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Research paper

Rat intestinal drug permeability: A status report and summary of repeated determinations

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A B S T R A C T

Intestinal permeability is a key biopharmaceutical variable in pharmaceutical research and development, and regulatory assessment. In situ rat models are often used to predict the corresponding human intestinal permeability data. The rat single-pass intestinal perfusion (SPIP) and intestinal closed loop (ICL) models are commonly applied. The primary objective of this study was to collect, summarize, and evaluate all the available intestinal permeability data for drugs that have been obtained using these two in-situ rat models. The permeability data were also investigated for variability between the experimental designs. The literature survey found 635 permeability determinations for 90 drugs. The studies were performed on the jejunum (n = 284), whole small intestine (n = 111), colon (n = 108), ileum (n = 101), and duodenum (n = 30). All the SPIP (n = 484) and ICL (n = 147) permeability values were summarized in an easily accessible database. There was wide variability in the intestinal permeability to each drug between studies, which was unrelated to the permeability class of the drug. There was no relationship between rat intestinal permeability and luminal pH, luminal drug concentration, rat strain, experimental method, or intestinal region. There was, however, a correlation between permeability values determined in the same laboratory. This report showed that the SPIP and ICL methods are important in situ models for understanding and predicting intestinal drug absorption. However, conclusions based on permeability values sourced from different laboratories may not be reliable. Because each permeability study is unique and because between- and even within-laboratory variability can be substantial, data from individual studies should preferably be interpreted separately.

1. Introduction

Intestinal permeability is a biopharmaceutical variable that reflects a drug's potential to permeate the intestinal epithelial barrier. This variable can be used to predict and increase understanding of the intestinal drug absorption rate, the fraction of the dose absorbed (f_{abs}) and the bioavailability [1]. Whether the intestinal permeability of a drug is low or high has a direct impact on the drug development process, the drug formulation, and the potential success of the drug product. Access to reliable data on intestinal permeability is consequently highly requested during the drug development process. For example, optimal pharmacokinetics and dosage regimens might require development of an oral modified-release dosage form, which will need regional intestinal permeability data as the drug will be released throughout the gastrointestinal tract (GIT).

Experimental in-situ methods in the rat are considered suitable for assessing intestinal drug permeability, and the rat has become a useful model for predicting biopharmaceutical values [2–6]. These preclinical methods have also been used to generate data for different parts of the GIT so as to assess regional differences in intestinal drug permeability [7]. Over the last three decades, several studies have reported high correlations between human and rat small intestinal permeability, regardless of the transport mechanism [3,7]. This has revitalized the study of rat permeability data, and the number of rat permeability studies from various research groups, using different experimental designs and methods, is steadily increasing. The same model drugs are being repeatedly studied by different laboratories, using different rat strains and different designs and methods, and in different regions of the GIT [8–10].

The main experimental designs used for in-situ determination of rat intestinal drug permeability can be categorized into two general setups, the single-pass intestinal perfusion (SPIP) method and the intestinal closed loop (ICL) method [10]. Both methods include the administration of a drug solution (i.e. the perfusate) into the lumen of a defined intestinal segment. In the SPIP method, the selected intestinal segment is perfused continuously under steady-state conditions and the permeability is typically determined by measuring the difference in the perfusate drug concentration as it enters and leaves the intestine [11]. In the ICL method, the drug solution is either recirculated through the intestinal segment or is introduced as a bolus solution, and the permeability is determined by measuring the disappearance of drug from the luminal solution over time [12,13].

It is well recognized in experimental biopharmaceutical research and development that there is wide inter-and intra-laboratory variability that must be considered when comparing results from different studies [14,15]. This variability was the basis for this analysis of

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reported rat intestinal permeability values for a series of drugs. The main objective of this study was to compile and analyze the large quantity of reported experimental rat intestinal permeability data. We collected, summarized, and evaluated all available intestinal permeability data for the study drugs, measured using intestinal in-situ methods in the rat. A pooled value was calculated for each of the investigated drugs, for the different regions of the intestine, using SPIP or ICL methods. The data variability was investigated for the different experimental designs. It is hoped that, in the long term, this analysis will increase understanding of underlying physiological factors and their effects on epithelial drug transport.

2. Methods

2.1. Data collection

The six literature searches used the following search terms in PubMed: 1: ("single pass intestinal perfusion" OR "SPIP" OR "single-pass intestinal perfusion") OR ("closed loop" OR Doluisio) AND rat AND (permeability OR absorption); 2: (SPIP or "single pass intestinal perfusion" or "intestinal perfusion") AND rat AND (Peff or permeability); 3: Bermejo AND absorption AND rat; 4: Lennernäs AND absorption AND rat; 5: Dahan AND absorption AND rat; 6: Amidon AND absorption AND rat; 7: "Yamashita S." AND absorption AND rat AND permeability; 8: Langguth AND absorption AND rat AND permeability. Results were selected on availability of full text and English language. This resulted in 572 articles of interest (Supplementary Information 1). The abstract or full text of these articles were reviewed for the search terms and whether the investigated drugs were registered marketed drugs (not herbal medicine). Only data from tables and text were collected, data from graphs were not included. Non-permeability data (such as k_{abs}) were not taken into account in this analysis.

2.2. Data handling and analysis

From each article we collected information on: (i) the strain, sex, age and weight of the rats; (ii) the method: SPIP or ICL, segment perfused, segment length, perfusion flow or dose volume, duration of experiment and collection intervals; (iii) the drug perfusate: composition, pH, osmolarity, non-absorbable marker and reference marker; (iv) the drug: concentration in the perfusate, and effective permeability (P_{eff}); (v) the water flux; and (vi) the source of information (Supplementary Information 2).

When historical intestinal P_{eff} data were presented as means ± SEM or SE, the error was recalculated to SD when possible, using the number of replicates given (the smallest number when it was not clear how many). In reports where the variability estimate was unclear or unavailable, it was assumed to be the mean ± SD. The collected intestinal permeability data were finally recalculated to present P_{eff} values as 10^{-4} cm/s. The applied concentration in the perfusion solution entering the segment was recalculated to μM using the molecular mass of the base drug (i.e., not the salt form) if previously given in grams.

Variability was described as the fold difference between the highest and lowest reported P_{eff} value.

When mean, SD, and number of replicates were available, the pooled mean (Eq. (1)) and the pooled SD (Eq. (2)) for the intestinal P_{eff} were calculated for all combinations of drug, method and segment, e.g. intestinal P_{eff} for metoprolol measured with the SPIP method in the colon.

$$\overline{x_p} = \frac{\sum_i \overline{x_i} * n_i}{\sum_i n_i} \quad (1)$$

$$\sqrt{s_p^2} = \frac{\sum_i (n_i - 1) s_1^2}{\sum_i n_i - k} \quad (2)$$

When a P_{eff} value was determined in only one study, the mean and SD from that study are given. When data were not complete, i.e. no mean, SD, or number of replicates, the study was excluded from the meta-analysis. When the same mean and SD were obtained from different reports, the mean and SD from the study published first were chosen. If the number of replicates was unavailable in that study, the next oldest was chosen, and so on.

The focus in the data analysis was on intestinal P_{eff} data for the nine most extensively studied drugs, with special focus on metoprolol and atenolol, as these two passively transported drugs have been investigated most often using both the SPIP and ICL methods.

3. Results

3.1. Reported P_{eff} values

During the literature search, 572 peer-reviewed articles were identified (Supplementary Information 1: PubMed search results). After reviewing the title, abstract and/or main text, 93 articles were selected for in-depth analysis [5,8–10,12,16–103]. This resulted in 636 P_{eff} values for 90 drugs; these can be found in the database (Supplementary Information 2).

Intestinal P_{eff} values have mostly been determined for the high permeability drugs metoprolol (n = 74), antipyrine (n = 41), propranolol (n = 32), ketoprofen (n = 26), verapamil (n = 23), and ibuprofen (n = 20), and the low permeability drugs atenolol (n = 49), cimetidine (n = 19), and furosemide (n = 18) (Table 1, Fig. 1). More P_{eff} values have been generated using the SPIP method (484 P_{eff} values) than the ICL method (147 P_{eff} values). The most commonly investigated intestinal region for P_{eff} determination was the jejunum (284 P_{eff} values), followed by the whole small intestine (111 P_{eff} values), the colon (108 P_{eff} values), the ileum (101 P_{eff} values), and the duodenum (30 P_{eff} values). Pooled permeability values are summarized in Table 2. In addition, a subset of drugs, for which there were significant differences in P_{eff} between in-situ methods or intestinal segments, are presented in Supplementary Information 3, Table 1.

3.2. Variability in reported intestinal P_{eff} values

The compilation of collected historical intestinal P_{eff} data for the top nine drugs showed variability between the reports (Table 1, Fig. 1).

In SPIP studies, there was wide variability in P_{eff} values (> 10-fold error between the lowest and highest values) for atenolol in the jejunum (83-fold), ileum (79-fold), and colon (27-fold), and for metoprolol in the jejunum (14-fold), ileum (11-fold) and colon (35-fold). Most rat intestinal P_{eff} values have been reported for these two drugs.

The variability differed with the drug, ranging between 1.1- and 4.1-fold for antipyrine, 1.1- and 83-fold for atenolol, 1.1- and 6.7-fold for cimetidine, 1.1- and 14-fold for ibuprofen, 1.2- and 5.8-fold for ketoprofen, 1.1- and 14.4-fold for metoprolol, 2.3- and 4.1-fold for propranolol, and 2- and 2.5-fold for verapamil, with a value of 7.3-fold for furosemide.

The intestinal region which generally had the widest variability in reported P_{eff} values was, unexpectedly, the jejunum. This variability (between 2.5- and 14.0-fold) was unaffected by the permeability class of the drugs, with the exception of SPIP data for the low permeability drug atenolol (83-fold difference). This was also reflected in the high coefficient of variation for the nine most extensively studied drugs (84% for atenolol, based on pooled data) (Table 1).

A plausible explanation for the variability in SPIP P_{eff} values

Table 1
Range of reported values for the effective permeability (P_{eff}) ($\times 10^{-4}$ cm/s) of rat intestine to the nine most extensively studied drugs. The P_{eff} values were determined using the intestinal closed loop (ICL) or single-pass intestinal perfusion (SPIP) methods in different segments of the intestinal tract. The data are presented as the lowest to highest P_{eff} value (fold difference; number of reported values included in the analysis).

	SPIP					ICL		
Drug/Site	Duodenum	Jejunum	Ileum	Small intestine	Colon	Jejunum	Small intestine	Colon
Antipyrine		0.49–2 (4.1; 22)	0.67–1.6 (2.4; 7)		0.75–1.2 (1.7; 7)			
Atenolol		0.006–0.5 (83; 22)	0.01–0.79 (79; 6)	0.11–0.41 (3.9; 2)	0.019–0.51 (27; 7)	−0.33–0.23 (−0.69; 2)	0.1–0.11 (1.1; 3)	0.21–0.23 (1.1; 4)
Cimetidine		0.072–0.48 (6.7; 8)	0.037–0.041 (1.1; 3)	0.062–0.069 (1.1; 2)				
Furosemide		0.045–0.33 (7.3; 11)						
Ibuprofen	0.39–5.5 (14; 4)	0.39–2.3 (5.9; 7)	0.44–0.48 (1.1; 3)		0.5–1.4 (2.7; 4)			
Ketoprofen		0.69–4 (5.8; 14)	1.1–2.9 (2.6; 4)		1.3–1.5 (1.2; 3)			
Metoprolol		0.11–1.5 (14; 30)	0.15–1.6 (11; 13)	0.14–0.16 (1.1; 2)	0.09–3.2 (35; 8)		0.38–0.77 (2; 13)	0.81–0.81 (1; 3)
Propranolol	0.77–0.77 (1; 2)	0.25–0.72 (2.9; 12)	0.31–0.93 (3; 10)	0.31–1.3 (4.1; 3)				
Verapamil		0.43–1.1 (2.5; 9)					0.55–1.1 (2; 11)	

between studies in all the intestinal segments was sought for the two most extensively investigated drugs, atenolol and metoprolol. The effects of entering concentration, entering pH of the luminal drug solution and the rat strain on intestinal P_{eff} values are shown in Fig. 2. No correlations were observed between the concentration of drug in the luminal solution and the P_{eff} [atenolol: R^2 0.3 (jejunum & colon), 0.2 (ileum); metoprolol: R^2 0.3 (jejunum), 0.6 (ileum), 0.2 (colon)] or the pH of the luminal drug solution and the P_{eff} [atenolol: R^2 0.06 (jejunum), 0.2 (ileum), < 0.01 (colon); metoprolol: R^2 0.06 (jejunum), < 0.01 (ileum), 0.4 (colon)] (Fig. 2, Supplementary Information, Table 2). No correlations were observed between the concentration of drug in the luminal solution and the jejunal P_{eff} for antipyrine (R^2 0.06, 20 values), cimetidine (R^2 0.4, 20 values), furosemide (R^2 0.35, 9 values), ibuprofen (R^2 0.6, 5 values), ketoprofen (R^2 0.003, 13 values), propranolol (R^2 0.3, 9 values) or verapamil (R^2 0.6, 8 values) (Supplementary Information 3, Fig. 1 and Table 2). Studies that used the SPIP method mostly used the Sprague-Dawley rat strain (atenolol 16 P_{eff} values; metoprolol 16 P_{eff} values), followed by Wistar (atenolol 8 P_{eff} values; metoprolol 14 P_{eff} values), Wistar Han (atenolol 9 P_{eff} values; metoprolol 9 P_{eff} values), and Albino Wistar (metoprolol 10 P_{eff} values). Overall, there were no differences in P_{eff} values from any intestinal area between the strains. However, the variation in jejunal P_{eff} value between studies tended to decrease for each strain: Sprague-Dawley (atenolol 38-fold; metoprolol 7-fold), Wistar (atenolol 11-fold; metoprolol 14-fold), Wistar Han (atenolol 2-fold; metoprolol 2-fold), and Albino Wistar (metoprolol 2-fold).

The P_{eff} values of atenolol and metoprolol differed with the laboratory reporting the data (Fig. 3). Within-laboratory repeated measurements of P_{eff} values for a specific drug in a specific intestinal segment tended to conform. Variability decreased to < 3-fold for within-laboratory comparisons of P_{eff}, except for atenolol P_{eff} values measured by laboratory 4a (jejunum, 9-fold) and metoprolol P_{eff} values measured by laboratory 2 (ileum, 6-fold).

3.3. Differences between single-pass intestinal perfusion and intestinal closed loop methods

In total, 484 SPIP and 147 ICL intestinal P_{eff} values for drugs were collected from the literature. The number of drugs studied varied with the intestinal region. For the SPIP method, the number of drugs studied for each region was: jejunum (67) > ileum (40) > colon (22) > small intestine (13) > duodenum (11). For the ICL method, the number of drugs per region was: colon (28) > small intestine (27) > jejunum (10) > ileum (6) > duodenum (2). In total, pooled P_{eff} values were calculated for 87 drugs where the SPIP method was used and 12 drugs where the ICL method was used. There were few drugs where P_{eff} was determined with both SPIP and ICL; the pooled P_{eff} values for these drugs are shown in Fig. 4. The number of data points falling within a 2-fold difference was three of seven for the jejunum, two of three for the ileum, one of four for the whole small intestine, and 12 of 16 for the colon. Metoprolol and atenolol measurements fell within the area of 2-fold error in the jejunum, ileum and colon (Fig. 4).

The significant differences in pooled P_{eff} between the SPIP and ICL methods are shown in Table 2. Significant differences in pooled jejunal P_{eff} values were observed for acyclovir, ganciclovir, ketoprofen, metoprolol, and talinolol. For pooled ileal P_{eff} values, a significant difference was only observed for ketoprofen. For the pooled colonic P_{eff} values, significant differences were observed for cimetidine, codeine, digoxin, furosemide, hydrocortisone, ibuprofen, ketoprofen, paracetamol, and propranolol.

3.4. Regional differences

Studies investigating the permeability of multiple intestinal regions have been carried out for 42 drugs. Significant differences between regions for the (pooled) P_{eff} values for each drug are shown in Table 2.

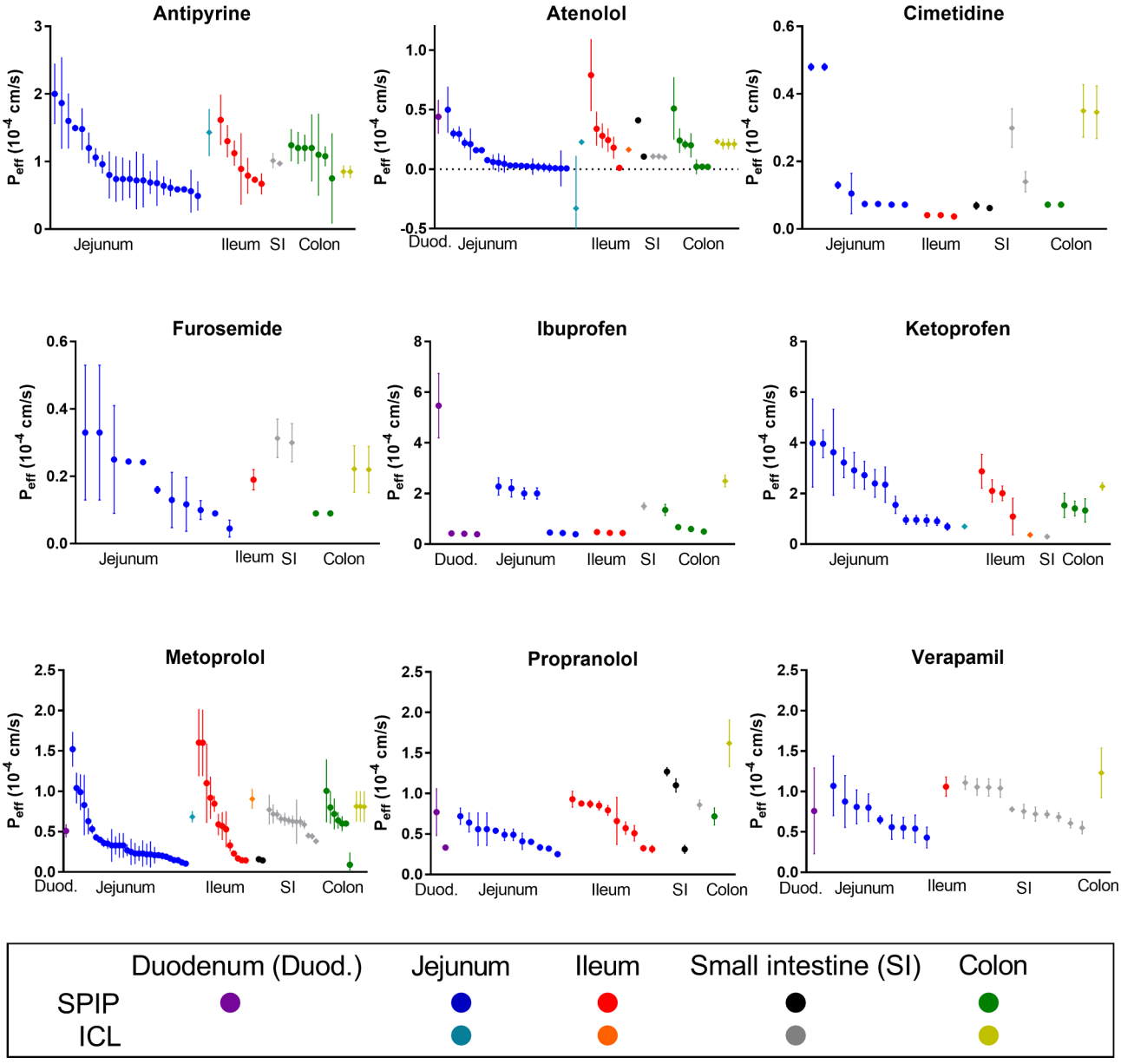


Fig. 1. The effective permeability (P_{eff}) of the rat intestine to the nine most extensively studied drugs. The P_{eff} values for each drug are shown for the studied intestinal segment and the experimental method (SPIP: single-pass intestinal perfusion, ICL: intestinal closed loop). All data are sorted from highest to lowest reported value.

The following text reports differences between intestinal regions based on individual papers, i.e. not based on pooled P_{eff} values. The results of the individual studies are presented in Fig. 5. Drugs for which more than two studies have been performed to determine regional permeability included metoprolol (n = 13) [8,10,19,28–30,38,39,51,55,63,72,73], antipyrine (n = 6) [8,10,49,51,55,96], atenolol (n = 5) [8,51,55,72,73], propranolol (n = 4) [10,30,59,94], and cimetidine (n = 4) [10,28,30,51]. The results varied between reports and laboratories when the SPIP method was used (Fig. 5). There were no significant differences in P_{eff} between jejunum and ileum for antipyrine [8,96]. Statistical differences in P_{eff} between jejunum, ileum and colon have been observed, but the direction of the difference differs with the study [8,49,55]. These contradictory results have also been observed for atenolol [8,55,72] and metoprolol [8,28,30,38,39,55,63,72], with some (but not all) studies showing statistically significant differences in P_{eff} between the different regions (Fig. 5). For atenolol, cimetidine, metoprolol and propranolol, P_{eff} determined with the ICL method was lower in the small intestine than the colon (Fig. 5). A statistically significant difference between colon (0.211 ± 0.043) and small intestine (0.107 ± 0.024) was obtained for atenolol, but not for the other drugs [51].

4. Discussion

4.1. Reported P_{eff} values

This paper is the most complete collection of reported rat intestinal permeability values to date, resulting in a comprehensive database. It includes a large amount of published SPIP and ICL data from the small and large intestine, only excluding literature values that were not reported in text. The database also includes specifics of the methods used to obtain the P_{eff} values. The database can thus be used for further analysis of reported P_{eff} values. This report discusses the results of the analysis, without extensive discussion of the relevance of individual studies. Discussions and analysis primarily concern SPIP data, as this

Table 2
The (pooled) effective permeability (P_{eff}) of the rat intestine to the studied drugs in different intestinal regions. The data are divided to show the method used [single-pass intestinal perfusion (SPIP) or intestinal closed loop (ICL) methods]. Data are shown as the mean (pooled) P_{eff} values ± SD × 10^{-4} cm/s (number of studies that reported values used in the pooled analysis; total number of individual rats).

	SPIP			
	Jejunum	Ileum	Small intestine	Colon
Acyclovir	0.347 ± 0.218 (2; 11)[w]	0.07 ± 0.02 (1; 3)[y]	0.15 ± 0.039 (1; 5)[z]	1.83 ± 0.68 (1; 6)
Amiodarone	20 ± 0.2 (1; 4)			
Amoxicillin	0.12 ± 0.11 (1; 4)			
Antipyrine	1.17 ± 0.354 (19; 152)	1.03 ± 0.284 (7; 31)		1.07 ± 0.463 (6; 26)
Artemisinin	1.31 ± 0.351 (2; 12)			
Atenolol	0.0922 ± 0.0784 (18; 106)[u]	0.324 ± 0.16 (6; 31)	0.258 ± 0.0165 (2; 12)	0.189 ± 0.126 (7; 36)
Candesartan cilexetil			2.53 ± 0.05 (1; 6)	
Carbamazepine	2.17 ± 0.3 (2; 8)			1.6 ± 0.1 (1; 4)
Carvedilol	0.262 ± 0.0429 (2; 6)	0.594 ± 0.175 (2; 6)		
Celecoxib	0.639 ± 0.064 (1; 3)			
Cephalexin	0.448 ± 0.144 (3; 23)	0.26 ± 0.06 (1; 3)		
Chloramphenicol	1.1 ± 0.096 (1; NAv)			
Cimetidine	0.091 ± 0.0277 (5; 20)	0.0397 ± 0.0043 (3; 12)	0.0655 ± 0.00836 (2; 8)	0.072 ± 0.0072 (2; 8)
Clopidogrel	0.517 ± 0.0701 (3; 9)	0.558 ± 0.0815 (3; 9)		
Codeine	0.49 ± 0.05 (1; 4)			0.355 ± 0.0705 (2; 8)
Colchicine	0.0855 ± 0.276 (2; 8)	0.06 ± 0.008 (1; 4)		
Cyclosporine	2.32 ± 0.611 (5; 20)			
Dexamethasone	0.4 ± 0.02 (1; 4)[u]	1.57 ± 0.21 (1; 3)		
Diclofenac			0.629 ± 0.112 (8; 32)	
Digoxin	0.231 ± 0.0674 (4; 15)[w]	0.0112 ± 0.00309 (2; 9)[y]		0.775 ± 0.0568 (2; 8)
Docetaxel	0.215 ± 0.053 (1; 5)			
Domperidone		0.68 ± 0.1 (1; 6)		
Edaravone	0.00318 ± 0.000271 (1; 3)			
Enalaprilat	0.488 ± 0.694 (4; 22)	0.49 ± 0.198 (3; 18)		0.343 ± 0.109 (3; 18)
Famotidine	0.037 ± 0.0087 (1; 4)	0.01 ± 0.0038 (1; 4)	0.03 ± 0.0099 (1; 4)	
Fexofenadine		0.476 ± 0.0357 (3; 12)		
Fluvastatin	0.894 ± 0.313 (4; 20)	0.837 ± 0.197 (4; 20)		0.866 ± 0.4 (4; 18)
Furosemide	0.106 ± 0.0535 (5; 26)	0.19 ± 0.03 (1; 3)		0.09 ± 0.0051 (1; 4)
Gabapentin	0.136 ± 0.042 (2; 8)			
Ganciclovir	0.154 ± 0.039 (1; 7)		0.023 ± 0.005 (1; 7)	
Glipizide	0.75 ± 0.06 (1; 4)			
Griseofulvin	0.909 ± 0.243 (1; 4)	1.24 ± 0.293 (1; 4)		1.3 ± 0.094 (1; 4)
Hydrochlorothiazide	0.0728 ± 0.0684 (4; 16)	0.07 ± 0.03 (1; 3)		
Hydrocortisone				1.2 ± 0.19 (1; 4)
Ibuprofen	0.89 ± 0.174 (4; 16)[u]	0.452 ± 0.0701 (3; 12)		0.779 ± 0.121 (4; 16)
Indinavir		0.09 (1; NAv)		
irinotecan			8.17 ± 2.83 (1; 6)	
Isoniazide	0.15 ± 0.0315 (4; 24)			
Ketoprofen	2.49 ± 0.872 (12; 56)[u w]	2.15 ± 0.524 (4; 21)[y]		1.42 ± 0.419 (3; 18)
Labetalol	0.38 ± 0.06 (1; 6)	0.64 ± 0.3 (1; 6)		
Lamivudine	0.194 ± 0.0195 (2; 13)		0.33 ± 0.01 (1; 6)	
L-dopa	2 ± 0.63 (1; 8)[u w]	1.2 ± 0.27 (1; 6)[y]		0.02 ± 0.04 (1; 6)
Losartan	0.487 ± 0.295 (2; 12)	0.944 ± 0.156 (2; 12)		
Lumefantrine	0.333 ± 0.0433 (2; 8)	0.182 ± 0.025 (1; 5)		
Metformin	0.252 ± 0.0642 (2; 9)	0.296 ± 0.036 (1; 5)		0.295 ± 0.0955 (2; 8)
Methyldopa (α-Methyl dopa)	0.016 ± 0.004 (1; 4)			
Metoprolol	0.386 ± 0.145 (26; 149)[u w]	0.684 ± 0.256 (13; 64)[x y]	0.155 ± 0.0101 (2; 12)[z]	1.02 ± 0.514 (7; 38)
Midazolam		1.15 ± 0.4 (2; 6)		
Minoxidil	0.225 ± 0.07 (2; 8)	0.17 ± 0.06 (1; 4)		
Mitragynine			1.11 ± 0.17 (1; 6)	
Nadolol		0.043 ± 0.007 (1; 3)		
Naproxen	1.47 ± 0.247 (4; 19)[w]	1.78 ± 0.523 (3; 13)		2.06 ± 1.04 (2; 10)
Nifedipine	7 ± 1 (1; 4)			
Paracetamol	0.46 ± 0.0772 (3; 12)[w]			1.4 ± 0.3 (1; 4)
Phenytoin		0.615 ± 0.067 (1; 4)		
Pindolol	0.293 ± 0.11 (1; 4)			
Piroxicam	2.62 ± 0.37 (1; 4)			
Pravastatin	0.023 ± 0.002 (1; 4)			0.57 ± 0.0072 (1; 4)
Progesterone	8.24 ± 2.5 (1; 4)			
Propranolol	0.431 ± 0.0671 (8; 32)[v]	0.684 ± 0.0981 (10; 41)	0.967 ± 0.064 (3; 16)	0.717 ± 0.106 (1; 3)
Pseudoephedrine	0.19 ± 0.08 (1; 4)			
Quinidine		0.56 ± 0.13 (1; 3)		
Ranitidine	0.073 ± 0.06 (1; 4)			
Rapamycin	0.126 ± 0.02 (1; 4)			
Salicylic acid	2.13 ± 0.183 (1; 4)			
Sanquinavir	0.23 ± 0.104 (1; 3)			
Sotalol	0.039 ± 0.004 (1; 4)			
Stavudine	0.396 ± 0.047 (1; 7)			
Sulfasalazine	0.116 ± 0.0228 (2; 9)	0.06 ± 0.03 (1; 3)		
Tacrolimus	0.296 ± 0.0418 (6; 24)	0.17 ± 0.02 (1; 4)		0.12 ± 0.02 (1; 4)

(*continued on next page*)

Table 2 (*continued*)

	SPIP			
	Jejunum	Ileum	Small intestine	Colon
Talinolol	1.73 ± 0.211 (2; 12)	1.75 ± 0.498 (2; 12)		
Terbutaline	0.045 ± 0.08 (1; 8)	0.12 ± 0.09 (1; 3)		
Testosterone	0.38 ± 0.046 (1; 4)			
Theophylline	0.68 ± 0.19 (1; 4)			
Valsartan	0.06 ± 0.0067 (1; NAv)			
Verapamil	0.714 ± 0.221 (9; 37)	1.06 ± 0.123 (1; 3)		
Vinblastine	0.504 ± 0.236 (1; 3)	0.802 ± 0.196 (1; 3)		
Zidovudine	0.417 ± 0.043 (1; 7)			
	ICL			
	Jejunum	Ileum	Small intestine	Colon
Acyclovir	−0.42 ± 0.48 (1; 4)*			
Amoxicillin			0.167 ± 0.041 (1; 5)	0.299 ± 0.051 (1; 5)
Antipyrine	1.43 ± 0.34 (1; 4)[b c]		1.01 ± 0.108 (1; 5)	0.848 ± 0.086 (1; 5)
Atenolol	0.0042 ± 0.27 (2; 10)[a c]	0.163 ± 0.012 (1; 6)	0.107 ± 0.024 (1; 5)*	0.222 ± 0.0327 (2; 11)
Carbamazepine			1.28 ± 0.12 (1; 5)	1.42 ± 0.23 (1; 5)
Chloramphenicol			0.98 ± 0.034 (1; NAv)	
Cimetidine			0.299 ± 0.057 (1; 5)*	0.35 ± 0.078 (1; 4)*
Ciprofloxacin	0.115 ± 0.0248 (1; 3)	0.114 ± 0.0267 (1; 3)	0.0981 ± 0.039 (8; 40)	
Codeine			0.545 ± 0.023 (1; 5)	0.583 ± 0.091 (1; 5)*
Colchicine			0.25 ± 0.039 (1; 5)	#NUM!
Dexamethasone			1.57 ± 0.21 (1; 3)	
Digoxin			0.26 ± 0.096 (1; NAv)[f]	0.49 ± 0.18 (1; 4)*
Famotidine				0.51 ± 0.07 (1; 4)
Fexofenadine			0.169 ± 0.0119 (6; 34)[f]	0.44 ± 0.03 (1; 4)
Furosemide			0.313 ± 0.057 (1; 5)	0.222 ± 0.069 (1; 5)*
Ganciclovir	−0.24 ± 0.34 (1; 4)*			
Grepafloxacin			0.441 ± 0.0674 (11; 55)	
Hydrocortisone				0.69 ± 0.08 (1; 4)*
Ibuprofen			1.5 ± 0.13 (1; NAv)[f]	2.49 ± 0.23 (1; 4)*
Ketoprofen	0.695 ± 0.0159 (1; 6)[a b c *]	0.365 ± 0.0331 (1; 6)[e *]	0.3 ± 0.0103 (1; 6)[f]	2.28 ± 0.149 (1; 6)*
Labetalol			0.34 ± 0.061 (1; NAv)	
Metformin				0.45 ± 0.1 (1; 4)
Metoprolol	0.685 ± 0.0685 (1; 6)[a *]	0.905 ± 0.123 (1; 6)[d]	0.604 ± 0.117 (12; 51)[f *]	0.812 ± 0.188 (2; 9)
Nadolol	−0.41 ± 0.38 (1; 4)			
Naproxen			1.84 ± 0.116 (1; 5)	1.67 ± 0.123 (1; 5)
Oxprenolol				1.11 ± 0.09 (1; 4)
Paracetamol			0.82 ± 0.16 (1; NAv)	0.91 ± 0.115 (2; 8)*
Pravastatin		0.042 ± 0.0058 (1; 4)[e]		0.55 ± 0.1 (1; 4)
Propranolol			0.86 ± 0.06 (1; 4)[f *]	1.62 ± 0.29 (1; 4)*
Ranitidine			0.135 ± 0.032 (1; 5)	0.295 ± 0.073 (1; 5)
Rosuvastatin				0.58 ± 0.09 (1; 4)
Sildenafil			0.184 ± 0.07 (1; 6)	
Sparfloxacin			0.438 ± 0.0371 (1; 5)	
Talinolol	2.6 ± 0.465 (2; 8)*			2.42 ± 0.213 (2; 8)
Telmisartan	1.1 ± 0.34 (1; 4)			
Terbutaline			0.159 ± 0.027 (1; 5)[f]	0.352 ± 0.071 (1; 5)
Theophylline			0.986 ± 0.067 (1; 5)	1.06 ± 0.081 (1; 5)
Valsartan			0.262 ± 0.022 (1; 5)	0.328 ± 0.062 (1; 5)
Verapamil			0.83 ± 0.0812 (11; 45)[f]	1.23 ± 0.308 (1; 5)

NAv: total number of individuals was not available.
a–f indicate a significant difference ($p < 0.05$) in P_{eff} determined with the intestinal closed loop (ICL) method between segments [a = jejunum vs. ileum, b = jejunum vs. small intestine, c = jejunum vs. colon, d = ileum vs. small intestine, e = ileum vs. colon, f = small intestine vs. colon].
u-z indicate a significant difference in P_{eff} determined with the single-pass intestinal perfusion (SPIP) method between segments [u = jejunum vs. ileum, v = jejunum vs. small intestine, w = jejunum vs. colon, x = ileum vs. small intestine, y = ileum vs. colon, z = small intestine vs. colon].
* Indicates a significant difference in P_{eff} determined in the same segment between methods (ICL vs SPIP).

method was used more frequently than the ICL method (484 vs 148 P_{eff} values). The summary displays the published data, with a discussion emphasizing transport mechanisms, sources of data variability, and regional and experimental differences.

4.2. Intestinal transport mechanisms and their relevance for in vivo predictions

The concept of intestinal permeability is often applied in the regulatory assessment of bioequivalence using the well established Biopharmaceutics Classification System [1]. The permeation mechanism(s) may be a single process or several acting together; they include processes such as passive transcellular diffusion, passive paracellular diffusion, and/or carrier-mediated uptake and efflux [104]. Regardless of the permeation mechanism, the drug must be transported across the aqueous boundary layer before reaching the membrane. This transport step is, however, very rapid in vivo, and the epithelial membrane controls the transport rate for both low and high permeability low-molecular mass drugs [105]. Passive transcellular diffusion is the most common transport mechanism, as supported by a large number of reports and indicated by the large mucosal membrane area. The surface area available for paracellular diffusion has been estimated

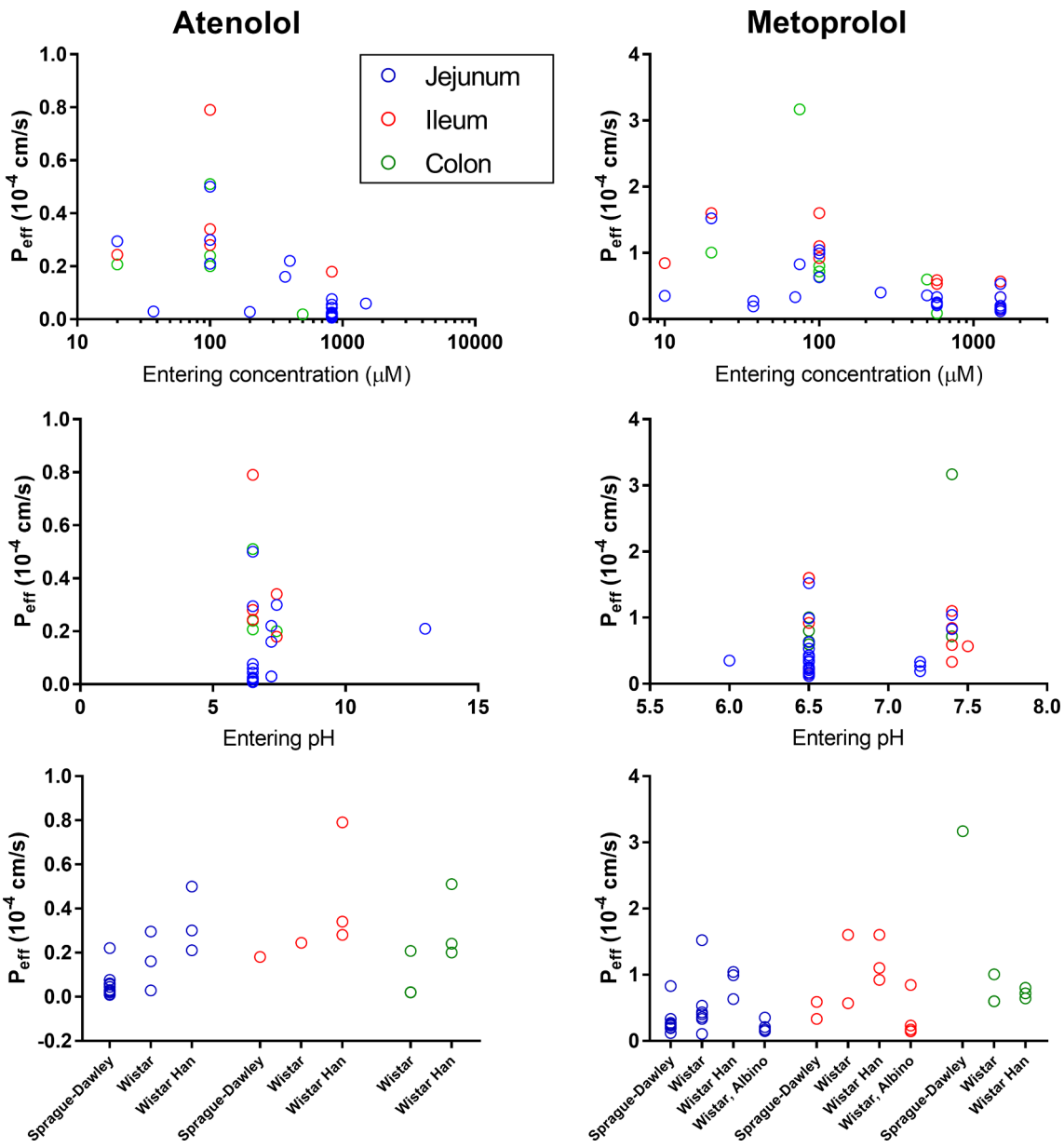


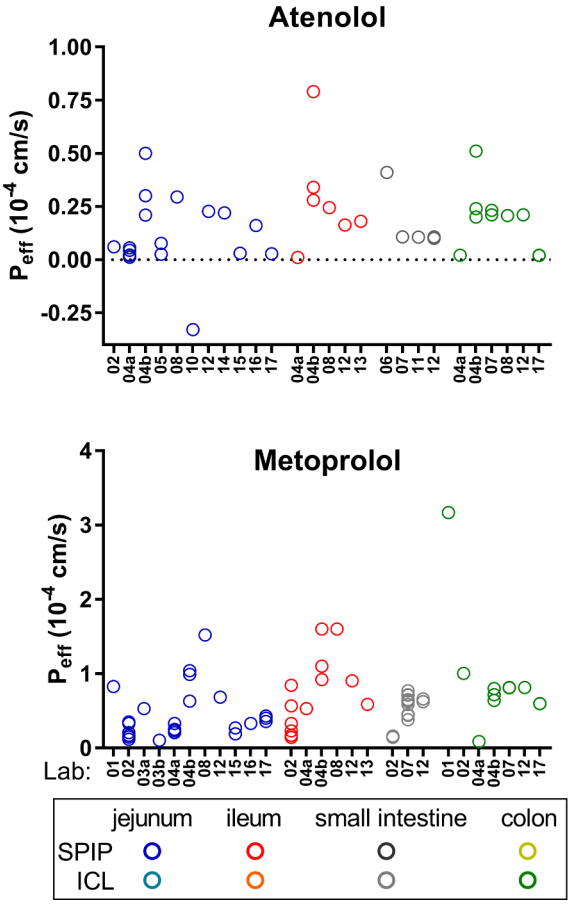
Fig. 2. Investigation into possible reasons for the variations in rat intestinal effective permeability (P_{eff}) values for atenolol and metoprolol between studies. Reported jejunal, ileal and colonic P_{eff} values are plotted against entering concentration, entering pH, and the reported rat strain. The statistics for these comparisons are available in Supplementary Information 3, Table 2.

to be less than 0.01% of the total area [106]. However, the paracellular transport route may still be quantitatively important for large, hydrophilic molecules, even if their bioavailability is low and highly variable. Transporter proteins can have a large effect on cell membrane permeability. The relationship between concentration and permeability, using the SPIP method, deviates from the linear for drugs such as L-dopa and D-glucose, which are transported by a carrier protein [14,107]. The transport rates for such drugs are related to the expression profiles of transporter proteins, and the drug affinity to the transporter. This demonstrates that drugs transported via carrier protein(s) must be individually investigated in order to elucidate and characterize the transport mechanism(s).

4.3. Variability

There was substantial variability in the permeability values between those determined with the SPIP method and those determined with the ICL method, regardless of the intestinal segment, as illustrated in Fig. 1 for the nine most extensively studied drugs. The difference between the lowest and highest values for each drug in each intestinal segment was typically between 2- and 10-fold, when more than two studies were compared (Fig. 1). The exceptions were atenolol and metoprolol, where differences larger than 10-fold were observed in all intestinal segments when the SPIP method was used. This is most likely related to the large number of studies (> 49) investigating these two drugs, rather than to any intrinsic molecular properties increasing absorption variability. This clearly illustrates the substantial inter-laboratory variability, as discussed below.

There were no obvious differences in the degree of variability between the drugs with low permeability (atenolol, cimetidine, and furosemide) compared to the high permeability drugs (antipyrine, ibuprofen, ketoprofen, metoprolol, propranolol, verapamil), regardless of intestinal segment or experimental method (Fig. 1, Table 1). This was surprising, given the generally higher variability in rate and extent of



Lab	Rat strain	In-situ method	pH of luminal drug solution
01	Sprague-Dawley	SPIP	7.4
02	Albino Wistar, Sprague-Dawley, Wistar	SPIP	6.5
03a	Wistar	SPIP	6.5
03b	Wistar	SPIP	6.5
04a	Sprague-Dawley	SPIP	6.5
04b	Wistar Han	SPIP	6.5, 7.4
05	Sprague-Dawley	SPIP	6.5
06	Sprague-Dawley	SPIP	7.2
07	CD/IGS, Long Evans, Sprague Dawley, Wistar, Wistar Han, Wistar Unilever	ICL	7
08	Wistar	SPIP	6.5
09	Sprague-Dawley	SPIP	7.4
10	Sprague-Dawley	ICL	6.8
11	Wistar	SPIP	N/A
12	Wistar	ICL	6.5, 7, 7.4
13	Sprague-Dawley	SPIP	7.4
14	Sprague-Dawley	SPIP	7.2
15	Sprague-Dawley	SPIP	7.2
16	Wistar	SPIP	7.2
17	Wistar	SPIP	6.5

Fig. 3. Investigation into possible reasons for the variations in rat intestinal effective permeability (P_{eff}) values between studies. Reported jejunal, ileal and colonic P_{eff} values for atenolol and metoprolol are plotted against the laboratory conducting the experiment. The laboratories were differentiated by physical location. The Table shows the rat strain, the in-situ method and the initial pH of the luminal drug solution for each laboratory.

absorption for low permeability drugs [108]. Further, accurate determinations of intestinal permeability for low permeability drugs is notoriously challenging [109]. This is related to the small amount of these drugs that is absorbed from the intestinal lumen during SPIP and ICL experiments, although this is less important for the ICL method as luminal exposure times are longer. However, the lack of difference in variability between the low and high permeation drugs indicates that sources of variability other than those discussed above appear to dominate. This could be explained by other experimental factors, such as the experience of the laboratory personnel in surgery, the type and dosage of the anesthetic and pain medication, the choice of luminal buffer and the buffer strength [110–112]. We investigated the impact of the initial drug concentration and the pH of the luminal drug solution, the rat strain, and the laboratory on the variability of the P_{eff}, with an emphasis on atenolol and metoprolol (Figs. 2 and 3).

The concentration of the drug in the luminal solution may affect its permeability if it is a substrate for an influx and/or efflux transporter protein. However, no such trends were observed in the global analysis of verapamil and cimetidine, which are accepted intestinal transporter substrates [61]. Drug concentration could also have an effect on the permeability of passively absorbed drugs, if the drug itself has an effect on the intestinal physiology or mucosal barrier [7]. However, these effects have not been frequently reported, and are most likely of limited importance for in vivo drug permeation studies. There were no such effects noted for the two passively transported drugs, atenolol and metoprolol, in our study.

There are several reports showing a relationship between intestinal permeability and luminal pH for ionizable weak acids and bases [113,114]. However, we observed no correlations between the pH of the administered luminal solution and the permeability of atenolol and metoprolol in this global analysis. The overwhelming majority of the collected data were studied using luminal solutions with an entering pH ranging from 6 to 7.5, which is close to the regional luminal pH values: jejunum 6.5, ileum 7.4, colon 6.0 to 7.4 [115]. Both metoprolol and atenolol (pKa 9.7 and 9.6, respectively) are ionized to between 99.0 and 99.9% in this intraluminal pH range. It should be noted that the luminal solutions were not always matched to the pH of the perfused region and it was often the entering pH that was reported. The pH of the exiting luminal drug solution might be more relevant, as the rat intestine has a substantial ability to normalize the pH of the perfusate to the physiological pH in any given region. One study showed that a phosphate buffer strength of 67 mM was necessary to keep the pH constant in the jejunum and ileum at pH values other than 6.5 and 7.4, respectively [110]. Investigating the pH-permeability relationship should thus be carefully planned, using a sufficiently high buffer strength. However, regardless of luminal pH and buffer strength, the microclimate pH adjacent to the epithelial barrier may not have been affected, and this is most likely a better predictor of permeability [116].

Differences in P_{eff} between different strains of rat (Wistar Unilever, Wistar Han, CD*IGS, Long Evans, and Sprague-Dawley) have been observed previously: male Sprague-Dawley rats, female Sprague-Dawley rats and female CD*IGS rats had lower intestinal absorption rates for metoprolol, and male Sprague-Dawley rats, male Wistar Han rats and female Wistar Unilever rats had lower absorption rates for verapamil [9]. Conversely, there were no differences in mucosal permeability to [50] Cr-EDTA between Sprague-Dawley and Dark Agouti rats using the SPIP method [117]. This is supported by this report; we found no general correlation between rat strain and intestinal permeability to

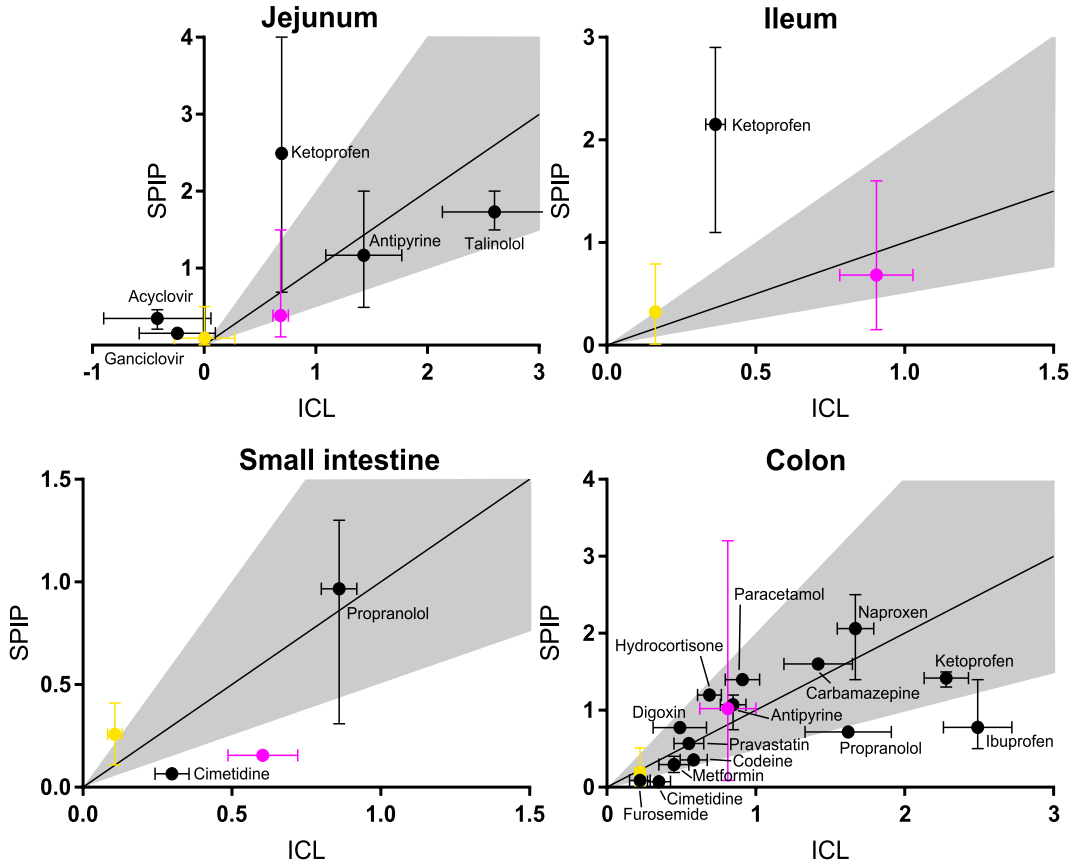


Fig. 4. Effective permeability (P_{eff}) determined using the single-pass rat intestinal perfusion (SPIP) method versus the intestinal closed loop (ICL) method. Data are shown as (pooled) means with minimum–maximum values (SPIP) or (pooled) standard deviations (ICL). The line of unity (black solid line) and the area of 2-fold difference from the line of unity (grey area) are shown. Metoprolol and atenolol are shown in pink and yellow, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

low molecular weight drugs (Fig. 2). Although Wistar Han rats tended to be associated with higher P_{eff} values for both low and high permeation drugs in all intestinal regions, this was most likely associated with the laboratory performing the P_{eff} determination; Wistar Han rats were only used in laboratory 4b.

It is well known that individual laboratories generate different results for almost any in vitro or in vivo method. This has been shown for a range of drug transport methods, such as Caco-2 monolayers and excised intestinal segments in the Ussing chamber [15,118]. It has also been demonstrated in other biopharmaceutical research fields, such as solubility and dissolution assays [119]. Accordingly, the laboratory that was responsible for the permeability studies was the only parameter that we identified that could explain the variabilities in drug permeability. This was well illustrated for atenolol and metoprolol, where the permeability values tended to cluster if they were determined repeatedly in the same laboratory (Fig. 3). By extension, this shows that any conclusions based on comparison of permeability values between laboratories will have a low evidence value. Preferably, data from individual studies should be interpreted separately, as within-laboratory variability can also be substantial.

4.4. SPIP vs ICL

There were more rat intestinal permeability determinations using the SPIP method (484 P_{eff} values) than using the ICL method (147 P_{eff} values). Only a few studies investigated the intestinal P_{eff} values of drugs using both methods in the same segment. The whole small intestine or colon is usually used in the ICL method, while specific regions of the small intestine are usually used in the SPIP method. This explains the small number of drugs available for comparison. For example, colon tissue was used in both methods for only 16 drugs. Of these drugs, only cimetidine, furosemide, ibuprofen and propranolol had significantly different (> 2-fold) P_{eff} values. Interestingly, these differences were from one reported P_{eff} value from both SPIP and ICL determinations. There were no systematic differences in the permeability or variability, regardless of intestinal site. Therefore, the selected method will most likely depend primarily on the experience of the laboratory personnel, and the methods of surgery and anesthesia.

4.5. Regional differences

Many individual studies reported regional differences in permeability along the rat intestine [3,10,110]. However, our analysis did not identify any consistent regional intestinal pattern, regardless of the permeability class of the drug; regional differences varied between segments, drugs, and studies. This was expected for the high permeability drugs, where mucosal surface area and paracellular space have little effect on their permeability as they permeate through the transcellular route at the tip of the villa. However, the finding of a similar pattern for the low permeability drugs was unexpected, as the same physiological processes are expected to result in reduced permeability in the colon. The absence of a declining permeability profile for low permeability drugs does not reflect the epithelial properties along the intestine, as individual studies have demonstrated regional differences in permeability. The lack of a general trend from the combined reports is possibly explained by interstudy differences in the experimental conditions. For instance, a short rat colon will result in poor, highly variable absorption, with subsequent uncertainty in the permeability

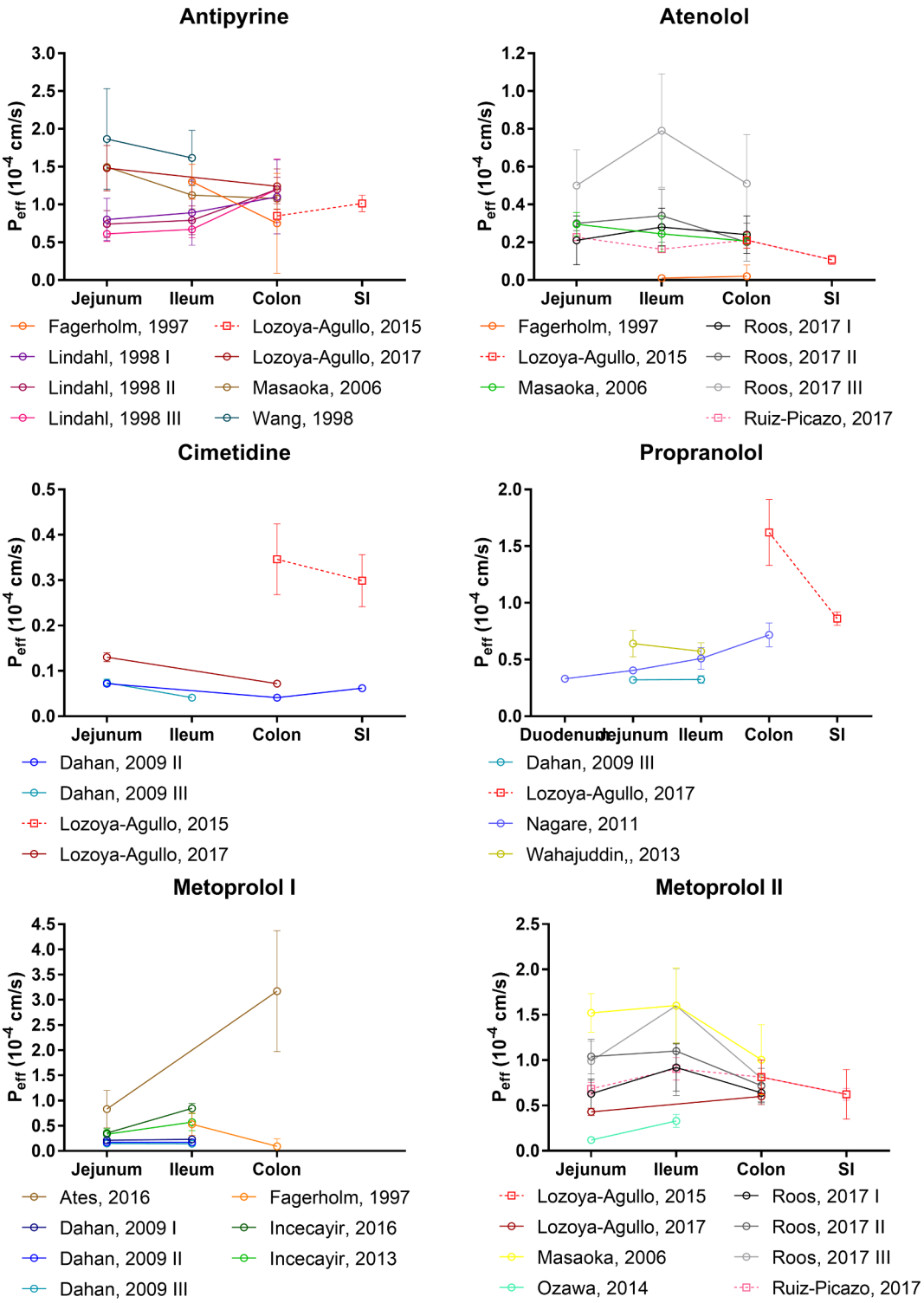


Fig. 5. Drugs for which more than two studies have reported regional rat intestinal effective permeability (P_{eff}) data. The regions studied included the jejunum, ileum, small intestine (SI) and colon. Data from single-pass intestinal perfusion (SPIP) methods are shown with circles and continuous lines, data from intestinal closed loop (ICL) methods are shown with squares and dashed lines. Data were gathered from: Dahan, 2009 I [29], Dahan, 2009 II [28], Dahan, 2009 III [30], Incecayir, 2016 [38], Lozoya-Agullo, 2015 [51], Lozoya-Agullo, 2017 [10], Masaoka, 2006 [55], Nagare, 2011 [59], Ozawa, 2014 [63], Ruiz-Picazo, 2017 [73], Wahajuddin, 2013 [94], and Wang, 1998 [96].

estimate (as discussed above). Therefore, it is likely that permeability determinations based on the appearance of drugs in the plasma will provide reliable regional permeability data, especially for low permeability drugs [109,120]. In summary, the data collected by us did not demonstrate consistent intestinal regional differences in for low permeability drugs, in contrast to the results of individual studies in rats, humans and dogs [3,106,121]. This highlights a need for these studies to be carried out in the same laboratory, with trained personnel, well treated animals, and reliable equipment. Investigation of regional differences in intestinal permeability in the rat is probably best based on the appearance of drugs in the plasma rather than the disappearance of drugs from the lumen.

4.6. Conclusions

This paper has summarized and analyzed all available rat intestinal drug permeability data derived from the rat SPIP and ICL methods. This has resulted in a comprehensive database, which can be used for further analysis of P_{eff} data. The database contains P_{eff} values for 90 drugs, determined in the duodenum, jejunum, ileum, small intestine or colon. Specific details of the methods used are also included in the database.

Comprehensive analysis of the data demonstrated that there was no relationship between the P_{eff} variability and the entering drug concentration, the pH of the drug solution, the rat strain, the experimental method, or the intestinal region. The variability between permeability values appears, however, to be associated with the laboratories carrying out the studies. This shows that conclusions cannot be based on comparison of permeability values between laboratories. Preferably, data from individual studies should be interpreted separately, as results from repeated studies within the same laboratory can also vary substantially. This report concludes and emphasizes that the key requirements for obtaining useful permeability data are trained personnel, high quality handling of the animals, a well equipped laboratory, and careful choice of the permeability calculation method.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2019.07.005.

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