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Toward an Increased Understanding of the Barriers to Colonic Drug Absorption in Humans: Implications for Early Controlled Release Candidate Assessment

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Abstract: The purpose of this study was to increase the understanding of in vivo colonic drug absorption in humans by summarizing and evaluating all regional in vivo human absorption data with focus on the interpretation of the colonic absorption data in relation to intestinal permeability and solubility. In addition, the usefulness of the Biopharmaceutics Classification System (BCS) in early assessment of the in vivo colonic absorption potential of controlled release drug candidates was investigated. Clinical regional absorption data (Cmax, Tmax, and AUC) of 42 drugs were collected from journal articles, abstracts, and internal reports, and the relative bioavailability in the colon ($Frel_{colon}$) was obtained directly or calculated. Bioavailability, fraction dose absorbed, and information if the compounds were substrates for P-glycoprotein (P-gp) or cytochrome P450 3A (CYP3A) were also obtained. The BCS I drugs were well absorbed in the colon ($Frel_{colon} > 70\%$), although some drugs had lower values due to bacterial degradation in the colon. The low permeability drugs (BCS III/IV) had a lower degree of absorption in the colon ($Frel_{colon} < 50\%$). There was a clear correlation between in vitro Caco-2 permeability and $Frel_{colon}$, and atenolol and metoprolol may function as permeability markers for low and high colonic absorption, respectively. No obvious effect of P-gp on the colonic absorption of the drugs in this study was detected. There was insufficient data available to fully assess the impact of low solubility and slow dissolution rate. The estimated in vivo fractions dissolved of the only two compounds administered to the colon as both a solution and as solid particles were 55% and 92%, respectively. In conclusion, permeability and solubility are important barriers to colonic absorption in humans, and in vitro testing of these properties is recommended in early assessment of colonic absorption potential.

Keywords: Colon; absorption; controlled release; relative bioavailability; solubility; permeability; BCS

Introduction

The clinical utility of oral controlled release (CR) products may have several benefits compared to immediate release (IR) products; a prolonged exposure may enable once daily dosing of compounds with short elimination half-life, reduction of side effects related to peak plasma concentration, as well as increased effect duration and patient compliance.[1] There is a need to reduce the pharmaceutical development time, and accordingly, it is crucial to assess if a certain drug candidate possesses the necessary characteristics to become a successful oral CR product early in the development process. As a CR formulation often is designed to release its drug content between 12−24 h, it is obvious that release and absorption from the small intestine alone will be

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insufficient in order to attain the desired plasma profiles due to the limited small intestinal transit time of 2−5 h.[2] In contrast, the residence time in the colon is often more than 24 h,[3] which makes the different regions of the colon critical for release and subsequent absorption.[4−6] It is therefore of particular interest to understand the limitations and assess the potential for absorption in the colon.

Colonic absorption of drugs may differ significantly compared to the small intestine as a consequence of several physiological, physicochemical, and biopharmaceutical factors.[1,6−10] In general, permeability and solubility are considered to be the two most fundamental determinants of intestinal absorption, regardless of region. These two parameters also constitute the basis of the Biopharmaceutics Classification System (BCS) for IR products.[11] Since the purpose with a CR formulation is to control the absorption rate, the plasma concentration profile, and the pharmacodynamics through the release from the formulation, it is desirable that neither permeability nor solubility/dissolution should limit the absorption of the compound, as this would affect the in vivo performance of the formulation. From a pharmaceutical point of view, it also has an impact on establishment of in vitro in vivo correlations (IVIVCs). The applicability of BCS for CR products and colonic absorption has been discussed previously.[6,8,12] The passive permeability has been suggested to be lower in the colonic tissue due to smaller surface area and tighter junctions in the epithelial cell layer, and in addition the expression of efflux and uptake transporters, such as P-glycoprotein (P-gp) and the human di/tripetide transporter (hPepT1), has been reported to increase and decrease, respectively, in the colon, which could limit the membrane transport in the colon.[6,8,13−19] Solubility and dissolution has also been suggested to be more restricted in the colon as a consequence of several factors, including lower water content, irregular motility and lack of bile salts.[6−8,10,20] A drug may also be subject to bacteria-mediated degradation in the colon,[21] and the distribution of cytochrome P450 3A (CYP3A) and phase II enzymes in the gut wall has also been reported to vary between regions, which will result in regional differences in bioavailability.[22,23] Since the absorption throughout the entire gastrointestinal tract needs to be taken into account, it is clear that the evaluation of a

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CR candidate is more challenging and complex. Indeed, it was recently reported that development of CR formulations was considered difficult for up to 60% of the drugs in development as a consequence of limitations in colonic drug absorption.[24]

Ideally, early assessment of the potential for colonic drug absorption and CR feasibility of a drug candidate should be performed using in vivo predictive in vitro methods and in silico tools, enabling cost-effective and rapid assessment with high accuracy. Today, in vitro based predictions of colonic permeability are mainly performed using well established cell models, such as Caco-2, regional permeability studies using excised tissues, and regional in situ perfusion studies in rats,[1,16,25−28] although there is, to our knowledge, no published report where in vitro permeability data are directly correlated to human in vivo colonic drug absorption data. Similarly, the in vitro tests used to assess the effect of solubility and dissolution on colonic absorption of drugs tend to be performed in pH adjusted buffers using conventional methodologies, which do not take colon relevant volumes, in vivo constituents, and hydrodynamics into consideration. However, recent efforts have been made to increase the biorelevance of such tests.[10,29] Furthermore, it is also obvious that the models of colon used in the commercial absorption simulation softwares available today are too simple in terms of predictions of colonic drug absorption,[30,31] partly due to lack of the in vivo colonic absorption data needed to build more relevant models. Based on this, it is clear that there is a need to increase the fundamental understanding of the drug absorption from the different regions of colon. Such data are obtained from mechanistic relative bioavailability studies where the drug is administered to different intestinal regions, including different parts of the colon using different capsule, intubation, and colonoscopy techniques.[4,5] These studies have contributed significantly to our current understanding of colonic absorption in humans; however, there is no published report where the colonic absorption data from all these studies are summarized and related to the biopharmaceutical properties of the drugs.

The main objective of this report was to increase the understanding of in vivo colonic drug absorption in humans by summarizing and evaluating all to us available regional in vivo human drug absorption data, along with the relevant physicochemical, biopharmaceutical, and pharmacokinetic properties with special emphasis on the interpretation of the colonic absorption data in relation to intestinal permeability, solubility, and BCS class. The second objective was to investigate the usefulness of biopharmaceutical in vitro data in early assessment of the in vivo colonic absorption potential in humans for CR drug candidates.

Material and Methods

Collection of In Vivo Colonic Absorption Data in Humans. The area under the plasma concentration time curve (AUC), the maximum plasma concentration (Cmax), and the time at which it occurred (Tmax) of 42 compounds, which was used to assess regional absorption in colon, were obtained from journal articles, abstracts, and AstraZeneca internal reports of relative bioavailability studies in humans. The study drugs were administered to different regions of the gastrointestinal tract, including the colon, using various intubation and capsule techniques as well as colonoscopy investigations. The regions within the colon where the administrations had occurred, that is, cecum, ascending colon (AC), transverse colon (TC), or descending colon (DC), were also noted, if specified. In 16 of the studies, it was not specified within which area the study drug was administered. Studies where administration had occurred in the rectum were excluded from this investigation. The corresponding data obtained from any small intestinal region of administration was also collected if available. For clarification purposes, all the different sites of administration were divided into three groups, namely, oral, small intestine (SI), and colon, in some of the figures. Other study characteristics, such as dose, number of subjects, type of formulation, and if the dose was administered as a bolus or as an infusion, was also included in this investigation. The majority of the compounds had been administered to the colon as solutions. The only exceptions were almokalant, diclofenac, and glibenclamide/glyburide, which were administered as suspensions, and cyclosporin A, which was administered as an emulsion. Moreover, AZ6 and dexloxiglumide were the only com-

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pounds where colonic absorption data were available both after colonic administration of a solution and as solid material. The formulations used in the M100240 and oseltamivir studies were not stated in the reports. Rouge et al. previously commented on the regional absorption of 17 of the compounds included in this study, although the focus of that article was not to understand colonic absorption and no colonic absorption data were presented.[32]

The bioavailability (F) in humans after oral dosing, either as a solution or as various solid formulations, was obtained from published journal articles and AstraZeneca internal reports. The fraction absorbed after oral administration (FA) in humans was estimated from

(1) human absorption data given in literature;[33−35]
(2) plasma pharmacokinetic data after oral and intravenous administration (F, plasma clearance (CL), hepatic extraction ratio (E_H), and blood to plasma ratios (CB/CP) if available), assuming no contribution of gut wall metabolism in accordance to Amidon et al.;[11]
(3) urinary pharmacokinetic data after oral and intravenous administration;
(4) mass balance studies using radiolabeled compounds.

Biopharmaceutical Compound Characteristics and BCS Classification. Solubility data were obtained from published journal articles and AstraZeneca internal reports, if available. The data included intrinsic solubility data (S_0), solubility in buffers in the pH range of 5−7.5, as well as solubility in water. The solubility data together with the suggested clinical dose, or the dose applied in the regional absorption study if no clinical dose was reported, were used to tentatively classify the compounds according to BCS. AstraZeneca in-house in vitro apparent permeability (Papp) data from the Caco-2 model, published in vitro data, and previously published permeability classifications were used to classify the investigated compounds as high or low permeability compounds.[34,36−38] If no permeability data were available, the permeability classification was either based on log *P*, according to Kasim et al.,[38] or from degree of metabolism, as recently proposed by Benet et al.,[39] The AstraZeneca in-house Caco-2 permeability data were also used to relate in vitro permeability to human colonic absorption data. In addition, literature and internal AstraZeneca data were used to identify compounds that are substrates for P-gp and/or are mainly eliminated through CYP3A-mediated metabolism. The above biopharmaceutical and related characteristics relevant to colonic absorption of drugs are summarized in Table 1.

Data Analysis. The relative bioavailability in the colon ($Frel_{colon}$) was calculated by the ratio $AUC_{colon}/AUC_{reference}$, and the fraction absorbed after colon administration (FA_{colon}) was estimated by $FA \times Frel_{colon}$. These variables were either obtained directly from the reports or calculated using dose-corrected AUC data after administration to the stomach ($n = 2$) and jejunum ($n = 6$) or after oral administration ($n = 34$), as reference. Since no applicable reference administration were available for atenolol, cimetidine, furosemide, and hydrochlorothiazide, published AUC values after oral administration of a solution was used in the calculations of $Frel_{colon}$.[40] The calculations of $Frel_{colon}$ were based on ratios for individual subjects when such data were available while the ratio was otherwise based on mean data.

Results and Discussion

Human colonic absorption data of 42 drugs and the corresponding data after administration to other intestinal regions are presented in Table 2 in relation to the assigned BCS class to identify the biopharmaceutical property most likely to limit the absorption process. The drugs in this study span over a wide range of biopharmaceutical properties (Table 1), which suggest that conclusions drawn from this in vivo data set are likely suitable for making general recommendations regarding assessment of colonic drug absorption and its importance for the development of oral CR products.

Colonic Absorption of High Permeability/High Solubility (BCS Class I) Compounds. Thirteen of the compounds (31%) in this study were assigned BCS class I based on the available permeability and solubility data (Table

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Table 1. Physicochemical and Biopharmaceutic Properties of 42 Drugs with Available Human Colonic Absorption Data

compound	MW	*c* log *P*	charge type(pK_a)[a]	PSA (static)	human F[b]	human FA[c]	permeability Class	solubility (mg/mL)	solubility media[d]	BCS class	CYP 3A4 substrate	P-gp substrate
acrivastine	348	1.46	Z	60	70	83	low			IV	(no)	yes
almokalant	353	2.21	B	81	55	95	high	23.8	S_0	I	no	
amoxicillin	365	−1.99	A(2.61;6.93)	132	72suspension	88	low	3.96	water	III	no	yes
atenolol	266	0.16	B(9.6)	92	50	57	low	13.3	S_0	III	no	no
benazepril	425	2.04	A	103		37capsule	low	33	ns	III		
BMS-181101			B									
budesonide	430.5	3.14	N	93	9.6micronized	100	high	0.016	buffer pH 7.2	I	yes	yes
AZ1	611	6.69	B	143			high			II	no	
AZ2	440	4.79	B	119	42solution	61/43	high	0.0005	buffer pH 6.5	II	no	
captopril	217	1.19	Z3.45;9.80	96	62	71	low			III		
cimetidine	252	0.4	B(14.2;6.7)	90	76	96	low	24/13	buffer pH 6.8/7.4	III	no	yes
ciprofloxacin	331	−1.08	Z(6.15;8.66)	84	63	84	low	0.17	S_0	IV	no	yes
cyclosporin A	1191	13.39	N	263	27	65	high	0.0066	water	II	yes	yes
dexloxiglumide	461		A(4.48)	96	48capsule	82	high	0.533	buffer pH 7.5	II	yes	yes
diclofenac	296	4.4	A(4.18)	54	51EC tablet	100	high	0.5/3.8	buffer pH 6.5/7.4	II	no	no
diltiazem	415	2.8	B(7.7)	68	41	100	high	10/3	buffer pH 6.8/7.4	I	yes	yes
fexofenadine	538	6.26	Z	124	30	30	low	1	ns	III	no	yes
furosemide	331	2.03	A(3.04)	122	64solution	66	low	2.25	buffer pH 7.2	IV	no	no
glibenclamide	494	4.24	A(6.5)	118	63tablet	67	high	0.01	buffer pH 7.4	II	no	yes
AZ3	430	−0.62	Z(2;7;11.5)	153	7.7	9	low	215	ns	III	no	
AZ4	474	1.77	B(4.5;5.2)	149	21	70	high	0.16−0.58	ns	I	no	yes
hydrochlorothiazide	298	−0.07	A(9.96;8.87)	127		63	low	0.595	S_0	III	no	no
isorbide-5-mononitrate	191	−0.4	N	104	93	100	high	100	ns	I		
lefradafiban	439	3.01	B	132			high	0.0046	buffer pH 6.5	II		
lumiracoxib	294	4.66	A(4.7)	54	74	82	high	0.03	water	II	no	
M100240	481	3.61	A	100		49Tablet	low			III	yes	
metoprolol	267	1.88	B(9.7)	59	50	95	high	43	S_0	I	no	no
AZ5	318	3	B(7.4)	58	50.5capsule	57	high	70	buffer pH 3.6	I		
nifedipine	346	2.86	N	113	51	91	high	0.0044	buffer pH 7.4	II	yes	no
nisoldipine	388	4.53	N	112	8.4	88	high			II	yes	
nitrendipine	360	4.15	N	117	19tablet/23solution	73/88	high	0.001	buffer pH 5−7.4	II	yes	no
omeprazole	345	2.23	N	83	48solution	97	high	0.13	S_0	II	yes	yes
ondansetron	309	3.31	B	36	57	84	high			I	yes	yes
oseltamivir	312	2.33	B	91	79	81	low	500	water	III	no	yes
oxprenolol	265	2.1	B(9.5)	60	55	90	high			I		yes
ranitidine	314	0.27	B(8.4)	88	56	64.5	low	1000	ns	III		yes
rivastigmine	250	2.1	B	39	35	96	high			I	no	
AZ6	469	2.59	N	93	0.5	100	high	0.03	water	I	yes	
salicylic acid	138	2.26	A(3.0)	59	90	100	high	7.22	buffer pH 7.2	I	yes	no
sumatriptan	295	0.93	B	74	14	78	low	100	ns	III	no	no
theophylline	180	−0.02	A(8.4)	75	96	100	high	11.6	ns	I	no	no
zafirlukast	576	7.09	A	122			high	0.01	ns	II	no	

[a] A = acid, B = base, N = neutral, Z = zwitterion. pK_a value indicated within parenthesis. [b] Human oral biovailability data. Oral formulation indicated if stated. [c] Estimated human fraction absorbed after oral administration. Oral formulation indicated if stated. [d] Media used in the solubility test; pH of any buffer is indicated; S_0 = intrinsic solubility; ns = not stated.

1). As shown in Table 2 and Figure 1, all BCS I compounds, except almokalant and AZ4, were highly absorbed throughout the gastrointestinal tract with $Frel_{colon}$ values in the range of 68−127%, which indicates high extent of absorption in the colon. Accordingly, for the majority of the BCS I compounds, no statistically significant difference was observed in the extent of absorption after colonic administration compared to oral or proximal small intestinal administration (Table 2). The mean plasma profiles after administration to different regions of the gastrointestinal tract of the BCS I compound metoprolol are shown in Figure 2.[5] Interestingly, this borderline high permeability drug did not only have the same extent of absorption in the colon compared to the proximal small intestine, but the rate of absorption was also very similar between the two regions (Figure 2). For some of the compounds (diltiazem, AZ5, salicylic acid, and

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Table 2. Human Regional Absorption Data of 42 Drugs According to BCS Class

compound[reference]	dose (mg)	n^a	formulation[b]	type[c]	region[d]	technique[e]	Cmax (nmol/L)	ratio (%)	Tmax (h)	ratio (%)	AUC (nmol h/L)	Frel (%)[f]	FA_{colon} (%)[g]
BCS Class I													
almokalant[71]	10	5	suspension	oral			40.3	100	0.97	100	190	100	
	8	5	suspension	bolus	distal ileum	HF capsule	26.7	66	0.85	88	122.1	63	
	8	5	suspension	bolus	TC	HF capsule	19.2	48	1.2	124	100.2	**53**^{ns}	**50**
budesonide[65]	3	8	solution	infusion	jejunum	intubation	4.14	100	0.66	100	8.97	100	
	3	8	solution	infusion	ileum	intubation	5.75	139	0.67	102	12.3	137	
	3	8	solution	infusion	colon	intubation	2.72	66	0.47	71	9.05	**101**^{ns}	**101**
diltiazem[71]	120	9	solution	oral	oral		491	100	0.6	100	1816	100	
	120	9	solution	bolus	cecum/TC	HF capsule	188	38	3.2	533	1529	**82**$^{P<0.05}$	**82**
AZ4[71]	50	9	solution	oral	oral		423	100	1.5	100	2074	100	
	50	8	solution	bolus	ileum	intubation	606	143	1.3	87	2842	136	
	50	7	solution	bolus	colon	intubation	219	52	1.2	80	1124	**56**$^{P<0.05}$	**39**
isorbide-5-mononitrate[72]	20	6	solution	oral	oral		2315	100	0.81	100	15 500	100	
	20	6	solution	bolus	jejunum	intubation	3298	142	0.28	35	14 900	97	
	20	6	solution	bolus	ileum	intubation	2518	109	0.28	35	12 400	80	
	20	6	solution	bolus	AC	intubation	2052	89	0.68	84	10 600	**68**nd	**68**
metoprolol[5]	25	7	solution	bolus	jejunum	intubation	90	100	1.1	100	386	100	
	25	7	solution	bolus	ileum	intubation	130	144	0.8	73	380	98	
	25	7	solution	bolus	colon	intubation	110	122	0.8	73	405	**105**^{ns}	**100**
AZ5[71]	3	6	solution	oral	oral		147	100	0.5	100	357	100	
	3	9	solution	bolus	prox SI	Intelisite capsule	110	75	0.5	100	242	70	
	3	5	solution	bolus	dist SI	Intelisite capsule	113	77	1	200	371	94	
	3	6	solution	bolus	AC	Intelisite capsule	63	43	1	200	267	**88**^{ns}	**50**
ondansetron[73]	8	6	solution	oral	oral		129	100	1.3	100	728	100	
	8	6	solution	bolus	colon	intubation	91	70	1.1	85	764	**105**^{ns}	**88**
oxprenolol[74]	80	3	suspension	oral	oral		1849	100	0.75	100	4192	100	
	80	3	suspension	bolus	colon	colonoscopy	1136	61	0.5	67	3208	**82**^{ns}	**74**
rivastigmine[75]	3	7	solution	oral	oral		51.2	100	0.87	100	83.6	100	
	3	7	solution	bolus	jejunum	intubation	52.4	102	0.44	51	66	85	
	3	7	solution	bolus	ileum	intubation	59.2	116	0.4	46	85.6	113	
	3	7	solution	bolus	AC	intubation	53.6	105	0.58	67	89.6	**107**^{ns}	**103**
AZ6[71]	6.4	8	solution	oral	oral		0.20	100			0.997	100	
	6.4	8	solution	bolus	colon	intubation	0.32	159			1.108	**111**nd	**111**
	6.4	8	granules	bolus	colon	intubation	0.16	79			0.613	**66**nd	**66**
salicylic acid[76]	500	6	solution	oral	oral		331 160	100	0.85	100	1.85	100	
	500	1	solution	infusion	jejunum	intubation	376 800		0.75		3.22		
	500	5	solution	infusion	cecum	intubation	246 380	74	3.2	376	2.34	**127**nd	**127**
theophylline[77]	80−120	3	solution	oral	oral		14 890	100	0.89	100	91 110	100	
	80−120	3	solution	bolus	stomach	HF capsule	12 500	84	0.54	61	90 560	98	
	80−120	3	solution	bolus	ileum	HF capsule	10 440	70	1.35	152	85 560	91	
	80−120	3	solution	bolus	colon	HF capsule	7720	52	3.05	343	76 670	**85**^{ns}	**85**
BCS Class II													
AZ1[71]	0.4	3	solution	bolus	prox jejunum	intubation	33.2	100	1.83	100	447.5	100	
	0.4	4	solution	bolus	AC/TC	intubation	0	0	0	0	0	**0**nd	
AZ2[71]	5	9	solution	bolus	prox jejunum	intubation	155.8	100	1.53	100	999	100	
	5	9	solution	bolus	term ileum	intubation	118	76	1.17	76	699.3	70	
	5	7	solution	bolus	AC/TC	intubation	79.2	51	0.96	63	569.7	**57**$^{P<0.05}$	**25**
cyclosporin A[78]	150	10	capsule	oral	oral		740	100	1.5	100	2582	100	
	150	9	emulsion	bolus	duodenum	intubation	910	123	1	67	2382	93	
	150	8	emulsion	bolus	jejunum	intubation	971	131	1	67	3647	147	
	150	9	emulsion	bolus	ileum	intubation	411	56	1	67	1238	52	
	150	10	emulsion	bolus	DC	intubation	280	38	2.2	147	1156	**53**$^{P<0.05}$	**34**
dexloxiglumide[79]	200	11	tablet	oral	oral		6725	100	0.75	100	15 401	100	
	200	11	powder	bolus	jejunum	Enterion capsule	6074	90	0.5	67	15 835	104	
	200	11	powder	bolus	colon	Enterion capsule	1779	26	1.5	200	10 195	**69**nd	**57**
	200	9	solution	bolus	colon	Enterion capsule	3254	48	0.5	67	9978	**75**nd	**62**
diclofenac[74]	100	6	tablet	oral	oral		7905	100			10 439	100	
	100	6	suspension	bolus	colon	colonoscopy	5439	69			7669	**83**^{ns}	**83**
glibenclamide[80]	1.75	8	suspension	oral	oral		362	100	1.7	100	966	100	
	1.75	8	suspension	bolus	duodenum	intubation	364	101	1.3	76	962	100	
	1.75	6	suspension	bolus	AC	intubation	206	57	2.8	165	984	**102**^{ns}	**68**
lefradafiban[81]	10	11	solution	oral	oral		77	100	1.7	100	975	100	
	10	11	solution	bolus	jejunum	intubation	82	106	1.4	80	1048	108	
	10	11	solution	bolus	ileum	intubation	87	113	1.4	80	1000	103	
	10	11	solution	bolus	DC	intubation	41	52	2.3	135	661	**68**$^{P<0.05}$	
lumiracoxib[82]	100	10	solution	oral	oral	Intelisite capsule	4728	100	2	100	20 905	100	
	100	10	solution	bolus	prox SI	Intelisite capsule	5537	117	1	50	22 459	104	
	100	10	solution	bolus	dist SI	Intelisite capsule	8207	174	1	50	23 272	110	
	100	10	solution	bolus	AC	Intelisite capsule	3772	80	1	50	16 949	**85**^{ns}	**68**

Table 2. Continued

compound[reference]	dose (mg)	n^a	formulation[b]	type[c]	region[d]	technique[e]	Cmax (nmol/L)	ratio (%)	Tmax (h)	ratio (%)	AUC (nmol h/L)	Frel (%)[f]	FA_{colon} (%)[g]
nifedipine[70]	10	4	solution	oral	oral		201.7	100	0.46	100		100	
	10	4	solution	bolus	stomach	HF capsule	216.5	107	0.42	91	331.4	106	
	10	4	solution	bolus	jejunum	HF capsule	342.2	138	0.25	71	436.1	100	
	10	4	solution	bolus	AC	HF capsule	89.3	44	1	222	372.7	**110[nd]**	**100**
	10	4	solution	bolus	DC/TC	HF capsule	130.1	85	0.88	251	441.4	**126[nd]**	**115**
nisoldipine[64]	10	4	solution	oral	oral						50 mg h/mL	100	
	10	4	solution	bolus	stomach	HF capsule					62	125	
	10	4	solution	bolus	jejunum	HF capsule					49	99	
	10	4	solution	bolus	AC	HF capsule					143	**289[nd]**	**260**
	10	4	solution	bolus	DC	HF capsule					155	**313[nd]**	**281**
nitrendipine[64]	10	6	solution	oral	oral						215 mg/mL h	100	
	10	6	solution	bolus	stomach	HF capsule					326	152	
	10	6	solution	bolus	jejunum	HF capsule					223	104	
	10	6	solution	bolus	ileum	HF capsule					206	96	
	10	6	solution	bolus	AC	HF capsule					125	**58[nd]**	**51**
	10	6	solution	bolus	DC	HF capsule					120	**56[nd]**	**49**
omeprazole[71]	5	7	solution	bolus	jejunum	intubation	250	100	0.25	100	170	100	
	5	7	solution	bolus	ileum	intubation	300	120	0.25	100	180	102	
	5	7	solution	bolus	colon	intubation	140	56	0.25	100	90	**68[nd]**	**66**
zafirlukast[83]	40	5	solution	oral	oral		1210	100	2	100	3604	100	
	40	5	solution	bolus	colon	intubation	337	28	1.3	65	1045	**29[$P<0.05$]**	
BCS Class III													
amoxicillin[60]	375	4–9	solution	oral	oral		21 644		1		50 301	100	
	375	4–9	solution	infusion	duodenum	intubation					50 137	96	
	375	4–9	solution	infusion	jejunum	intubation					43 918	83	
	375	4–9	solution	infusion	ileum	intubation					30 301	60	
	375	4–9	solution	infusion	colon	intubation					82	**0[$P<0.05$]**	**0**
atenolol[76]	25	6	solution	oral	oral		126.6	100	2.6	100	0.001	100	
	20	1	solution	infusion	jejunum	intubation	672.9		2		0.005		
	20	5	solution	infusion	cecum	intubation	78.9	62	1.6	62	0.0007	**48[nd]**	**28**
benazepril[84]	20	13	solution	bolus	oral		638	100	0.5	100	520	100	
	20	13	solution	infusion	SI	intubation	128	20	2.9	537	420	90	
	20	7	solution	infusion	colon	intubation	38	6	3.4	630	106	**23[$P<0.05$]**	**9**
captopril[85]	100	9	solution	oral	oral		5037	100	0.7	100	7249	100	
	100	9	solution	infusion	AC	intubation	263	5	3.6	514	991	**14[$P<0.05$]**	**10**
	100	9	solution	infusion	AC	intubation	369	7	1.6	229	1502	**21[$P<0.05$]**	**15**
cimetidine[76]	200	7	solution	oral	oral		5032	100	1	100	0.019	100	
	200	1	solution	infusion	jejunum	intubation	11 111		2		0.018		
	200	5	solution	infusion	cecum	intubation	833	17	5	495	0.004	**20[nd]**	**19**
fexofenadine[44]	60	6	solution	bolus	jejunum	intubation	530	100	0.67	100	2026	100	
	60	6	solution	bolus	ileum	intubation	286	54	0.42	62.5	751	37	
	60	6	solution	bolus	colon	intubation	288	54	0.33	50	885	**44[$P<0.05$]**	**13**
AZ3[71]	50	10	solution	oral	oral		140	100	1.4	100	673	100	
	50	10	solution	bolus	ileum	HF capsule	80	58	2.2	156	416	62	
	50	10	solution	bolus	cecum/AC	HF capsule	50	32	2.2	153	186	**28[$P<0.05$]**	**3**
hydrochlorothiazide[76]	25	6	solution	oral	oral		282	100	3	100	0.0015	100	
	25	1	solution	infusion	jejunum	intubation	1211		2		0.0037		
	25	5	solution	infusion	cecum	intubation	57	20	1.6	53	0.0003	**20[nd]**	**13**
M100240[86]	25	10	tablet	oral	oral		27.9	100	0.75	100	94	100	
	25	10	?	bolus	prox SI	Enterion capsule	22.5	81	1	133	87	94	
	25	10	?	bolus	dist SI	Enterion capsule	36.6	131	0.5	67	89	97	
	25	10	?	bolus	AC	Enterion capsule	3.7	13	1.5	200	40	**41[nd]**	**20**
oseltamivir[48]	150	8	?	bolus	stomach	Enterion capsule	319	100	0.5	100	606	100	
	150	8	?	bolus	jejunum	Enterion capsule	333	105	0.61	122	564	93	
	150	8	?	bolus	ileum	Enterion capsule	423	133	0.56	112	564	94	
	150	8	?	bolus	AC	Enterion capsule	156	49	0.84	168	503	**83[ns]**	**67**
ranitidine[87]	150	8	solution	bolus	stomach	intubation	1849	100	2.7	100	7240	100	
	150	8	solution	bolus	jejunum	intubation	1361	74	2.9	109	6563	91	
	150	8	solution	bolus	cecum	intubation	167	9	2.7	100	1054	**15[$P<0.05$]**	**9**
sumatriptan[88]	50	8	solution	oral	oral		106	100	1	100	400	100	
	50	8	solution	bolus	jejunum	intubation	132	124	0.9	90	366	90	
	50	3	solution	bolus	cecum	intubation	30	28	2.3	230	121	**28[nd]**	**15**
BCS Class IV													
acrivastine[89]	12	6	syrup	oral	oral		514	100	0.85	100	1655	100	
	12	6	syrup	bolus	colon	colonoscopy	40	8	3.6	424	299	**18[$P<0.05$]**	**15**
ciprofloxacin[69]	180	4	solution	oral	oral		2296	100	0.59	100	2779	100	
	180	3	solution	bolus	jejunum	HF capsule	483	20	0.5	77	1148	37	
	180	4	solution	bolus	ileum	HF capsule	362	15	0.5	77	725	23	
	180	4	solution	bolus	AC	HF capsule	91	4	0.64	98	242	**7[$P<0.05$]**	**6**
	180	3	solution	bolus	DC	HF capsule	181	7	0.32	49	151	**5[$P<0.05$]**	**4**
furosemide[76]	20	6	solution	oral	oral	intubation	973	100	1	100	0.0019	100	
	20	1	solution	infusion	jejunum	intubation	2538		1		0.0026		
	20	5	solution	infusion	cecum	intubation	94	10	4.8	483	0.0007	**35[nd]**	**22**

Table 2. Continued

compound[reference]	dose (mg)	n^a	formulation[b]	type[c]	region[d]	technique[e]	Cmax (nmol/L)	ratio (%)	Tmax (h)	ratio (%)	AUC (nmol h/L)	Frel (%)[f]	FA_{colon} (%)[g]
Unclassified													
BMS181101[90]	15	13	solution	oral	oral		9.43 ng/mL	100	3 h	100	55.9 ng h /mL	100	
	15	5	solution	infusion	jejunum	intubation	4	41	3.5	117	23	51	
	15	5	solution	infusion	ileum	intubation	9.52	76	3	100	54.4	65	
	15	9	solution	infusion	colon	intubation	9.66	64	3.25	108	54.2	**60[nd]**	

[a] Number of subjects. [b] Type of formulation used in the regional absorption study. [c] Type of administration in the different intestinal regions. [d] The different intestinal regions into which the drug were administered. AC = ascending colon; DC = descending colon; prox/dist SI = proximal/distal small intestine. [e] Technique used to administer the drug to different gastrointestinal regions. [f] The relative bioavailability in the colon; calculated as $AUC_{colon}/AUC_{reference}$. Observed significant statistical differences between colonic and reference administration is indicated: $P < 0.05$ = observed significant statistical difference; ns = no observed statistical difference; nd = not determined. [g] Estimated fraction absorbed after colonic administration; calculated by FA × $Frel_{colon}$.

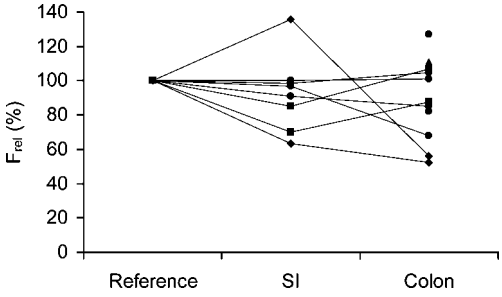

Figure 1. Regional absorption of BCS class I compounds in humans indicating that high permeability compounds have good colonic absorption properties ($Frel_{colon}$ > 70%, range = 68−127%). The somewhat lower $Frel_{colon}$ values of 53 and 56% observed for almokalant and AZ4, respectively, are most likely caused by bacterial degradation in the colon.

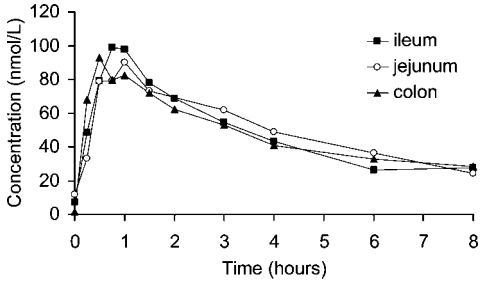

Figure 2. Mean plasma concentration−time profiles after administration of 25 mg of metoprolol (BCS class I) as a solution to the ileum, jejunum, and colon in humans, which indicates that no regional differences in rate and extent of absorption exist for this compound.[5]

theophylline), there was a decrease and increase in Cmax and Tmax, respectively, after colonic administration compared to the small intestine, suggesting a slower absorption rate in the colon compared to the small intestine (Table 2). This suggests that more in vivo studies may be needed to increase the understanding of the factors affecting the absorption rate in the colon, however, these findings may also be attributed to a delayed drug release from the devices used in these studies (Table 2).

The somewhat lower $Frel_{colon}$ values of 53% (ns) and 56% ($P < 0.05$) observed for almokalant and AZ4, respectively, are most likely caused by bacterial degradation in the colon, as these compounds are degraded in the colonic environment in vitro (AstraZeneca, unpublished data) (Table 2 and Figure

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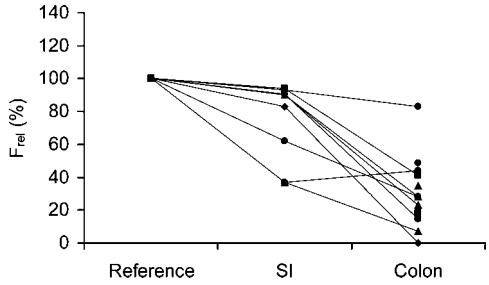

Figure 3. Regional absorption of BCS class III and IV compounds in humans indicating that low permeability compounds have poor colonic absorption properties ($Frel_{colon}$ well below 50%, range = 0−48%). The reason for the apparent high $Frel_{colon}$ of oseltamivir, a moderate permeability compound and a P-gp substrate, is currently unknown.

1). This is further supported by the regional absorption data, where Cmax decreased after colonic administration while Tmax was unchanged, suggesting a decrease in extent of absorption rather than in the rate (Table 2 and Figure 1). This demonstrates the importance of investigations of the luminal degradation in the colon, using in vitro tests mimicking the colonic environment, in early CR feasibility testing.

The data presented in the current study clearly show that compounds with high and mainly passive permeability and high stability against the microflora will have a high rate and extent of colonic absorption in man, and should be considered as good CR candidates provided that dissolution and/or solubility does not limit the absorption process significantly.

Colonic Absorption of Low Permeability (BCS Class III/IV) Compounds. Twelve compounds in this study were assigned BCS class III (29%) and three were classified as BCS class IV (7%) based on the available permeability and solubility data (Table 1). As shown in Table 2 and Figure 3, the extent of absorption was significantly lower in the colon compared to the small intestine for all low permeability compounds except oseltamivir. Accordingly, for the majority of the BCS III/IV compounds, a statistically significant decrease in the extent of absorption was observed after colonic administration compared to oral or proximal small intestinal administration (Table 2). The $Frel_{colon}$ values were generally well below 50% (ranging between 0 and 48%), except for oseltamivir for which the corresponding value was 83% (Table 2 and Figure 3). The lower fraction of the dose absorbed from colon of these low permeability compounds can be attributed to the lower surface area and more tight junctions in the colon, which has been suggested to be of greater importance for low permeability compounds.[25] The general trend toward a decrease and increase in Cmax and Tmax, respectively, after colonic administration compared to the small intestine was consistent with a slower absorption rate in the colon for the low permeability compounds (Table 2). Similar results have previously been reported for other low permeability drugs when investigated in human and rat

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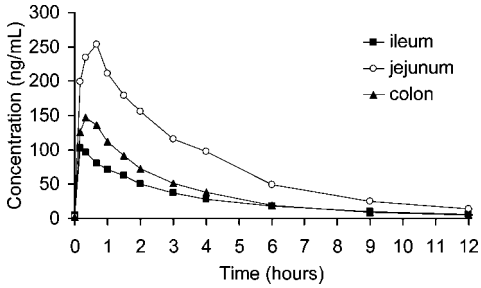

Figure 4. Mean plasma concentration−time profiles after administration of 60 mg of fexofenadine (BCS class III) as a solution to the ileum, jejunum, and colon in humans, showing that the extent of colonic absorption is significantly lower compared to the jejunum for this low permeability compound.[44]

excised tissues.[25,41] The mean plasma profiles of fexofenadine after administration to different regions of the gastrointestinal tract are shown in Figure 4 as an example of regional absorption of a low permeability drug; the jejunal effective permeability is low ($0.1-0.2 \times 10^{-4}$ cm/s) and variable, which classifies fexofenadine as a low permeability compound according to the BCS.[42,43] The $Frel_{colon}$ value of 44% for the efflux transporter substrate fexofenadine is in accordance with the hydrophilic nature and low passive permeability of the compound.[42−45] Notably, the plasma exposure of fexofenadine is linear from the microdose scale up to 800 mg.[46,47] This is in agreement with the linear permeability in the absorptive direction for fexofenadine seen in the Caco-2 model, but it is in contrast to the high efflux ratio observed in this in vitro model.[35,45] This shows that the absorption process of fexofenadine, both from the small intestine and the colon, is complex and that the role of P-gp, or other efflux transporters, is not fully understood. It also demonstrates the limited usefulness of Caco-2 in vitro efflux ratios in predictions of the in vivo importance of efflux proteins.

The reason for the apparent high $Frel_{colon}$ of the P-gp substrate oseltamivir, which is the only compound in the current study reported to have a moderate in vitro permeability, is currently unknown.[48,49] Although the available data may suggest that a moderate permeability drug, which in addition is a substrate for P-gp with an efflux ratio between 4 and 8 in the Caco-2 model,[50] may be completely absorbed from the colon, it is vital to thoroughly investigate all the factors affecting the regional absorption of the compound before any conclusions can be drawn regarding the colonic absorption potential of this class of compounds. For example, the fact that oseltamivir is an ester prodrug, which potentially may undergo luminal degradation in the proximal part of the intestine, also needs to be taken into consideration when these results are evaluated.[49]

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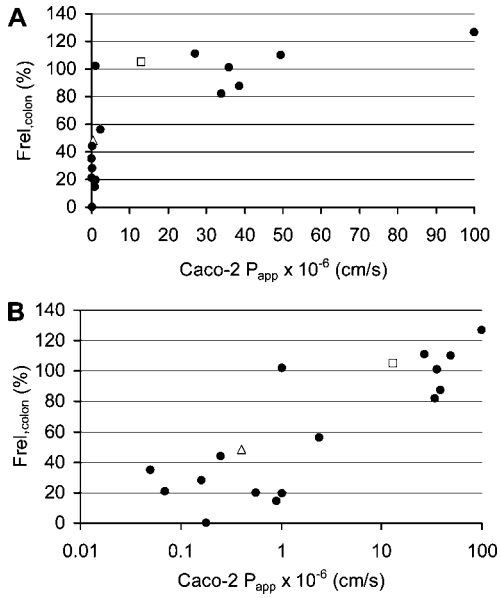

Figure 5. Relationship between Caco-2 permeability (Papp) and colonic absorption in humans ($Frel_{colon}$) ($R^2 = 0.74$). Atenolol (open triangles) and metoprolol (open squares) may be suitable permeability markers for low and high extent of colonic absorption.

The data presented above clearly show that the rate and extent of colonic absorption in humans of low permeability compounds generally will be slow, incomplete, and highly variable, suggesting that compounds with low passive permeability will be poor CR formulation candidates, as there is a significant risk that membrane transport rather than release from the formulation will control the bioavailability. However, additional in vivo evaluation is warranted for moderate permeability drugs to better understand their extent and rate of colonic absorption.

Caco-2 In Vitro Permeability Data Can Be Used to Predict Colonic Absorption in Man. A sigmoidal relationship ($R^2 = 0.74$) was obtained when the Caco-2 in vitro apparent permeability (Papp) of 18 of the compounds (AstraZeneca, unpublished data) in Table 2 was correlated to their corresponding $Frel_{colon}$ data (Figure 5). This suggests that the Caco-2 cell model and possibly other in vitro models, which are widely used in early assessment of small intestinal absorption, also may be valuable tools in early assessment of colonic absorption potential of CR candidates. This is not surprising, since the Caco-2 model is of colonic origin and the tight junctions in the Caco-2 model more closely resembles that of the colon than that of the small intestine.[51] The data also suggested that atenolol and metoprolol may be used as permeability markers for low and high colonic absorption, respectively (Figure 5). Both compounds are well established BCS permeability markers with complete dissolution and passive diffusion as the main membrane transport mechanism.[11] In addition, the fact that metoprolol has been successfully developed as a commercial CR formulation with even plasma exposure over 24 h provides a high degree of confidence in the colonic absorption potential for candidates with in vitro permeability above that of metoprolol.[52] The use of these markers would divide the correlation curve into three well-defined regions: (a) one low (passive) permeability region ($Papp \leq Papp_{atenolol}$) where low/poor colonic absorption is predicted and high risk for development failure for such CR candidates is expected; (b) one high (passive) permeability region where complete colonic absorption is predicted ($Papp \geq Papp_{metoprolol}$) and compounds in this region are expected to be good CR candidates; and finally, (c) a moderate permeability region ($Papp_{atenolol} < Papp < Papp_{metoprolol}$), in which it currently is difficult to predict the rate and extent of colonic absorption with any accuracy due to lack of in vivo data in the database. Regional absorption studies in humans will thus primarily be beneficial for moderate permeability compounds in order to assess their colonic absorption potential and CR feasibility. Moreover, the transport mechanisms should be thoroughly investigated for low-moderate permeability compounds to assess the passive permeability, which may be masked by high efflux in several in vitro models, before any decision regarding CR development is made.

The correlation presented in Figure 5 is in very good agreement with the Caco-2 Papp criteria presented recently by Thombre, but an actual correlation was not presented.[1] Although the correlation between the in vitro and in vivo colonic absorption data in this study is encouraging, it is also important to evaluate other in vitro permeability methods than Caco-2. For example, Collett et al. recently reported that human in vivo colonic absorption data of a drug in development could be better predicted using permeability data obtained from excised human colonic tissue compared to Caco-2 data.[28] Excised human tissue experiments have the advantage that regional absorption can be investigated in vitro and that the enzyme and transporter expression levels are more relevant compared to those of cell lines.[15,18,53,54] Indeed, it has been shown that the permeability decreased in the colon compared to the small intestine in both rats and humans for low permeability compounds.[25,41]

Effect of P-gp-Mediated Efflux and Carrier-Mediated Uptake on On Vivo Colonic Absorption in Humans. Currently, there is some degree of uncertainty regarding the expression levels of P-gp and other efflux transporters in different regions of the gastrointestinal tract.[14,15,18,53] For instance, some reports suggest that P-gp expression is higher in the colon while other reports suggest lower expression levels in the colon, compared to the small intestine.[14,15,18,53] No difference in the extent of colonic absorption was observed when the $Frel_{colon}$ data for compounds that have been shown to be subjugated to P-gp-mediated efflux were compared with the $Frel_{colon}$ data for nonsubstrates, irrespective of permeability class (Tables 1 and 2). Thus, the in vivo data presented in this study do not indicate any clear limiting effect of P-gp-mediated efflux on colonic absorption, which suggests that the intrinsic passive permeability, rather than efflux, is the

major determinant of colonic absorption in humans. Collet et al. recently made a similar observation, which is consistent with the conclusions drawn regarding the limited effect of efflux transporters on the absorption in the small intestine.[28,34,42,43,55−58] However, it should be noted that many of the regional absorption studies were performed at rather high concentrations, often in the mM range, and therefore it cannot be excluded that P-gp and other efflux transporters located in the colon were saturated during the regional absorption studies. In contrast, the local lumenal concentration will remain low in the colon during release of a CR formulation, suggesting that efflux may potentially limit colonic absorption when a substrate is administered as a CR formulation. Currently, it is recommended that additional mechanistic in vitro investigations should be performed for low−moderate permeability CR candidates, which are P-gp substrates, to delineate their passive permeability in order to accurately assess the colonic absorption potential. Assessment of other efflux transporters was beyond the scope of this study.

Another aspect is carrier-mediated active uptake. Amoxicillin, benazepril, and captopril, which were included in the present study, are all substrates for hPepT1.[59] A clear decrease in absorption was observed for all three compounds when they were administered to the colon (Table 2), which is consistent with the fact that the expression levels of uptake transporters, such as hPepT1, generally decrease in the distal intestine.[15,19] The results clearly suggest that compounds which are highly absorbed in the small intestine due to carrier-mediated uptake will be poorly absorbed in the colon and be unsuitable for CR development, since the passive permeability is low. However, these compounds will be correctly classified as low permeability compounds in the colon based on in vitro methods such as Caco-2 cells, as a consequence of their low passive diffusion and the low in vitro activity of the carrier-mediated uptake because of the low hPepT1 expression in these models.[60,61]

Colonic Absorption of High Permeability/Low Solubility (BCS Class II) Compounds. Since only 3 of the 13 BCS class II compounds were administered as solid material to the colon while the rest were formulated as solutions (Table 2), it is obvious that there currently is insufficient data available to assess the impact of low solubility and slow dissolution on the colonic absorption humans. To be able to predict the effect of low solubility and slow dissolution on the colonic absorption in humans and to set dose:solubility ratio targets early in the development process, more mechanistic in vivo absorption studies are needed, either in humans or dogs, where low solubility compounds have been administered to the colon both as a solution and as solid material in order to assess the in vivo fraction dissolved in colon and the corresponding in vivo dissolution profiles.[8] Such data would also guide the development of in vivo predictive dissolution methods and media for the colon.

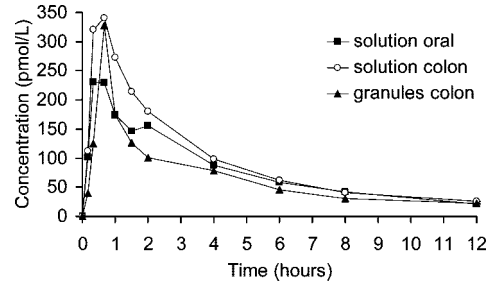

Figure 6. Mean plasma concentration−time profiles after administration of 6.4 mg of AZ6 as an oral solution or a solution and as granules to the colon in humans, showing the effect of low solubility/slow dissolution on colonic absorption in humans.[71]

The estimated in vivo fraction dissolved for AZ6 (solubility 30 μg/mL) and dexloxiglumide (solubility 533 μg/mL), which were the only low solubility compounds where colonic absorption data were available both for a solution and solid material in the current study, were 55% and 92%, respectively (Table 2). The corresponding dose:solubility ratios were 213 and 375 mL (Tables 1 and 2). Colonic administration of solid material also resulted in decreased Cmax and, in the case of dexloxiglumide, a longer Tmax compared to the solution, which also suggests a slow dissolution rate (Table 2 and Figure 6). In contrast, no difference in AUC, Cmax, or Tmax and complete dissolution in the colon were obtained when 40 mg of fasudil was administered to the colon both as a powder and as a solution, which is consistent with the high solubility (200 mg/mL) and the low dose:solubility ratio (0.2 mL) of the compound.[62] These results together with the low water content in the colon, a most recent report suggests that the fluid volume in the colon may be below 50 mL,[7] suggest that the dose:solubility criteria of 250 mL for high solubility in the small intestine may not accurately reflect the colonic environment and that lower values may be needed to ensure complete dissolution in the colon. Indeed, many marketed CR products, such as metoprolol, diltiazem, verapamil, diclofenac, isorbide-5-mononitrate, and theophylline, have dose:solubility ratios well below 50 mL. In addition, the use of dose:solubility ratios as initial criteria in CR feasibility assessment was recently proposed, where ratios $<$100 mL, 100−1000 mL, and $>$1000−10 000 mL would suggest straightforward CR development, challenging CR development and difficult/impossible CR development, respectively,[1] which is in accordance with the findings in the current study. However, it is obvious that there is a need for an improved understanding leading to more robust solubility criteria with respect to colonic absorption.

It was noted that some of the BCS II compounds had an unexpected low degree of absorption in the colon despite being administered to the colon as solutions (Table 2). This suggests that in vivo precipitation in the colon may occur for some compounds within this class during regional absorption studies and that there is a need to assess the risk for in vivo precipitation and guide

formulation selection prior to conduction of a regional absorption study, since this may affect data interpretation. This is consistent with data previously reported in regional absorption studies in dogs.[8]

The Applicability of BCS in Early Assessment of Colonic Absorption Potential in CR Formulation Candidates. The applicability of BCS for CR products and colonic absorption has been discussed previously.[6,8,12] Corrigan proposed a modified BCS classification for CR products but concluded that more data were needed regarding regional dependency in permeability and how to predict it, while Wilding thought that BCS would be a too simple approach, proposing that regional differences in gut wall metabolism needed to be considered as well, and that vitro tests would be of limited value in CR assessment.[6,12] In the present study, we related the collected colonic absorption data to BCS class to indicate the expected limiting barrier to the colonic absorption process, not for classification purposes, and because permeability and solubility data easily can be used to investigate colonic absorption potential of CR candidates early in the development process.

In the present study, it was shown that the compounds, which were classified as high permeability drugs, had $Frel_{colon}$ values >70%, while the low permeability drugs had $Frel_{colon}$ values <50% (Table 2 and Figures 1 and 3). This is in good agreement with a recent proposal based on the results from 37 human regional drug absorption studies; that is, CR development would be straightforward for drugs with $Frel_{colon} > 60\%$, very difficult or impossible at $Frel_{colon} < 30\%$, and challenging in between.[24] This clearly suggests that permeability classification, based on established in vitro Caco-2 permeability−in vivo colonic absorption correlations and/or permeability markers for high and low colonic absorption, will be very valuable in early assessment of the colonic absorption potential of CR candidates, especially since the Caco-2 model is likely to correctly classify compounds undergoing carrier-mediated uptake in the small intestine as low permeability compounds in the colon. Further in vivo data in humans and additional in vitro investigations may be needed for proper assessment of the colonic absorption potential for compounds with moderate passive permeability, especially when such compounds are substrates for efflux transporter(s).

Although much less is known of the effect of low solubility and slow dissolution rate on the colonic absorption due to lack of human in vivo studies, the data presented in this paper show that solubility/dissolution may limit the colonic absorption process, even for low solubility compounds classified as BCS I according to the criteria for the small intestine (Table 2 and Figure 6). This suggests that the lower colonic fluid volume (compared to the small intestine) needs to be taken into account when setting solubility or dose:solubility targets. Currently, we suggest the use of a dose:solubility ratio of ≤50 mL as criterion for high solubility in early assessment of colonic absorption potential for CR candidates, as this also is a relevant colonic fluid volume.[7] It is very important to increase the in vivo understanding in this area, since the number of low solubility compounds in development is increasing. Regional absorption studies in humans using solid material should be considered in the CR assessment of low solubility compounds.

The Effect of Regional Differences in Gut Wall Enzyme Distribution on Interpretation of Colonic Absorption Data. The expression levels of the different enzymes present in the enterocytes are highly regional dependent. For example, the protein levels and catalytic activity of CYP3A and phase II enzymes are highest in the proximal small intestine and decline in the distal small intestine and colon.[14,22,23,53] Although this may have positive consequences regarding the bioavailability and in vivo performance of a CR formulation of a drug,[63] which is substrate for such enzymes, the involvement of gut wall metabolism will confound the assessment of the extent of colonic absorption from a biopharmaceutical viewpoint, by suggesting a falsely high extent of absorption in the colon. This may in turn mask the effect of other factors limiting the colonic absorption, such as low permeability, solubility, precipitation, and bacterial degradation. The most obvious example in the present study is the CYP3A substrate nisoldipine with $Frel_{colon}$ values well above 200% (Table 2).[64] Moreover, coadministration of the CYP3A inhibitor ketoconazole increased the bioavailability of budesonide twofold after jejunal administration, most likely by inhibiting intestinal CYP3A, resulting in an apparent decrease in $Frel_{colon}$, although the actual exposure after colonic administration remained unchanged.[65] $Frel_{colon}$ values of other CYP3A substrates with moderate−high liver extraction ratios are most likely affected as well (Table 2). It is currently not possible to quantify the contribution of gut wall metabolism to first-pass extraction with high accuracy, especially not in different regions of the intestine. However, it is important to assess this possibility as early as possible. Combined transport and metabolism experiments using human tissues from different intestinal regions may provide useful information in this matter. Other processes, which also may confound the assessment of the extent of colonic absorption, include degradation and precipitation.

Additional Aspects. The majority of the studies in this report were performed using various intubation techniques ($n = 26$), while the number of studies performed using capsule and colonoscopy techniques were 13 and 3, respectively (Table 2). Some reports have suggested that the intestinal motility may be affected by the intubation procedures.[4,12,66] The data presented in this report do not support this hypothesis. As shown in Figure 7, the relative bioavailability after jejunal administration is not significantly altered compared to the reference oral administration regardless if the compound was administered using a capsule or intubation technique (Figure 7). This suggests that both techniques provide human in vivo data with high accuracy regarding this aspect. Similarly, Naslund et al.

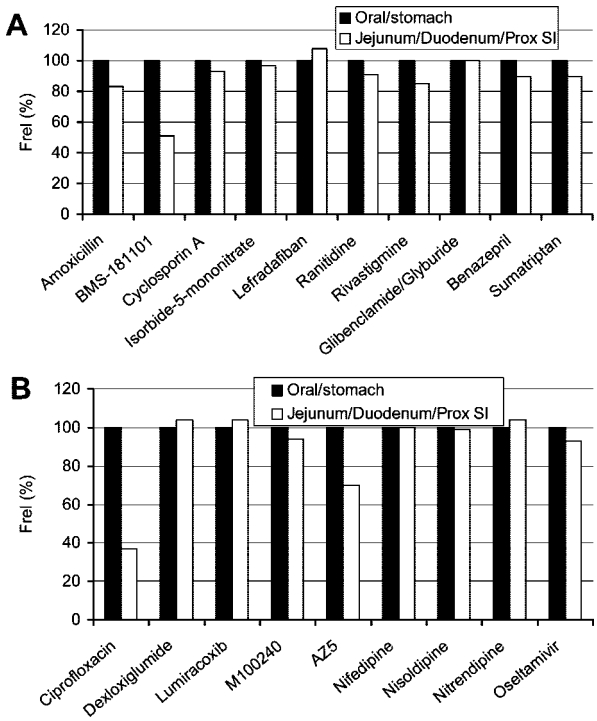

Figure 7. Relative bioavailability (Frel) after jejunal administration using intubation (A) or different capsule techniques (B). Atenolol, cimetidine, furosemide, hydrochlorothiazide, and salicylic acid were excluded from this comparison, although data after jejunal administration were available due to an insufficient number of subjects ($n = 1$).

clearly showed that there was no difference in gastric emptying between the following three methods: scintigraphy, oral dosing of paracetamol tracer and subsequent plasma sampling, and polyethylene glycol (PEG) dilution methods using intubation tubes.[67] Interestingly, although it may be argued that absorption after infusion of a drug solution into the colon may more closely reflect the absorption from a controlled release formulation, the majority of the local administrations into colon intubation has been performed using bolus administrations ($n = 32$) (Table 2).

The majority of the compounds were administered to either the cecum or ascending colon (Table 2), most likely because the proximal colon has been suggested to be more relevant from a drug absorption point of view; the luminal content is more liquid, the surface area compared to that of the distal colon is somewhat larger and the bacterial activity is higher.[10,68] Ciprofloxacin, nifedipine, nisoldipine, and nitrendipine were the only compounds where absorption has been investigated in both the ascending and descending colon.[64,69,70] Considering the physiological differences, it was somewhat surprising that no obvious difference seemed to exist regarding the rate and extent of absorption between these two colonic regions for these compounds. This may suggest that there are no major regional differences in absorption within the colon (Table 2). This observation may have important implications for the design of future regional absorption studies as well as in the development of physiology based pharmacokinetic models. However, further in vivo investigations in humans are needed before a more general conclusion and recommendation can be drawn. For example, the fact that all these low solubility compounds were administered as solutions may, at least in part, explain the absence of any differences in the rate and extent of absorption between the two colonic regions.

Conclusions

In this report, we have provided, summarized, and evaluated fundamental data in order to better understand the barriers limiting the colonic absorption in humans to be able to better predict colonic absorption potential during early CR candidate assessment. Not surprisingly, it can be concluded that both permeability and solubility/dissolution are important factors for colonic drug absorption in humans and that in vitro testing of these properties should be mandatory along with colonic stability tests in early assessment of colonic absorption potential. The data provided in this report do not support the hypothesis that P-gp-mediated efflux is a major barrier to colonic absorption of drugs in humans, but additional in vitro tests are warranted for P-gp substrates to assess their passive permeability. To enable better predictions of colonic absorption in the future, more in vivo human data are needed for compounds with moderate permeability, especially if they are substrates for efflux transporters and/or low solubility. The data presented in this report will also likely be useful in the development and validation of future in vitro and in silico methods with the aim to predict colonic absorption.

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