The use of gastrointestinal intubation studies for controlled release development

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Regional gastrointestinal absorption and toleration studies to support controlled release (CR) development have been limited to naso-intubation and telemetric capsule methods.
- The use of intubation for this purpose has been individually reported for single CR candidates; viz rivastigmine, and UK-338,003.
- Nyberg *et al.* have recently published a report on 13 Phase I studies using a telemetric capsule.

WHAT THIS STUDY ADDS

- When the highest ethical standards were employed in the recruitment, treatment, compensation and follow-up care given to all participants, subjects were easily recruited, and clinical intubation studies were uncomplicated to complete.
- From the eight Phase I studies, improved methods are proposed for unequivocal interpretation of the results.
- When required, clinical intubation studies are uniquely designed to aid in the CR development of drug candidates.

AIMS

This review describes clinical results of gastrointestinal intubation studies of eight controlled release (CR) candidates under development during the 1990s and offers suggestions for determining why, when and how to conduct human intubation studies.

METHODS

Experience with the administration of the following eight compounds to various regions of the gastrointestinal tract is described: CJ-13,610, CP-195,543, CP-331,684, CP-409,092, CP-424,391, azithromycin, sertraline, and trovafloxacin. Also included are human pharmacokinetic studies with prototype CR dosage forms for CJ-13,610 and CP-424,391.

RESULTS

Intubation studies, while appearing invasive, are safe and not unpleasant procedures that have been found to be valuable in the development of CR formulations.

CONCLUSIONS

The following recommendations are made regarding intubation studies: (i) no intubation study is recommended for compounds with high permeability, since these compounds are likely to be well absorbed from the colon; (ii) compounds with moderate permeability may require an intubation study if the dog colon and *in silico* models predict a marginally acceptable CR concentration–time profile; (iii) use a dose that approximates 1 h of the intended CR delivery rate; (iv) use the smallest volume possible; (v) define and record tubing placement; (vi) use a thermodynamically stable solution or/and suspension.

Introduction

Controlled release (CR) formulations are designed to deliver and maintain the active plasma concentration above some minimum value for a specified length of time,

with a dosing regimen that is less frequent than the immediate release (IR) formulation. The primary purpose of gastrointestinal (GI) intubation studies is to support CR formulation development by determining whether the permeability of the CR candidate [active pharmaceutical

Figure 1

Compound structures

ingredient (API)] in the distal GI tract is adequate to achieve the desired concentration–time profile. Additionally, the safety and toleration of distal intestinal delivery can be definitively accessed. The interpretation of this colon permeability may depend on dose, solubility and residence in the GI tract. The preferred CR candidate must dissolve and be absorbed before intestinal propulsion moves it out of the region (i.e. excretion). Thus a permeability that may be acceptable for a small dose may be too slow for a larger dose. Likewise, a permeability determined from a solution may be acceptable for a CR dosage form that delivers a solution, unless the delivered solution precipitates in the GI tract and slowly re-dissolves. Finally, although the solubility of a weak base may be adequate after ingestion of an IR formulation—due to the large volume of fluids, bile acids and pH in stomach and duodenum—the low volume of fluids, lack of bile acids and higher pH may not be sufficient for its solubility when delivered by a CR formulation in the colon. The likely influence of these factors on CR may one day be predicted from a combination of *in vitro* and *in silico* models.

The Biopharmaceutics Classification Scheme (BCS) adopted by the Food and Drug Administration is an attempt to simply classify compounds based on (i) minimal

solubility throughout the GI tract and (ii) the fraction absorbed in humans following an IR dosage form. A high permeability (HP) designation applies to a compound with >90% of the IR dose absorbed [1].

While a CR candidate designated as low permeability (LP) may exhibit a small value for k_a which is too slow for an extended-duration CR dosage form, a short-duration (e.g. $T_{80\%} \leq 8$ h) CR dosage form may be acceptable. By ‘acceptable’ is meant that the CR dosage form would exhibit good bioavailability relative to the IR dosage form (RBA). *In silico* modelling can be a useful tool to predict the RBA likely for a CR formulation. Accurate absorption data from carefully designed intubation studies lead to more accurate pharmacokinetic simulations, which in turn lead to more efficient and optimized CR formulation development.

During the 1990s, the following compounds were studied in humans by our laboratories using a variety of GI intubation protocols: CJ-13,610, CP-195,543, CP-331,684, CP-409,092, CP-424,391, azithromycin, sertraline and trovafloxacin (for structures, see Figure 1; for selected biopharmaceutical properties, see Table 1). Throughout this period of time, the same compounds were also evaluated in the dog colon model [2]. This study summarizes the intubation results and provides guidelines for future intubation

Table 1

Selected biopharmaceutical characteristics

Compound	MW* (dalton)	Solubility† (mg ml ⁻¹)	Log P‡	pK _a	k _a (min ⁻¹)	RBA§ (%)
CJ-13,610	490	<0.1	2.9	7.3	0.003, 0.015¶	47
CP-195,543	428	<0.1	4.7	2.9	0.035 ± 0.008	–
CP-331,684	331	2.5	–2.2	3.9, 5.5, 8.1	0.006 ± 0.017	27
CP-409,092	334	0.9	1.8	9.1	0.014 ± 0.003	69 (33)
CP-424,391	656	1.7	2.0	7.7	0.011 ± 0.018	93
azithromycin	749	5	–2	–9 (base)	<0.003	15
sertraline HCl	343	≈0.18	4.9	9.1	0.02	9.3 (4.4)
trovafloxacin	513	<0.1	1.9	5.6, 9.5	ND††	25 (1.5)

*Molecular weight. †Aqueous thermodynamic, at pH 6.5, RT. ‡Octanol/pH 7 water. §From intravenous and oral clinical studies. ¶Value of k_a 0.003 min⁻¹ calculated at 5 µg ml⁻¹; k_a 0.015 min⁻¹ calculated at 10 µg ml⁻¹ concentrations, respectively. CJ-13,610 is a substrate for intestinal efflux (personal communication: Rob Polzer, Pfizer, Inc., Ann Arbor, MI, USA). ††ND, not determined. Trovafloxacin has a mean absolute bioavailability in humans of >90% [18].

studies designed in support of CR candidate development. This study is a tool for assigning value to human intubation studies and includes recommendations and an algorithm for when to consider an intubation study.

Methods

Materials

CJ-13,670 mesylate¹ (a 5-lipoxygenase inhibitor with oral activity in animal models of inflammation and airway obstruction [3]), CP-195,543² (a LTB₄ antagonist for the treatment of inflammation), CP-331,684³ (a β₃-agonist for the treatment of obesity), CP-409,092⁴ (a GABA_A partial agonist developed for the treatment of anxiety), CP-424,391 tartrate⁵ (a growth hormone secretagogue for the treatment of frailty [4], [5]), azithromycin, sertraline, and trovafloxacin were synthesized at Pfizer, Inc. (Groton, CT, USA).

Solubility determination

The compounds examined in this study are weak bases or zwitterions, and their pH-solubility profiles were determined using classical methods [6].

Permeability determination

Depending on the availability of data, the permeability classification of a compound may be supported by clinical

studies {e.g. absolute bioavailability studies [i.e. intravenous (i.v.) and oral], or mass balance studies}. When clinical data are not available, a preliminary estimate of a compound's permeability classification can be determined from *in vitro* models (e.g. cell monolayer) and rat intestinal models. In our lab, the rat single-pass intestinal perfusion model was used preclinically to preliminarily classify compounds [7, 8]

Assays

Analysis of CJ-13,610 [3], CP-195,543 [9], CP-331,684 [8], CP-409,092 [10], CP-424,391 [11], azithromycin [12], sertraline [13] and trovafloxacin [14] in plasma or serum was completed using published assays.

Human intubation studies

The Institutional Review Boards (azithromycin intubation: Medical and Technical Research Associates, Clinical Research Center, Boston, MA, USA; all others: Houston Institute for Clinical Research, Houston, TX, USA), in accordance with all applicable regulations, approved the final protocol, advertisement for the recruitment of subjects, pay scale and distribution and informed consent text. The pay scale did not include any 'balloon' payment. Instead, subjects were paid according to the minimum wage, over the length of time confined to the clinical unit, and adjusted nominally for each procedure (e.g. each sample collection). The studies were conducted in compliance with the ethical principles originating in or derived from the Declaration of Helsinki and in compliance with all International Conference on Harmonization Good Clinical Practice Guidelines. In addition, all local regulatory requirements were followed, in particular those affording greater protection to the safety of trial participants.

Although these eight studies were completed over a period of 10 years, and therefore no two studies were completely alike, some typical protocol details are provided. Additional details are summarized in Table 2.

¹CJ-13,670: 4-[3-{4-(2-Methylimidazol-1-yl)phenylthio}]phenyl-3,4,5,6-tetrahydro-2H-pyran-4-carboxamide methanesulfonate.

²CP-195,543: 2-(3-Benzyl-4-hydroxy-chroman-7-yl)-4-trifluoromethylbenzoic acid.

³CP-331,684: (4-(2-(2-(6-aminopyridin-3-yl)-2(R)-hydroxyethylamino)-ethoxy)-phenyl)-acetic acid.

⁴CP-409,092: 4-Oxo-4,5,6,7-tetrahydro-1H-indole-3-carboxylic acid (4-methylaminomethyl-phenyl)-amide.

⁵CP-424,391: 2-amino-N-[2-(3aR-benzyl-2-methyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridin-5-yl)-1R-benzoyloxymethyl-2-oxoethyl]-isobutyramide L-tartrate.

Table 2

Details and results of the clinical intubation protocols

Compound	Dose (mg)	n	Formulation	Route	Region	Apparatus*	Dosing volume (ml)	Rinse volume (ml)	Cmax (µg ml ⁻¹)	T _{max} (h)	AUCinf (µg h ⁻¹ ml ⁻¹)	RBA (%)
CJ-13,610	300	12	tablets	oral	–	–	240	–	1.6 (45)	2.6 (0.8)	14.5 (49)	100
	300	12	solution	5 min infusion	jejunum	M-A	100	40	1.14 (45)	1.7 (1)	13.5 (50)	93
	300	12	solution	5 min infusion	AC	W-C	100	40	0.212 (57)	7.5 (4.7)	5.00 (55)	34
CP-195,543	100	12	capsule	oral	–	–	240	–	0.003 (27)	1.9 (1.1)	0.013 (19)	100
	100	12	solution	5 min infusion	ICJ	M-A	100	40	0.003 (59)	0.7 (0.3)	0.012 (48)	100 (52)
	100	12	solution	5 min infusion	AC	W-C	100	40	0.002 (58)	1.5 (1.3)	0.011 (55)	93 (44)
CP-331,684	200	6	solution	oral	–	–	240	–	0.380 (100)	2.8 (1.5)	2.00 (106)	100
	200	6	solution	5 min infusion	ICJ	W-C	100	40	0.450 (63)	2.4 (0.7)	2.15 (56)	145 (118)
	200	6	solution	5 min infusion	AC	W-C	100	40	0.060 (69)	0.6 (0.2)	0.420 (41)	27 (17)
CP-409,092	100	6	solution	oral	–	–	240	–	20.1 (21)	1.2 (0.8)	78.0 (17)	100
	100	6	solution	5 min infusion	duodenum	W-C	60	180	46.7 (30)	0.5 (0)	109 (19)	141 (27)
	100	6	solution	5 min infusion	AC	W-C	60	40	20.6 (52)	0.5 (0)	81.7 (46)	126 (66)
CP-424,391	10	6	solution	oral	–	–	240	–	9.2 (30)	1.3 (0.7)	46.9 (43)	100
	10	6	solution	5 min infusion	AC	W-C	100	40	10.6 (51)	1.0 (0.4)	55.6 (54)	139 (92)
Azithromycin	500	6	solution	oral	–	–	240	–	0.35 (27)	1.9 (0.9)	3.4 (34)	43 (100)
	500	12	solution	5 min infusion	ICJ	nasoenteric tube	50	15–30	0.41 (105)	0.7 (0.5)	2.75 (48)	81
	500	6	solution	bolus	rectal	enema	12.5	0	0.11 (53)	1.1 (0.7)	0.69 (70)	20
Sertraline	200	12	tablets	oral	–	–	240	–	0.042 (41)	6.9 (2.3)	1.210 (41)	100
	200	6	solution	5 min infusion	ICJ	M-A	100	40	0.027 (49)	5 (1.1)	1.180 (67)	89
	200	5	solution	5 min infusion	TC	M-A	100	40	0.011 (49)	4.4 (1.7)	0.232 (70)	20
Trovafoxacin	300	16	capsule	oral	–	–	240	–	3.46 (16)	1.9 (1.1)	43.3 (22)	100
	300	16	solution	5 min infusion	ICJ	W-C	50	40	0.5 (57)	4.5 (3.0)	9.6 (55)	22
	300	16	solution	5 min infusion	AC	W-C	50	40	0.39 (43)	9.7 (2.2)	8.97 (61)	21

For Cmax and AUC, the geometric mean (%CV) are shown; for T_{max} and RBA, the average (SD) are shown.
 *M-A, Miller-Abbott double-lumen intestinal tube; W-C, Wilson-Cook (Marcon) Colon Decompression apparatus.

Healthy, male subjects aged 18–45 years, inclusive, weighing no more than 200 pounds (61–91 kg) and within 15% of their weight range for age, gender, height and frame as established in the '1983 Metropolitan Life Insurance Height and Weight Tables' [15], were considered for these studies. The investigator explained the nature, purpose, and risks of the study to each subject. Each subject was informed that he could withdraw from the study at any time and for any reason. Each subject was given sufficient time to consider the implications of the study before deciding whether to participate. Subjects who chose to participate signed an informed consent document.

Subjects were off all prescription drug therapy, over-the-counter drugs or drugs of abuse for at least 2 weeks prior to participation in the study and off any investigational drug for at least 4 weeks. Subjects with any condition possibly affecting drug absorption were excluded. The exclusion criteria also included subjects with evidence or history of clinically significant allergic (except for untreated, asymptomatic, seasonal allergies at time of dosing), haematological, renal, endocrine, pulmonary, GI, cardiovascular, hepatic, psychiatric, or neurological disease; subjects with history of drug or alcohol dependence or drug allergies; subjects intending to donate blood or blood components while receiving experimental drug or within 1 month of the completion of the study; subjects with supine blood pressure at screening >140/90 mmHg, or a heart rate on the screening ECG >100 bpm; subjects with a history or evidence of habitual tobacco or nicotine use within the 3 months preceding screening.

Subjects were admitted to the clinical research unit the night prior to study initiation. They were confined to the clinical research unit under continuous medical or paramedical observation for at least 12 h prior to and for at least 24 h following compound administration. When the compound was administered by nasogastric infusion, volunteers were confined to the clinical research facility for at least 48 h prior to dosing. Subjects were fasted for 12 h prior to drug administration and fasted a further 4 h following compound administration. Subjects were to abstain from ingestion of alcohol, drugs or caffeine for at least 3 days prior to and throughout each admission. Subjects were fed a repetitive, standardized, low-fat, caffeine-free diet at each confinement. Placement of the tube was confirmed by fluoroscopy. A total volume of 240 ml water accompanied each orally administered formulation (exceptions are noted). Subjects were required to refrain from lying down, eating, or drinking caffeinated beverages during the first 4 h after dosing. Subjects were restricted from walking or standing unattended for at least 6 h post dose. A wash-out period of at least 7 days separated each administration.

A final physical examination (including vital signs) was completed when a subject completed the study. If a subject dropped out or was terminated from the study by the investigator, a final physical examination was also provided.

Prior to administration of API to the duodenum and jejunum, subjects received a local anesthetic to the throat to minimize gagging. In addition, at the discretion of the investigator, a sedative of midazolam was administered as premedication for endoscopy. Subjects were placed in the lateral position; a fibre optic endoscope was inserted into the mouth and positioned in the duodenum or proximal jejunum. A Wilson-Cook Colon Decompression catheter (CDSM-8.5, 8.5 Fr, 350 cm long, disposable; Wilson-Cook Medical Inc., Winston-Salem, NC, USA) was guided through the endoscope and the API was infused through the catheter into the duodenum or proximal jejunum. Following drug administration, the catheter was clamped and removed within 2 h. If the physician was unable to pass the endoscope into the duodenum or proximal jejunum, then an alternative method was used. The subject was asked to swallow a lubricated enteric Miller Abbott tube (Double Lumen Intestinal 14 Fr, 320 cm long; Davol Inc., Cranston, RI, USA). This tube was fitted with a weighted end to promote delivery of the tube to the duodenum or proximal jejunum.

For administration to the distal portion of the small intestine in the region of the ileal-caecal junction (ICJ), a single-lumen, 4.5 m long nasoenteric tube was used. The tube terminated in a side port to be used for drug administration. A mercury weight was affixed to the end of the tube to promote delivery of the tube to the ICJ. Following the infusion of API or saline, the tube was rinsed with 12–30 ml of saline.

For administration of API to the ascending colon (AC) or rectum, subjects were on clear liquids starting 24 h prior to the scheduled procedure. On the evening prior to dosing, the subjects received a 240-ml dose of magnesium citrate and two enemas. Prior to the colonoscopy, the subject may opt to receive a sedative of midazolam intravenously to make the procedure more comfortable. After the sedative, the subject was positioned in the lateral position and a fibre optic colonoscope was introduced through the rectum to identify the AC. A Wilson-Cook (Marcon) Colon Decompression catheter was guided into the AC just proximal to the hepatic flexure. Following drug administration, the catheter was clamped and removed within 2 h. Rectal administration of the API in a 12.5-ml volume was completed in a study, following an 8-h fast. API administration was 7.5–10 cm into the rectum using an apparatus similar to a Fleets™ enema [16].

All of these procedures were well tolerated by subjects. No subject dropped out of a study because of the procedure. Rather, occasional drop-out occurred because of the side-effect of the API (e.g. nausea, headache).

API administration

Three 100-mg CJ-13,610 tablets were orally administered to each of 12 fasted, healthy male volunteers in this cross-over study. The same dose—as a 3 mg ml⁻¹ solution—was infused into the duodenum, jejunum and AC over a 5-min

period (total volume 100 ml) and followed with a 40-ml rinse. The choice of AC for the colon delivery region was intended to mimic an intermediate-duration CR formulation.

A dose of 100 mg CP-195,543 was administered orally to each of 12 fasted, healthy male subjects in the crossover study. The same dose and total volume (100 ml infused over 5 min, followed by a 40-ml rinse) was administered into the duodenum and AC.

A dose of 200 mg CP-331,684 was administered orally to six healthy male subjects in a three-way crossover study. The same dose was administered into the proximal jejunum and into the AC (100 ml, followed by two 20-ml rinses).

A dose of 100 mg CP-409,092 was administered orally to six healthy male volunteers in a three-way crossover study. The same dose was administered into the duodenum as a 60-ml volume infused over 5 min, followed by a 180-ml rinse, and into the AC, but with only a 40-ml rinse of the intubation tube.

CP-424,391 was administered orally and into the AC of six healthy male volunteers in a crossover fashion. The 10-mg dose was dissolved in 100 ml water and infused over a 5-min period. The intubation tube was then rinsed with an additional 40 ml water.

A 500-mg dose of azithromycin was administered orally to 12 fasted healthy male volunteers as two 250-mg capsules. The same dose was delivered in a 50-ml volume to the ICJ via a nasointestinal tube. The choice of ICJ was made in an attempt to find the limit of small intestinal absorption for this API. Rectal administration of this dose in a 12.5-ml volume was completed in a separate study [16]. Both groups also received an i.v. infusion of azithromycin. RBA to the oral dose in the first group was possible by correcting for i.v. clearance in each group.

A dose of 200 mg sertraline was orally administered to 12 fasted, healthy male volunteers in a crossover study. The same dose was also delivered by intubation into the ICJ and transverse colon (TC). The TC was selected for the evaluation of an extended-duration CR formulation. All intubation doses were administered in 100 ml (pH ~4) over 5 min, with a 40-ml rinse. The oral dose was administered to the entire group, which was then equally divided into the two subgroups.

A 300-mg dose of trovafloxacin was orally administered to a group of 16 healthy male and female subjects in a crossover study. The same dose was delivered by intubation into the duodenum and the ICJ and AC. All intubation doses were infused in a 50-ml volume (pH ~4) over 5 min, followed by a 40-ml rinse.

Controlled release formulations

Although most of the above compounds were formulated into CR dosage forms and administered to humans, only data collected for CR dosage forms for CJ-13610 and CP-424,391 are included in this study. CR formulations

Figure 2

Average in vitro dissolution profiles for CJ-13,610 release from the following controlled release (CR) formulations: short (SMT) and long (LMT) duration matrix tablets, AMT [SGN (no enzymes), 50 rpm, USP apparatus 2 (paddles)]. SMT (---◆---); LMT (---■---); AMT (—□—)

were made using hydrophilic matrix tablet or osmotically-driven asymmetric membrane technology (AMT) [17].

Compounds with good solubility were typical candidates for AMT technology. Briefly, the core, which consisted of the active drug, and tableting excipients, was compressed into a tablet and coated with an asymmetric coating. The asymmetric coating controlled the release of the active drug. The rate of release in matrix tablets was moderated by polymeric excipients (e.g. hydroxypropyl or hydroxypropylmethyl cellulose). Dissolution profiles of the CR formulations were obtained using US Pharmacopeia apparatus 2 with paddles rotating at 50 rpm. The dissolution profiles showing the release of CJ-13,610 from matrix tablet and AMT formulations are shown in Figure 2. The dissolution profiles showing the release of CP-424,391 from AMT formulations are shown in Figure 3.

Results

Solution formulations

CJ-13,610 (low solubility/high permeability) Figure 4 shows the solubility profile for CJ-13,610 in buffers of various pH. Also shown in Figure 4 is the approximate concentration of the intubation solution. Note that this is represented by a dotted line with a slope = 0 and extends over only the pH range of the region of intestine in which the intubation solution was administered. A compound with its 'intubation solution concentration line' below or close to the pH-solubility profile would be likely to remain in solution, even after delivery to the intestine, since any pH adjustment by the intestine would not result in supersaturating the intubation solution. In the case of CJ-13,610, however, the intubation solution concentration line was above the pH-solubility profile. The solution of CJ-13,610 was probably supersaturated after administration to the intestine, when the pH was brought close to the physiological pH. Furthermore, since

Figure 3

Average in vitro dissolution profile showing the release of CP-424,391 from a 12-h asymmetric membrane technology (AMT) formulation [SGN (no enzymes), 50 rpm; SIN (no enzymes), 50–150 rpm; USP apparatus 2]. SGN-50 rpm (—x—); SIN-50 rpm (—◆—); SIN-100 rpm (—◆—); SIN-150 rpm (—■—)

CJ-13,610 was supersaturated in the intubation formulation at colon pH, by three orders of magnitude, it probably precipitated. The average ($\pm$ SD) plasma CJ-13,610 concentrations following intubation to the jejunum and AC are shown in Figure 5. CJ-13,610 was well absorbed in the small intestine, where the pH, available water and bile acids were apparently sufficient to maintain solubility until absorption was completed (Table 2). However, the bioavailability after delivery to the AC compared with oral administration was incomplete (RBA = 34%). The lower solubility at colon pH, reduced volume of water and lack of surfactants increased the likelihood for precipitation and consequent incomplete absorption of CJ-13,610 in the colon.

CJ-13,610 was also shown *in vitro* in Caco-2 cell monolayers to be highly permeable (high apical-to-basolateral, A→B flux) with a slight asymmetry in the B→A vs. A→B values with an efflux ratio of 2.1.⁶ This slight asymmetry probably reflects the magnitude of efflux in the colon. However, the high absorptive flux (A→B) diminishes the likelihood that the lower RBA observed after delivery to the AC was due to a large efflux.

CP-195,543 (low solubility/high permeability) The solubility profile for this weak acid classified CP-195,543 as low solubility (LS), according to the definition of the BCS. However, since the 1 mg ml⁻¹ CP-195,543 concentration of the intubation solution is similar to its solubility at colon pH, it is unlikely that CP-195,543 precipitated from the intubation solution.

The average ($\pm$ SD) plasma CP-195,543 concentrations after oral administration and following intubation to the ICJ and AC were similar (data not shown). The RBA of

CP-195,543 administered to the AC was (mean $\pm$ SD) 93 $\pm$ 44% (Table 2). Since the RBA was high and the $T_{\max}$ and $C_{\max}$ values were similar for colonic and oral routes, absorption was probably rapid.

CP-331,684 (high solubility/low permeability) The human intubation solution concentration of CP-331,684 (2 mg ml⁻¹) was within its solubility (2.5 mg ml⁻¹). The average ($\pm$ SD) plasma CP-331,684 concentrations following intubation to the AC were significantly less than after oral administration (data not shown). The RBA of CP-331,684 administered to the AC was (mean $\pm$ SD) 27 $\pm$ 17% (Table 2). Since the RBA was low, and solubility was not limiting, permeability was likely too low for complete absorption of CP-331,684.

CP-409,092 (high solubility/high permeability) The concentrations of CP-409,092 in the oral solution (= 0.4 mg ml⁻¹) and in the intubation solution (=1.7 mg ml⁻¹) were in the range of its solubility at intestinal pH (0.9 mg ml⁻¹). Therefore, CP-409,092 probably remained in solution after oral administration, and after intubation into the duodenum and colon.

The average ($\pm$ SD) plasma CP-409,092 concentrations following oral administration and intubation were similar (data not shown). The RBA of CP-409,092 administered to the AC was (mean $\pm$ SD) 126 $\pm$ 66% (Table 2). Since the RBA was high and the $T_{\max}$ was equally short for colonic and oral routes, absorption was probably rapid.

CP-424,391 (high solubility/high permeability) CP-424,391 was found to be an extremely soluble compound. At pH values <7 it was impossible to saturate the solution and still maintain adequate pH control. The solubility at pH 7 was found to be 5 mg ml⁻¹. The free base (intrinsic, S_0) solubility, found at pH 10.7, was 0.65 mg ml⁻¹. Knowing the intrinsic solubility and the pK_a , a theoretical pH-solubility profile was constructed using the following equation, which relates total solubility of a weak base (S_T) with the aforementioned parameters:

$$S_T = S_0(1 + 10^{(pK_a - pH)})$$

The concentration of CP-424,391 in the intubation solution (=0.1 mg ml⁻¹) was well below the minimum solubility of CP-424,391 in the GI tract. Therefore, CP-424,391 probably remained in solution after intubation into the colon.

The average $\pm$ SD plasma CP-424,391 concentrations following administration of 10 mg CP-424,391 orally and by intubation to the AC are shown in Figure 6, and the pharmacokinetic parameters are summarized in Table 2. The RBA for CP-424,391 administered to the AC was complete (mean $\pm$ SD): 139 $\pm$ 92%. Since the RBA was high, the $C_{\max}$ values comparable and the $T_{\max}$ equally short for colonic and oral routes, absorption was rapid.

Azithromycin (high solubility/low permeability) In two separate studies, azithromycin was administered to the ICJ

⁶RM Shepard, Pfizer, Inc., Groton, CT, USA.

Figure 4

Solubility profiles of compounds in this study, showing the approximate range of gastrointestinal pH and concentration of the intubation solution (mg ml^{-1}). (A) CJ-13,610, (B) CP-195,543, (C) CP-331,684, (D) CP-409,092, (E) CP-424,391, (F) azithromycin, (G) sertraline, (H) trovafloxacin

and the rectum. The intubation and rectally administered solutions consisted of azithromycin concentrations of 10 and 40 mg ml^{-1} . The absolute bioavailability (F) of azithromycin administered to the ICJ and rectum was determined from intravenously administered azithromycin in both studies. As shown in Table 2, T_{max} were similar for oral (1.9 h) and rectal administration (1.1 h). C_{max} and $\text{AUC}_{0-\infty}$ however, reflected a lower bioavailability as the administration progressed distally: the bioavailability was 43% (oral), 35% (ICJ) and 8.6% (rectal). RBA was 81% at the ICJ, but only 20% after rectal administration. The data suggest that the azithromycin permeability would be adequate in the small intestine, but very low in the colon.

Sertraline (low solubility/high permeability) The sertraline k_a calculated from i.v. and oral clinical studies was 0.02 min^{-1} (data not shown); however, sertraline has been shown to be a substrate for active transport in Caco-2 (personal communication J. Bennett, Pfizer, Ltd., Sandwich, UK). When active transport is demonstrated for a CR candidate, reasonable efforts should be made to determine the magnitude of the passive component. It is the passive component of absorption that usually predominates in the large intestine. In the small intestine, the facilitated transport was probably the predominant component for sertraline, and therefore a low RBA for sertraline administered to the colon was expected.

Figure 5

Plasma CJ-13,610 concentrations following oral administration and intubation to the jejunum and ascending colon (AC). AC (—●—); Jejunum (—Δ—); Oral (—x—)

Figure 6

Plasma CP-424,391 concentrations following oral administration and intubation to the ascending colon (AC). AC (—■—); Oral (—●—)

The solubility plot for sertraline and the intestinal pH-intubation solution concentration suggest that the intubation solution was supersaturated in the ICJ and colon. Soon after the solution was administered into the colon, the solution was probably neutralized; in such an environment where pH approaches 7.5, sertraline might have precipitated.

The C_{max} from the intubation studies as shown in Table 2 were largest for oral and smallest for TC; after ICJ administration, C_{max} and $AUC_{0-\infty}$ values fell between the values for TC and oral routes of administration. The T_{max} of sertraline was 5 and 4.4 h in the ICJ and TC, suggesting that the absorption of sertraline in these regions was not rapid. The RBA of sertraline administered to the ICJ was ~90%, whereas it was only ~20% in the TC. The low RBA in the TC was probably due to a combination of specific regional differences in permeability, and precipitation. Therefore, an intermediate-duration CR formulation may be shown to have an acceptable RBA, whereas an extended-duration CR formulation would not.

Trovafloracin (low solubility/high permeability) Trovafloracin has a mean absolute bioavailability in humans of

Figure 7

Average plasma CJ-13610 concentrations in human volunteers following administration of 300 mg CJ-13610 in the following formulations: IR, immediate release, short (SMT) and long (LMT) duration matrix tablet, asymmetric membrane technology (AMT). SMT (—◆—); IR (—x—); LMT (—■—); AMT (—▲—)

>90% [18]. The solubility profile for trovafloracin reflects the significant common ion effect with physiological amounts of NaCl (Figure 4). Also shown in Figure 4 are the approximate regional pH of the small and large intestine and the concentration of the intubation solution. In this case, the pH-solubility curve for the free base and the intestinal pH-intubation solution concentration curves suggest that precipitation may be likely following delivery of the solution in the intestine.

As shown in Table 2, the RBA was only ~20% after intubation to the ICJ and AC. Additionally, the T_{max} was prolonged after administration to the distal bowel: 1.9 h (oral) vs. 4.5 h (ICJ) and 9.7 h (AC). As stated earlier, a compound such as trovafloracin, with a high absolute bioavailability, should be well absorbed from the colon. It is likely that the low RBA for trovafloracin in this study was due to precipitation of the drug from the intubation solution. Therefore, a CR formulation that delivered trovafloracin as a solution and prevented its precipitation may be successful.

Controlled release formulations

CJ-13610 matrix tablet and AMT The results from a clinical study of several 300-mg CJ-13610 CR formulations are shown in Figure 7. The short-duration matrix tablet (SDM) had a concentration-time profile that was similar to the IR formulation (RBA ~120%). However, both the 6-h matrix tablet (long-duration matrix tablet (LDM), RBA ~78%) and the 12 h AMT (RBA ~62%) showed profiles consistent with a reduced absorption beyond 4 h.

CP-424,391 AMT Figure 8 shows a comparison of the average CP-424,391 plasma concentrations following oral administration of a 10-mg dose as an aqueous solution and a 12-h AMT formulation to eight healthy, elderly male fasted subjects. The RBA of the 12-h AMT was 86%. Because the permeability and solubility of CP-424,391 were high, it

Figure 8

Average plasma CP-424,391 concentrations in human volunteers, following administration of 10 mg as an oral solution, 12-h asymmetric membrane technology (AMT) formulation. Oral Solution (—◆—); 12 hr AMT (—■—)

was not surprising that the exposure was high following administration of the 12 h AMT formulation.

Discussion

Despite numerous descriptions of intubation studies in the literature by various groups [19–24], there remains a common misconception that these studies are extremely unpleasant. The implication is that only by unethical means could recruitment be accomplished and study completion ensured [25, 26]. However, the highest ethical standards were employed in the recruitment, treatment, compensation and follow-up care given to all participants. Alternative methods to intubation are reviewed in the literature and will not be discussed here [27]. The purpose of this study is not to compare and contrast those methods or alternative intubation techniques [28–30] with those included in this manuscript. Rather by review of our experience, this study shows that some method to estimate a candidate's bioavailability after administration to the colon is helpful in the development for CR formulations. The compound's biopharmaceutical properties can in some cases predict the colon RBA of CR candidates. This discussion is presented in three parts: (i) impact of the intubation studies on the CR formulation development programme, (ii) recommendations based on the experience from the intubation studies, and (iii) correlation of a recently published dog colon model with the human intubation studies.

Impact of the intubation studies on the CR formulation development programme

For compounds with HP/high solubility characteristics and good absorption after intubation to the colon, exposure from the CR formulation is good (e.g. the RBA for the CP-424,391 12-h AMT was ~90%). In contrast, for those compounds with poor absorption after intuba-

tion to the colon, exposure from the CR formulation was poor (e.g. the RBA for the CJ-13,610 12-h LDM was ~60%). Intubation studies can therefore guide the CR formulation development programme by identifying the candidates with low colon absorption. For these candidates, either the $C_{\max}$ and/or $C_{\min}$ targets are reached with formulations of shorter duration, or CR may not be a viable option.

Recommendations based on the experience from the intubation studies

Following oral administration to fasted subjects, CR formulations with release duration of ≥ 8 h will deliver most of the total dose in the colon. Computer simulations can provide useful estimates of plasma concentration vs. time profiles for various durations of CR formulations, but the accuracy of these simulations is only as good as the parameter estimates used, and it is accurate estimates of the colon absorption parameters that are provided by intubation studies. In some cases an intubation study can be avoided without sacrificing the quality of predictions required for CR candidate development. Based on this review, the following recommendations are proposed for intubation studies in support of CR candidate development:

1. If an API has good permeability, an intubation study is probably not needed.

An API with this designation has a permeability comparable to metoprolol. Unless the compound exhibits unusual oral pharmacokinetics (e.g. saturable first-pass hepatic extraction, double peaking, active intestinal transport/secretion, degradation by colon contents) it is very likely that the CR formulation will have a high RBA. This statement is true even as in the case of CJ-13,610—where a slight asymmetry in B→A vs. A→B ratios suggested intestinal efflux—if the absorptive flux (A→B) is high at concentrations likely to be present in the colon.

2. An API with moderate permeability may require an intubation study if the dog colon and *in silico* models predict a marginally acceptable CR concentration–time profile.

For moderately permeable API, the purpose of the intubation is to improve the accuracy of *in silico* predictions. If the permeability is quite low, the prediction would be unequivocal—an unacceptable CR concentration–time profile. The results of numerous simulations seem to support the notion that, if the colon RBA in the dog intubation model is $>30\%$, a clinical intubation study is not needed. That is, the colon RBA in humans is also likely to be $>30\%$, and the predicted CR concentration–time profile is not limited to permeability. For CR candidates with RBA in the 10–30% range, simulation accuracy becomes more important, and there is less tolerance of error in the allometric scaling of pharmacokinetic parameters. These

factors are best evaluated in a pharmacokinetic simulation. If pharmacokinetic simulations (based on dog colon RBA) predict the need for too large a dose, then a confirmatory human intubation study is recommended.

3. The intubation dose should approximate the dose delivered by the CR formulation over a period of 1 h.

For example, if the expected IR formulation dose is 100 mg, but the CR formulation is designed to deliver 10 mg h⁻¹, then a 10–20-mg dose should be delivered via intubation. Such a dose is a better reflection of that expected from the CR formulation. If the subtle effects of hepatic extraction, intestinal metabolism, active absorption or secretion and the like are observed after the intubation at this dose, this observation has relevance to the CR programme. Obviously, there is a lower limit on dose based on the plasma assay LLOQ. Simulations may help select the proper dose. For example, simulations may predict little or no detectable plasma concentrations for a dose equivalent to 1 h of CR delivery, but reasonable levels for a dose equivalent to 2 h of CR delivery.

4. Intubation volume should be as small as practicable.

A small volume of the delivered solution is more likely to remain in close proximity to the intubation site. Provided the site is anatomically similar in all subjects, this will decrease the variability in the data. The ‘aliquot’ administration method should be used whenever practicable. This method does not require a tubing rinse: if the ‘dead volume’ in the intubation tube is X ml, and the desired delivery volume is Y ml, then the total amount introduced into the intubation tube is simply the sum of the two.

5. The tubing placement should be narrowly defined and recorded.

Incomplete recording of tubing placement may result in the appearance of high variability, whereas in fact the variability is due to regional differences in permeability. Warner [31] reported this for sumatriptan in humans. The placement of the tubing should be verified (e.g. fluoroscopy or scintigraphy) and recorded.

6. Intubation solution composition should be optimized to maintain drug in solution following intubation.

As shown in this review, interpretation of intubation results becomes difficult if drug precipitation is a possibility. The trade-off between keeping volumes small and keeping the drug in solution may require formulation development with solvents or complexation agents. A thermodynamically stable solution is preferred; if analytical limitations prevail, a thermodynamically stable suspension is recommended as an additional intubation.

Figure 9
Correlation between colon RBA in the dog colon model and human intubation studies [2] (with permission from Springer)

Table 3
Relative bioavailability (%): details of data shown in Figure 9, the Human Intubation vs. Dog Colon Model correlation

Compound	Human ICJ*	Human AC/TC†	Dog DC*	Human study References
Atenolol	15		67	[32]
Azithromycin	9		7.6	[16]
Nifedipine	97		66	[33]
Propranolol	>100		76.4	[34]
Sertraline	66	15†	5	this report
Theophylline	100		78	[35]
Trovaflaxacin	15		25	this report
CP-331,684	20		30	this report
CP-409,092	75		69	this report
CP-424,391	91		83	this report
CJ-13,610		35	43	this report

*ICJ, ileal–caecal junction; DC, descending colon. †Sertraline administration to the transverse colon (TC); CJ-13,610 administration to the ascending colon (AC).

Correlation of the dog colon model with the human intubation studies
The dog colon model is a simple, resource- and time-sparing model for predicting human colonic absorption. In this model, the test formulation is administered as a solution to the colon, 30 cm from the anal sphincter with a lubricated Schott Model VFS-5 flexible endoscope. The RBA was shown to predict the likelihood of acceptable human colon absorption for CR formulation candidates [2].
The results of the colon RBA from the above human intubation studies were correlated with the dog colon RBA [2] in Figure 9 and Table 3. From this review of human intubation studies it is clear that the dog colon model can be helpful in predicting the outcome of human GI intubation studies. Note that in retrospect, some of the formulations examined in the clinic were not optimal, and may have

resulted in precipitation. In hindsight, these formulations should be avoided, or evaluated along with a solution.

Competing interests

None declared.

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