known for decades.26,27,28,29,30 Holm and co-workers presented a mathematical model to describe the displacement of poorly soluble drugs from the HP-β-CD cavity by sodium taurocholate based on thermodynamic constants.31 To the best of our knowledge, however, displacement of albendazole from cyclodextrin has not been investigated before.

Permeation Screening

Table 2 shows the donor concentrations in the solutions and suspensions used for the permeation experiments. Even though the dispersions were sonicated for 10 min and incubated in a water bath for 16–18 h at 37°C before starting permeation experiments, the same concentrations as in the solubility test could still not be obtained. This is probably due to the poor wettability of albendazole crystals and the much lower amount of the drug used for the preparation of the solutions for permeation experiments as compared to the solubility test. The concentration of albendazole in the donor in plain phosphate buffer seems to exceed solubility at the end of the experiments, which may be due to the hypothesis that a tiny fraction of the HP-β-CD (used in the acceptor medium to generate sink) may be permeating from the acceptor compartment to the donor compartment. (Experimental data for HP-β-CD permeation across the barrier are presented in supplement S3). We regard this degree of permeation as negligible for the present study of permeability comparison.

Fig. 4 compares the permeation of albendazole (expressed as the concentration in the acceptor medium at a certain time point) from the different solutions and suspensions in phosphate buffer, FaSSIF and FeSSIFmod at different concentrations of HP-β-CD. The lowest permeation was observed in the cases with 10% HP-β-CD independently of which medium was used. This observation is consistent with the literature concerning overdosing with cyclodextrins which decreases the free fraction of the drug.7

Moreover, a higher permeation was observed in the 2 and 5% HP-β-CD dispersions of albendazole compared to the suspensions of albendazole without cyclodextrin, which we attribute to the higher concentrations.

In the cases of 2 and 5% HP-β-CD, where the suspensions and solutions contain amounts of solubilizers closer to that required to just completely dissolve the dose, we observed a significantly higher permeation of albendazole in FaSSIF in all cases compared to the phosphate buffer. While in the 5% HP-β-CD, the FeSSIFmod solutions showed similar tendencies to the FaSSIF solutions, was the enhancement less pronounced in the 2% HP-β-CD after 5 h, and we did not see any permeation enhancement after 24 h. However, it should be noted that almost 2-fold less albendazole was apparently dissolved in the 2% HP-β-CD FeSSIFmod medium compared to the phosphate buffer and FaSSIF (Table 2).

In literature, the competitive displacement of drugs from cyclodextrins has mainly been discussed regarding the dosing and the amount of cyclodextrin or competing excipients needed to completely dissolve the dose without over-solubilizing the drug.31,32 This assumption goes along with a recent rat study with albendazole, where the absorption was highest with a similar degree of overdosing with HP-β-CD as in the present study.33 We hypothesize therefore that competitive displacement from the hydrophobic cavity by bile salts might have released the drug to increase the
absorption. However, we could not show this effect in our permeation study. One explanation may be that rats contain a higher concentration of bile salts (≈ 50 mM in the upper jejunum under fasted conditions) than humans (≈ 3 mM) and thus the media we were using, a difference in experimental design, which was not further investigated here.33,34 According to Aungst rats have 3–4.6 mL present in their small intestine.35 Assuming 3 mL liquid with bile salt concentration at 50 mM and 20% CD solution administered in a volume of 0.5 mL results in a total volume of 3.5 mL. CD concentration of 2.8%, 43 mM bile salts and 14.3 mg/mL albendazole, it would fit with our observation that they see an increase in bioavailability with increasing HP-β-CD concentration under these conditions.

However, the dynamic interplay between the cyclodextrin/drug complex being exposed to the competing agent taurocholate might increase the absorption of the drug even further. This effect is not captured by our set-up dissolving all of the dose in the mixed media. We speculate that the competitive displacement might push out the drug compound of the hydrophilic cavity of cyclodextrins to generate a concentration of molecularly dissolved drug in a similar way as a supersaturation, which may be present for a longer time interval when simultaneously allowing for permeation. This would result in more API being able to permeate through the barrier, as illustrated in Fig. 5, and a dynamic permeation experiment was carried out.

**The Dynamic Scenario – HP-β-CD and FaSSIF Mixed Upon Administration**

The data presented in Fig. 4 represent a scenario with thermodynamically equilibrated donor solutions overnight in a water bath before the experiments, while in vivo the drug-cyclodextrin complex will not come in contact with bile components before reaching the GI tract. HP-β-CD and albendazole were incubated separately from the FaSSIF/FeSSIF/FaSSGF solution overnight and mixed when beginning the permeation experiments to investigate the dynamic effects. Fig. 6 shows that in FaSSIF, there are no statistically significant differences between co-incubation overnight and mixing of the media at the start of the experiment. For the high concentration of 10% HP-β-CD in the solutions, in both scenarios, the taurocholate concentration was too small to override the complexation by the competition and free enough albendazole to boost the permeation. In contrast, in FeSSIF, there are no statistically significant differences between co-incubation overnight and mixing of the media at the start of the experiment. For the high concentration of 10% HP-β-CD in the solutions, in both scenarios, the taurocholate concentration was too small to override the complexation by the competition and free enough albendazole to boost the permeation. In contrast, in FeSSIFmod, with the higher taurocholate concentration as compared to FaSSIF, the permeation was found to be increased in the dynamic scenario when mixing at the beginning of experiments in 2 and 5% HP-β-CD solutions. The taurocholate concentration, as compared to the HP-β-CD concentration, was high enough to remove the albendazole from the HP-β-CD complex to yield measurable increased permeation. It may be expected that the effect on
permeation would be even larger when using even higher taurocholate concentrations comparable to the rat intestine, possibly enhance the permeation from the 10 % HP-β-CD formulation. The measured increase in permeation with HP-β-CD concentration and the assumption regarding competing displacement in a dynamic scenario coincides with the findings of a recent rat study with albendazole, where the absorption was highest in overdosing with HP-β-CD.20

Conclusion

The taurocholate in the biomimetic media as well as HP-β-CD both increased the apparent solubility of albendazole. When albendazole was dissolved in media containing both compounds, a competitive displacement of albendazole from the hydrophobic cavity of HP-β-CD by bile salts caused that the apparent solubility of albendazole not to reach the additive value. In most cases, apparent solubility became even lower than if albendazole had been dissolved in the HP-β-CD alone. The interplay between the taurocholate and HP-β-CD reach the additive value. In most cases, apparent solubility became even lower than if albendazole had been dissolved in the HP-β-CD alone. The interplay between the taurocholate and HP-β-CD was found to enhance the permeation of albendazole at certain ratios of the two competitors. We thus suggest that the dynamic interplay between components in the biomimetic media and cyclodextrins might increase the amount of molecularly dissolved albendazole, thereby enhancing the permeation. This can be measured under carefully selected experimental conditions that not only regard the ratio of the components but also embrace the dynamic scenario by the kinetics of the competition and simultaneous permeation. Such experimental approach may explain bioavailability data and even allow to screen, predict and rank different formulations without the use of animal studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgments and Conflicts of Interest

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