Bile and excipient interactions directing drug pharmacokinetics in rats

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Abstract:

Bile solubilization plays a major role in the absorption of poorly water-soluble drugs. Excipients used in oral drug formulations impact bile-colloidal properties and their molecular interactions. Polymer-induced changes of bile colloids, e.g., by Eudragit E, reduced the flux of the bile interacting drug Perphenazine whereas bile non-interacting Metoprolol was not impacted. This study corroborates these *in vitro* findings in rats. Eudragit E significantly reduced systemic availability of Perphenazine but not Metoprolol compared to the oral administrations without polymer. This study confirms the necessity to carefully select polymers for bile interacting drugs whereas non-bile interacting drugs are more robust in terms of excipient choice for formulation. The perspective of bile interaction may introduce interesting biopharmaceutical leverage for better performing oral formulations of tomorrow.

Introduction

Bile plays a key role in the lipid digestion of vertebrates [1]. Furthermore, **p**oorly **w**ater-**s**oluble **d**rug**s** may be solubilized by bile, thereby enhancing their bioavailability [2]. Bile is essential for the absorption of drugs interacting with bile [2]. Nevertheless, some polymers used in oral drug formulations impact bile colloids. In fact, this polymer interaction with bile *in vitro* may reduce the flux of bile interacting drugs. The resulting hypothesis, bile interacting drugs may be preferentially formulated with bile-inert polymers whereas bile-non interacting drugs are more robust in terms of polymer choice, is now addressed *in vivo* in rats. For that, we focus on the pharmacokinetic profiles of Perphenazine (bile interacting) or Metoprolol (bile non-interacting), administered with and without (bile-interacting) Eudragit E [3]. The *in vivo* study conditions were selected in light of previously published *in vitro* conditions [3]. We also integrated another arm by applying a mixture of Eudragit E and Colesevelam, as Colesevelam is a therapeutic developed for binding bile acids thereby serving as a positive control. Plasma concentrations were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Materials and Methods

Materials

Eudragit E PO was gifted from Evonik Nutrition and Care GmbH (Essen, Germany). Colesevelam hydrochloride was acquired from BOC Sciences (Shirley, NY, USA). Perphenazine, Metoprolol tartrate, Metoprolol-d_7 tartrate, and Omeprazole were purchased from Sigma-Aldrich (Schnelldorf, Germany). Perphenazine-^{13}C3 was in-house synthesized and purified.

Methods

Media Preparation

Modified phosphate-buffered saline (PBS) pH 6.5 was prepared as reported [3]. For Colesevelam treatment groups, the PBS was adjusted with sodium hydroxide to neutralize acidic valency of the hydrochloride. Ionic strengths were kept constant in all treatment groups by adding sodium chloride if necessary.

Pharmacokinetic study in rats

A comparative **p**harmaco**k**inetic (**PK**) study in male wistar rats (Toxi Coop zrt., Budapest, Hungary) was conducted in compliance with the animal welfare directive 2010/63/EU at ATRC Aurigon Toxicological Research Center Ltd. (Dunakeszi, Hungary). 6 different treatment groups with 5 animals per group were included in this study (**Table 1**). The animals fasted overnight before application. Colesevelam and Eudragit E were equilibrated with respective PBS. Approximately 2 hours before administration, rats received 20 mg/kg Omeprazole in PBS to

minimize pH effects [4]. Metoprolol or Perphenazine was added as a DMSO stock solution to excipient/medium mixtures right before administration resulting in a drug concentration of 2 mmol/l. The amount of DMSO in applied solutions was 1% V/V. Sampling time points were selected from previous reports [5, 6]. Perphenazine and Metoprolol concentrations were selected to ensure a dissolved state throughout the gastrointestinal passage. Perphenazine did not precipitate in any medium for at least four hours (data not shown). Rats received solutions orally by gavage with an administered volume of 5 ml/kg. Perphenazine and Metoprolol-tartrate doses were 10 µmol/kg (4.04 and 3.42 mg/kg, respectively). The Eudragit E concentration was 2% w/V and Colesevelam 10% w/V.

Table 1*:* Study treatment groups and dose regime.

Group name	Drug [10 µmol/kg]	Colesevelam [mg/kg]	Eudragit E [mg/kg]
Perphenazine control	Perphenazine	-	-
Perphenazine + Eudragit E	Perphenazine	-	100
Perphenazine + Colesevelam + Eudragit E	Perphenazine	500	100
Metoprolol control	Metoprolol	-	-
Metoprolol + Eudragit E	Metoprolol	-	100
Metoprolol + Colesevelam + Eudragit E	Metoprolol	500	100

A Colesevelam concentration 10 times higher compared to *in vitro* experiments was chosen, as rats have roughly a tenfold higher basal intestinal bile salt concentration compared to humans [7]. Roughly 200 µl blood was withdrawn from e.g., tail, or sublingual vein, into lithium heparin tubes at 6 time points (1h, 2h, 3h, 5h, 10h, and 24h for Perphenazine; 15 min, 30 min, 45 min, 60 min, 120 min, and 270 min for Metoprolol), plasma was obtained by centrifugation (3000 g at room temperature for 10 min), and frozen until analysis. Animals were sacrificed after the last blood sampling.

Liquid chromatography-tandem mass spectrometry analysis

For liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, plasma was extracted with ice-cold Acetonitrile including internal standard, and vortexed. Samples were centrifuged for 15 min at 4 °C and 24,900 g (Universal 320 R, Andreas Hettich GmbH & Co. Kg, Tuttlingen, Germany). The supernatant was diluted with the respective mobile phase, vortexed and centrifuged again. Ultra-high performance liquid chromatography (UHPLC) was performed on an Agilent 1200 Series HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a XBridge C18 3.5 µm 2.1 x 50 mm (Waters Corporation, Milford, MA) column. The column compartment temperature was set to 20 °C and the injection volume was 5 µl. Mobile phase A was 10 mM NH_4HCO_3 in water/methanol 90/10 (V/V). Mobile phase B was 10 mM NH_4HCO_3 in 90/10 (V/V) methanol/water. The flow rate was set to 0.6 mL/min. The UHPLC effluent was channeled to an Agilent 6460 triple quadrupole

operating with an electrospray ionization interface, in multiple reaction monitoring and positive ion mode. Mass spectra were acquired using a transition of 404 to 143 m/z for Perphenazine and 407 m/z to 174.1 m/z for -Perphenazine $^{13}C3$ with a collision energy of 28 and 24 eV, respectively. For Metoprolol transitions from 268.1 to 159 m/z and 279.1 to 123 m/z for Metoprolol-d_7 with a collision energy of 17 and 20 eV were applied, respectively. Signal-to-noise ratios of 3 and 10 were used to estimate the limit of detection and limit of quantification , respectively [8]. The limit of detection and quantification was 0.53 nmol/l and 1.8 nmol/l for Perphenazine and 0.95 nmol/l and 3.15 nmol/l for Metoprolol, respectively. Drugs were quantified with respective internal standard pairing using calibration curves. Individual noncompartmental **PK** analysis was applied to plasma concentrations using R version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria) using the package 'NonCompart' version 0.4.9 (Kyun-Seop Bae). Area under the curve to last nonzero concentration using the linear up and down method was calculated (AUC_{last}). Rat number 41 (Perphenazine control) and 15 (Perphenazine + Eudragit E) were excluded from the analysis. Rat number 41 was not pretreated with Omeprazole and the 1 h plasma value of rat 15 was considered as an outlier.

Statistical Analysis

A double-sided Grubb's test was used for outlier testing. One-way ANOVA followed by *a post hoc* Tukey test was performed. Data were considered statistically significant at $p \leq 0.05$. OriginPro 2020 (OriginLab Corporation, Northampton, MA, USA) was used for statistical analysis.

Results and Discussion

Previously reported outcomes demonstrated the interaction of Eudragit E with taurocholate and lecithin in bile colloids (**Figure 1**; modified from [3]), and these interactions might impact drug solubilization, release rates, and ultimately bioavailability. For example, bile-solubilized Perphenazine had a reduced flux across cellulose membranes in presence of Eudragit E (**Figure 1A**; modified from [3]). Colesevelam, a polymeric bile acid sequestrant, increased Perphenazine flux. Metoprolol flux across cellulose membranes, a drug substance that is not relevantly solubilized by bile, was not affected by any of these conditions (**Figure 1B**). The *in vitro* study from which this data is shown [3] is now supplemented with in vivo pharmacokinetics in rats.

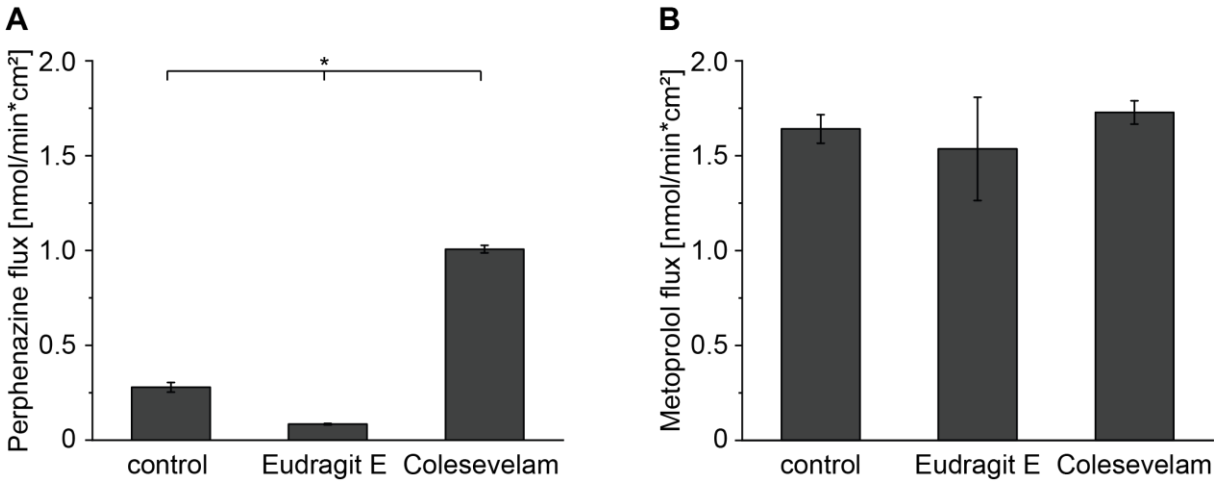

Figure 1: (A) Perphenazine and (B) Metoprolol flux over cellulose membrane in fasted state simulated intestinal fluid V1 (control) and with Eudragit E or Colesevelam. Data shown as mean ± SD, ANOVA considering $p \leq 0.05$ as statistically significant followed by Tukey post-hoc test for pairwise comparison (significant differences are shown by asterisks). The data was previously published: Schlauersbach et. al. "Leveraging bile solubilization of poorly water-soluble drugs by rational polymer selection", J. Control. Release, 330 (2021) 36-48 © Elsevier, 2020. Reprinted with license from Elsevier (license number: 5324180742375)

The plasma concentration profiles of Perphenazine (bile-interacting [9]) were biphasic with an initial absorption and subsequent elimination phase (**Figure 2A**). Co-administration of Eudragit E particularly impacted the absorption phase (**Figure 2B**). Exposure to both, Eudragit E and Colesevelam further flattened the plasma-concentration profile (**Figure 2C**). Overall, the Perphenazine **a**rea **u**nder the **c**urve (**AUC**) was significantly reduced in presence of Eudragit E and for both polymers (**Figure 2D, Table 2**). Metoprolol plasma concentration declined faster compared to Perphenazine (**Figure 2E-G**). Metoprolol (not interacting with bile [3]) plasma profiles and the AUC were neither impacted by Eudragit E nor Eudragit E/Colesevelam (**Figure 2H**). T_{max} was increased for Perphenazine groups with Eudragit E, which might reflect a release of drug in more distal parts of the gastrointestinal system (**Table 2**) as reported in the context of colonic drug delivery [10].

Taken together, Eudragit E induced changes to bile that significantly reduced flux and bioavailability of bile-interacting Perphenazine. From our in vitro experiments, one might have expected higher perphenazine exposure in the presence of Colesevelam and Eudragit E since flux increased in the presence of Colesevelam. However, the reduction in exposure to Eudragit E was still dominant. Colesevelam was thus unable to release Perphenazine from the strong interaction with Eudragit E and bile. In contrast, non-bile interacting Metoprolol's flux and bioavailability were not impacted.

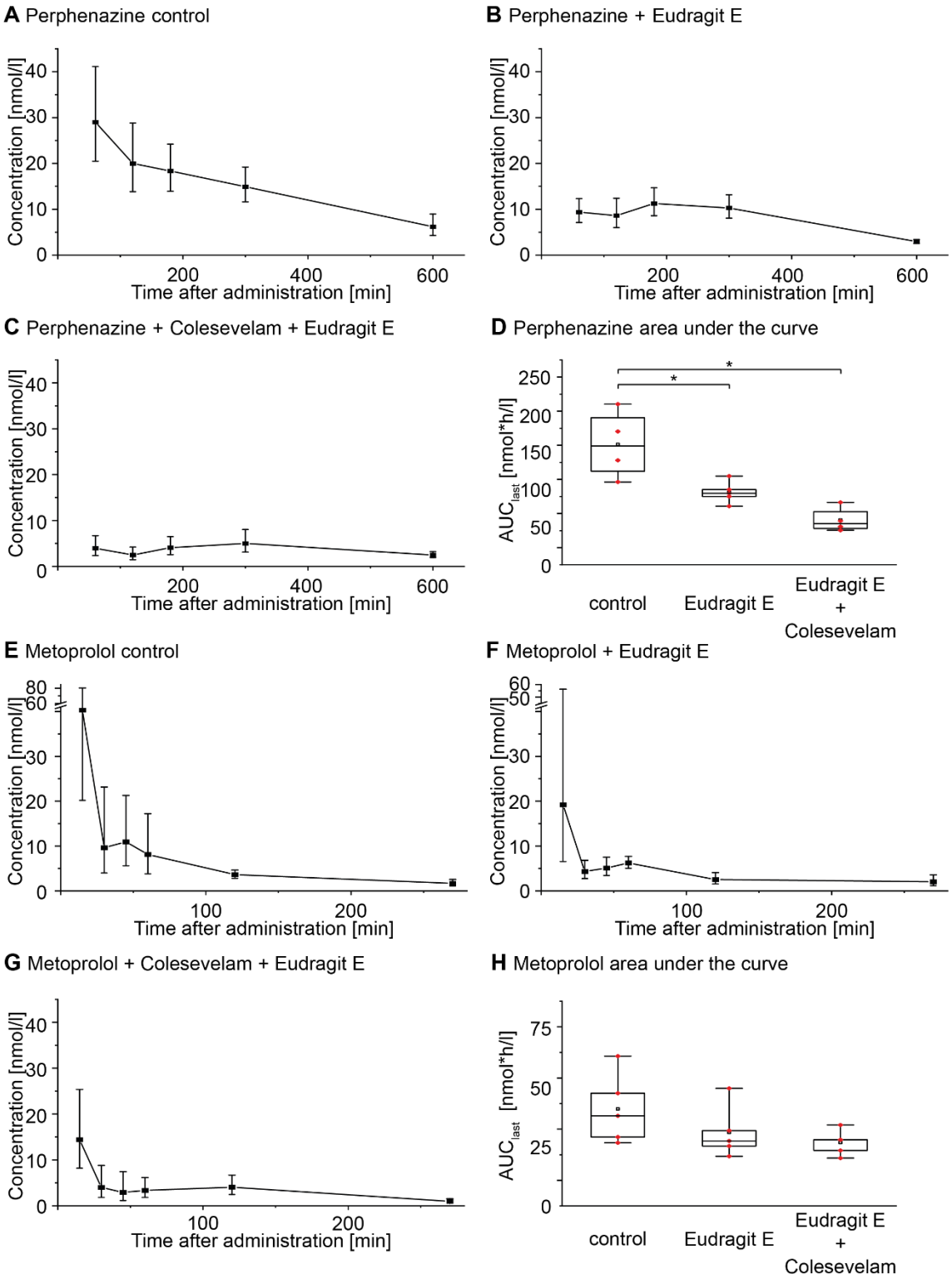

Figure 2: (A-C) Perphenazine and (E-G) Metoprolol plasma concentration over time (geometric mean ± geometric standard deviation) in rats (A, C n=4; B, E-G, n=5) after oral administration of 10 µmol/kg drug (A, E) in PBS, (B, F) with Eudragit E, and (C, G) Eudragit E and Colesevelam. Lines between points were linearly interpolated. AUC_{last} for (D) Perphenazine and (H) Metoprolol treatment groups. Data shown as red diamonds, boxplot in with mean as black dot, median as line, interquartile range from 25th percentile to 75th percentile as box, and range within minimum to maximum value as line. ANOVA considering $p \leq 0.05$ as statistically significant followed by Tukey *post-hoc* test for pairwise comparison (asterisks show significant differences).

In conclusion, Eudragit E and Eudragit E with Colsesevelam critically impacted Perphenazine absorption, but not Metoprolol. The *in vivo* data shown here corroborates previous *in vitro* data sets (shown again in part in **Figure 1**) and statements. Polymer selection for the formulation of bile-interacting drug substances might be critical to preserving the solubilization capacity of bile [3].

Table 2: Perphenazine and Metoprolol non-compartmental PK analysis for control, Eudragit E, and Colesevelam + Eudragit E treatment. T_{max} is shown as median and range in brackets. Data shown as mean ± SD. For Perphenazine groups with Eudragit E, no parameters dependent on λ_Z could be calculated, as observed excretion period was too short.

Treatment	c_{max} [nmol/l]	t_{max} [h]	AUC_{last} [h*mg/l]	λ_Z [1/h]	CL/F [l/h]
Perphenazine control	30.8 ± 12.1	1 (1-1)	151 ± 50	0.16 ± 0.02	57 ± 22
Perphenazine + Eudragit E	13.3 ± 3.1	3 (1-5)	81 ± 19	N/A	N/A
Perphenazine + Colesevelam + Eudragit E	5.8 ± 3.1	5 (3-10)	41 ± 18	N/A	N/A
Metoprolol control	50.3 ± 34.2	0.25 (0.25)	35 ± 17	0.48 ± 0.28	272 ± 90
Metoprolol + Eudragit E	35.3 ± 46.6	0.25 (0.25)	23 ± 13	0.29 ± 0.17	338 ± 144
Metoprolol + Colesevelam + Eudragit E	17.2 ± 10.4	0.25 (0.25 -2)	18 ± 6	0.44 ± 0.22	511 ± 189

Acknowledgments

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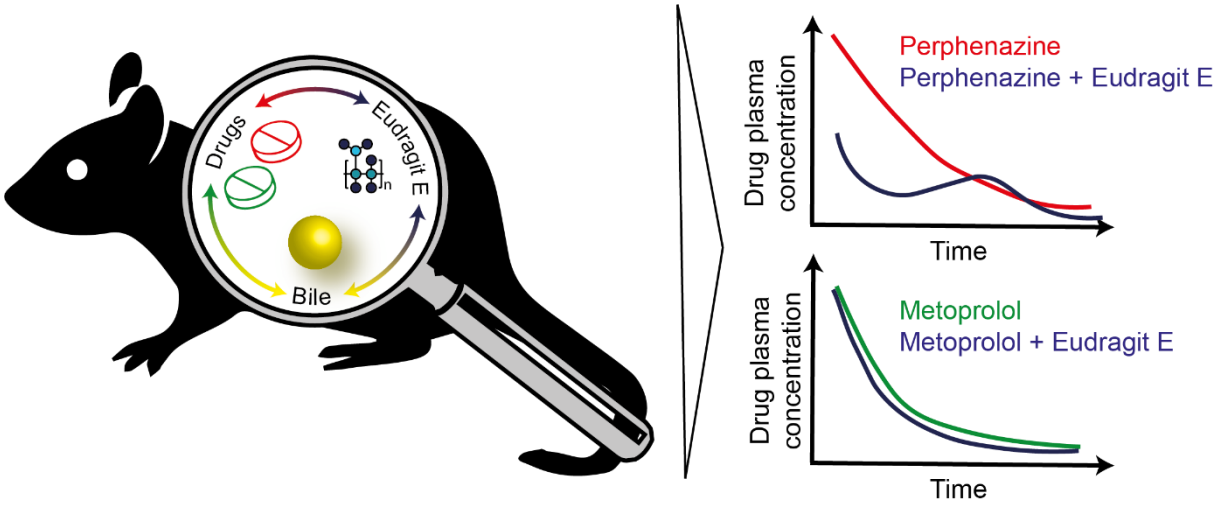

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