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RESEARCH PAPER

Characterization of the Ascending Colon Fluids in Ulcerative Colitis

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ABSTRACT

Purpose To characterize the fluid composition in ascending colon of fasted adults with ulcerative colitis in relapse and in remission with a view to predicting variations on dosage form performance in the lower inflamed gut.

Methods Twelve patients participated in a two-phase, cross-over study. Enrolment to the relapse phase (Phase A) and designation of the remission state for the second colonoscopy (Phase B) were based on Clinical Rachmilewicz Index values. Samples were analyzed for pH and buffer capacity immediately upon collection. After ultracentrifugation, osmolality, surface tension, soluble protein, soluble carbohydrates, and the levels of ten bile acids, seven short-chain fatty acids (SCFAs), three long-chain fatty acids, triglycerides, diglycerides, monoglycerides, phosphatidylcholine, and cholesterol were measured.

Results Total SCFAs are significantly decreased in relapse, but pH remains unaffected. Regardless of remission/relapse status, pH and isobutyric acid levels are lower than in healthy adults. Buffer capacity, osmolality, and soluble protein are higher than in healthy adults. Treatment with prednisolone increases the volume of intracolonic contents.

Conclusion Variations in fluid composition of the ascending colon with activity and severity of ulcerative colitis may have an impact on the performance of orally administered products that are targeted to release the therapeutic agent in the colon.

KEY WORDS ascending colon · colonic targeting · lumenal composition · ulcerative colitis

INTRODUCTION

Colonic targeting is often problematic, due to the variable colonic environment both in regard to the lumenal composition and transit times in the colon (1). The situation may be complicated further if inflammation is also present in the region. In Crohn's disease, an increased enteral bile acid loss may occur (due to lower bile acid reabsorption by the inflamed ileum) with subsequent cholegenic diarrhea (2). Both of these changes may affect dosage form and/or drug performance. It has also been observed that, regardless the mechanism to deliver 5-aminosalicylic acid (5-ASA) in the colon, i.e. as a prodrug or in a modified release dosage form, increasing the dose does not result in higher remission rates in patients with ulcerative colitis (3). The lack of clear dose-response relationship may reflect an upper limit to effective 5-ASA doses, above which little therapeutic benefit is observed. Alternatively, it may reflect the "saturation" of a drug delivery system, when doses above a certain levels are no longer delivered efficiently to the colon (3). Evaluation of the second possibility requires adequate knowledge of the luminal environment of patients with ulcerative colitis.

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Due to the substantial residence time in the ascending colon (1,4) and the limited free water volume in the transverse colon (5), the primary region of interest in regard to dosage form performance in the lower gut is the ascending colon.

The objective of the present study was two-fold. The first was to characterize the fluid content of the ascending colon of adults with ulcerative colitis in relapse and in remission on a sequential basis and, if possible, evaluate the effect of treatment type on the luminal contents. To date, apart from luminal pH (6), the physicochemical characteristics and composition of fluids in the ascending colon of adults with ulcerative colitis have not been investigated. Since bioavailability and bioequivalence studies are typically performed in healthy adults, the second objective was to identify differences in the characteristics and composition of fluids in the ascending colon between ulcerative colitis patients and healthy adults (7). To be able to make the second comparison, colon preparation and collection of colonic contents was done as in our recently published work in fasted healthy individuals (7).

MATERIALS AND METHODS

Human Study

The study was conducted in the Red Cross Hospital of Athens after receiving approvals by the Scientific and the Executive Committee of the Hospital (AP 151, 16 March 2007). The study was performed according to the Declarations of Helsinki for biomedical research involving human subjects. It comprised acquisition of colonic contents during colonoscopy and characterization of the fluid in the ascending colon of patients with ulcerative colitis in relapse and in remission on a sequential basis. Relapse and remission states were designated according to the Clinical Rachmilewitz Index [CRI (8)]. Number of stools, blood in stools, clinician's global assessment of symptomatic state, abdominal pain/cramps, temperature due to colitis, extraintestinal manifestations, and laboratory findings are taken into consideration for estimation of the value of CRI (8).

For each patient, the first colonoscopy was performed when the disease was in relapse (Phase A) and the second when disease was in remission (Phase B). Depending on the patient, Phase B was performed 1–3 months after Phase A.

Inclusion Criteria

Active ulcerative colitis (known or suspected) with CRI≥6 for patient in relapse and CRI≤3 for patient in remission, age between 18 and 65 y, and deviation from ideal body weight 20% at most.

Exclusion Criteria

Patient with colonic malignancy, toxic megacolon, CRI>14, and pregnant or lactating woman.

For 3 days prior to each phase, the patient had to abstain from cigarette smoking, alcohol, and over-the-counter medication(s). Patient work-up prior to either phase involved the administration of 10 mg of bisacodyl 50 h prior to colonoscopy plus 10 mg of bisacodyl 44 h prior to each colonoscopy. With this regimen, bisacodyl effects on the intracolonic environment have been shown to be non-significant (7). One patient received an additional 5 mg bisacodyl tablet 30 h prior to each colonoscopy. From the time of first bisacodyl administration until the night prior to each colonoscopy day, patients ate only liquified food (e.g. fish/chicken/rice soups, fruit juices) and white bread *ad libitum*. From the night prior to colonoscopy day until completion of colonoscopy, i.e. for at least 12 h, patients remained fasted. A few minutes prior to colonoscopy, 0.3 mg alphentanyl hydrochloride (0.5 mg/ml, Rapifen®) and 2.0 mg of midazolam (5 mg/ml, Dormicum®) were administered intravenously to induce conscious sedation. In four patients, a total of ~100 mg propofol (Diprivan®) had to be additionally administered by an anaesthiologist for unconcious sedation. In both phases, after placement of the colonoscope, collection of contents from the ascending colon was performed under anaerobic conditions. The collection lasted approximately 10 min. This period was sufficient to remove almost all of the contents from the region (7). After removal of the colonoscope, 2 mg of flumazenil (0.1 mg/ml, Anexate®) was administered as antidote to midazolam.

Fifteen patients gave their informed consent to participate in the study. Two did not return for the second colonoscopy, and one proved to have Crohn's disease. Table I shows the demographics, medications received by the patients during the study period, the values of CRI and the values of Endoscopic Rachmilewicz index (ERI) estimated after colonoscopy for the 12 patients who completed the study (8).

Depending on the patient and the severity of the disease, various therapeutic treatments had been applied (Table I). Assuming that colonoscopies were performed on Day 0, 12 noon, mesalazine had been administered orally as a modified release formulation (2.4–3.2 g/d) and/or rectally (suppositories 0.5–4 g/d; enemas 4 g/d) and last dose prior to colonoscopy was at Day 1, 8 pm or earlier. Prednisolone had been administered orally (10–20 mg/day) or iv (15–50 mg/d) and last dose prior to colonoscopy was Day 0, 8 am or earlier. Last doses of azathioprine (100–150 mg/d,

Table I Demographic Data, Medications Administered, and Clinical and Endoscopic Rachmilewicz Indices for the 12 Patients with Ulcerative Colitis Who Participated in this Study

Gender	Age	BMI[a,b]	Duration of disease at first colonoscopy	Medications received by the patients during the study period[a]	Rachmilewicz Index on colonoscopy day[a]	
					Clinical	Endoscopic
F	52	25.5	12 y	Prednisolone/Mesalazine	11	8
		23.4		Prednisolone/Mesalazine	0	1
M	18	18.9	1 y	Mesalazine	10	8
		20.5		Prednisolone/Mesalazine	2	3
M	61	25.9	50 y	Prednisolone/Mesalazine	14	10
		26.2		Mesalazine	3	3
F	43	26.0	13 y	Mesalazine	9	8
		26.0		Prednisolone/Mesalazine	0	3
M	26	21.9	3 mo	Mesalazine	7	8
		21.9		Mesalazine	0	1
F	34	18.4	3.5 y	Azathioprine/Mesalazine	9	10
		19.1		Azathioprine/Mesalazine	3	3
F	34	18.6	3 mo	Prednisolone/Mesalazine/Pantoprazole	7	8
		18.6		Prednisolone/Mesalazine	1	2
M	57	25.8	20 y	Prednisolone/Azathioprine/Tizanidine/Baclofen	13	12
		23.3		Prednisolone/Azathioprine	3	3
M	37	30.4	15 d	Mesalazine	6	7
		30.4		Mesalazine	0	1
F	22	19.1	15 mo	Mesalazine	6	8
		18.4		Mesalazine	0	1
M	30	24.4	15 d	Mesalazine	7	10
		25.1		Prednisolone/Mesalazine	2	3
M	58	26.8	20 d	Prednisolone/Mesalazine	8	10
		26.8		Mesalazine	2	3

[a] For each subject the upper row refers to relapse and the lower to remission. Details on therapeutic treatments are provided in the text

[b] Normal BMI is 18.5–25

orally), pantoprazole (40 mg/d, orally), tizanidine HCl (6 mg/d, orally), and baclofen (10 mg/d, orally) prior to colonoscopies were Day 1, 8 pm.

To estimate the ERI (Table I), granulation scattering reflected light, vascular pattern, vulnerability of mucosa and mucosal damage (mucus, fibrin, exudate, erosions, ulcer) were taken into account (8).

All twelve patients who participated in the study had pancolitis.

Sample Treatment and Analysis

Sample volume, pH and buffer capacity were measured immediately upon collection. After adding a methanolic cocktail of 50 mM diisopropylfluorophosphate, 50 mM diethyl(p-nitrophenyl)phosphate, 50 mM acetophenone, and 250 mM phenylboronic acid [2% (v:v)] to terminate the lipolytic and proteolytic activity (9), the sample was ultracentrifuged (30000 g, 15 min, 25°C) under anaerobic conditions, and the % aqueous content was measured. Ultracentrifugation conditions were appropriate for eliminating both solids and bacteria (10,11) without affecting the structure of bacteria, i.e. without liberating intracellular components which could contribute to degradation in the supernatant (unpublished data). The supernatant was divided in seven portions that were kept at −70°C until analysis of each of the following parameters was performed: surface tension, osmolality, soluble protein, soluble carbohydrates, short-chain fatty acids, bile acids, neutral lipids, and long-chain fatty acids.

All chemicals were of analytical grade and purchased from Sigma Aldrich Chemie GmbH (Germany), except for egg phosphatidylcholine (for constructing standard curves), which was donated by Lipoid GmbH (Germany). All solvents were of HPLC grade. All relevant analytical methods have been described recently (7).

Data Analysis

Data are plotted as Box-Whisker plots showing the median value, the 10th, 25th, 75th and 90th percentiles, and the individual outlying data points, with dotted lines indicating the mean value.

For each parameter, differences between relapse and remission were evaluated with the paired t-test or the Wilcoxon test, depending on the results of normality and equal variance tests.

Although mesalazine presence in the lumen may affect composition of lumenal contents (in few cases (part of) the dosage form was found in the ascending colon during sample collection), mesalazine effect(s) on physiological parameters of the lumen of ascending colon could not be evaluated, because it had been administered prior to almost all colonoscopies performed in this study (23 out of 24 cases, Table I). Based on day and time of administration of last dose prior to colonoscopy and plasma elimination half-lives of azathioprine (1.9 h (12) and 36 min for its active metabolite (13)), pantoprazole (1.9 h (14)), and tizanidine (1.6 h (15)) these agents had been almost eliminated from the body at colonoscopy time. Based on day and time of administration of last dose prior to colonoscopy and plasma elimination half-lives of baclofen and prednisolone half lives in plasma (3.4 h and 3.3 h, respectively (16,17)), these agents might still be active during colonoscopy. The effect of baclofen could not be evaluated in this study, because it had been adminitered prior to only one colonoscopy (Table I). The effect of prednisolone on composition of lumenal content was evaluated with unpaired t-test, by comparing the data in patients in relapse treated with prednisolone ($n=5$) with those in patients in relapse that were not treated with prednisolone ($n=7$) (Table I) and with those in healthy subjects (7). Only the significant effects are presented in the "Results and Discussion" section.

Differences to data in healthy adults (taken from (7)) were performed with an unpaired t-test or the Mann-Whitney test.

In all comparisons, Type I error was set to 0.05, and the desired power of the test was 0.800. All statistical comparisons were performed using Sigmastat 2.03 (SPSS Inc., USA).

RESULTS AND DISCUSSION

Characteristics of Contents of Ascending Colon

Mean ± SD volume of contents of the ascending colon in relapse and in remission was not statistically different ($p=0.196$, Table II). Likewise, volumes were not different ($p=0.321$) from volumes of colonic contents in healthy subjects in the fasted state [22.3 ± 7.7 ml (7)]. Interestingly, the volume of contents in the ascending colon of patients in relapse treated with prednisolone (37.4 ± 14.4 ml) was higher than the volume of contents in patients in relapse who were not treated with prednisolone (19.3 ± 6.3 ml) ($p=0.013$) and higher than the volume of contents in (untreated) healthy subjects ($p=0.012$).

Median pHs in relapse and in remission were similar (Table II). These values are significantly lower ($p\leq0.003$) than the median pH measured in the colonic contents of fasted healthy volunteers (pH 7.8 (7)). This observation is in accordance with previous data indicating a wide range of pH values in the right colon with a shift towards lower pH values in some patients with active disease (6).

When measured with hydrochloric acid solution, buffer capacities in relapse and in remission are similar ($p=0.391$, Table II). Compared with data in fasted healthy adults (21.4 ± 7.9 mmol/l/ΔpH, $n=12$ (7)), buffer capacity is significantly higher in patients in remission ($p=0.012$) and borderline higher in patients in relapse ($p=0.079$; power 0.303). Data generated by titrating with sodium hydroxide solution were also similar in remission and relapse. As in fasted healthy adults (7), titration with sodium hydroxide generated lower values than those generated with hydrochloric acid (Table II). However, no conclusions could be drawn on a statistical basis due to the limited volume of contents of the ascending colon available (Table II).

Characteristics of Supernatant After Ultracentrifugation of Contents of Ascending Colon

Mean percent (%) volume of supernatant after ultracentrifugation of samples collected in relapse and in remission (Table II) was lower than the mean value observed in fasted healthy volunteers (70.3 ± 17.0 (7)). No statistically significant differences were found, but it should be mentioned that the power of the tests was low (<0.167).

Surface tension values of supernatant of colonic contents in relapse and in remission (Table II) were low, indicating the presence of surface active components and similar to those measured in fasted healthy fasted volunteers (42.67 ± 2.80 mN/m (7)). It should be mentioned that values might have been affected by the presence of cocktail added after collection of lumenal contents to terminate lipid and protein digestion (7). However, comparison of data collected in this study with data in our previous study (7) is still possible, because sample treatment was the same with that in the previous study (7).

The difference in osmolality of supernatant of colonic contents between relapse and remission was not statistically significant ($p=0.155$, Table II). Values, however, are significantly higher ($p\leq0.029$) than those in the supernatant of contents of healthy volunteers (80.6 ± 102.5 mOsmol/Kg (7)). It may be noted here that, unlike with surface tension

Table II Physicochemical Characteristics of the Contents of the Ascending Colon and of Their Supernatant (after Ultracentrifugation) Measured in Patients with Ulcerative Colitis in Relapse and in Remission on a Sequential Basis[a]

	Relapse[b]	Remission[b]
Contents of ascending colon		
Volume (ml)	26.8 ± 13.5	21.2 ± 8.8
pH	6.6 (5.5–7.7)	6.5 (6.1–7.3)
Buffer capacity (mmol/l/ΔpH) measured with HCl solution [$n = 11$]	32.0 ± 18.1	37.7 ± 15.4
Buffer capacity (mmol/l/ΔpH) measured with NaOH solution [$n = 3$]	18.3 ± 10.4	16.7 ± 5.8
Supernatant of contents of ascending colon		
% of total contents	64.1 ± 14.9	60.4 ± 17.1
Surface Tension (mN/m)	41.6 ± 3.1	40.6 ± 3.4
Osmolality (mOsmol/Kg) [$n = 10$]	199.6 ± 127.4	290.1 ± 165.6
Soluble protein (mg/ml) [$n = 11$]	18.9 ± 8.1	19.0 ± 10.8
Soluble carbohydrates (mg/ml) [$n = 11$]	5.4 ± 2.7	6.4 ± 4.1

[a] Unless specified differently, $n = 12$.

[b] Values are mean ± SD or median (range)

data, osmolality data have been corrected for the presence of cocktail added for the terminating lipolysis and proteolysis (7). Previous data in fecal water have shown that osmolality is elevated in Crohn's patients but not in ulcerative colitis patients (18).

Mean level of soluble protein did not differ significantly between relapse and remission (Table II), but observed levels are significantly higher ($p \leq 0.016$) than those in the supernatant of colonic contents of fasted healthy fasted volunteers (9.8 ± 4.6 mg/ml (7)). The increased level of soluble protein observed in this study is in line with previous data in children with ulcerative colitis (according to which mucus protein concentration increases with the severity of disease (19)) and with events of protein losing enteropathies observed in association with ulcerative colitis (20,21). Since there are no data to support alteration of the lumenal fate of dietary and pancreatic proteins in ulcerative colitis, elevated levels of soluble proteins should be related to other endogenous factors or processes. For example, although the mucus layer is thinner and reduced in number in ulcerative colitis (22), mucosal cytokine profiles in the large bowel and in the terminal ileum are increased in ulcerative colitis patients (23). Also, epithelial leaks appear early due to micro-erosions resulting from upregulated epithelial apoptosis (24).

Mean level of soluble carbohydrates in relapse and remission was not statistically different (Table II) and similar to those measured in the supernatant of colonic contents of fasted healthy volunteers (8.1 ± 8.6 mg/ml (7)).

Compared to the remission state, total short-chain fatty acid (SCFA) concentration was significantly lower in relapse (23.2 ± 14.9 *vs.* 45.3 ± 26.8 mM, $n = 11$, $p = 0.041$). This difference is primarily due to differences in acetate levels (15.8 ± 9.3 mM *vs.* 32.7 ± 16.3 mM, $n = 11$, $p = 0.015$), because, although differences in caproate levels are also significant (0.01 ± 0.02 mM *vs.* 0.07 ± 0.05, $n = 11$, $p = 0.007$), caproate levels are too low to affect total SCFA levels (Fig. 1). Data from the present study are in agreement with previous data concerning the composition of fecal water, which show that SCFAs levels are high in quiescent and mild disease but significantly decreased in severe cases (25). Despite the significantly lower SCFAs levels in relapse, intracolonic pH is not affected (Table II), suggesting that, at least in patients with ulcerative colitis, intracolonic pH is not regulated exclusively by SCFAs levels. Fecal lactate levels are elevated in relapse (25) and could compensate for the effects of SCFAs levels on intracolonic pH. Although isobutyric acid levels (in both relapse (0.26 ± 0.27 mM) and remission (0.25 ± 0.28 mM)) and valeric acid levels (in relapse (0.07 ± 0.11 mM)) are significantly lower than those measured in healthy adults (0.48 ± 0.20 mM and 0.39 ± 0.36 mM, respectively, (7)); no significant differences in total SCFAs and major individual SCFAs levels (e.g. acetic acid, butyric acid, and propionic acid) between patients with ulcerative colitis and healthy adults were detected.

Mean total bile acid concentration in relapse was lower than that in remission, but the difference did not reach significance (75.83 ± 42.96 *vs.* 115.15 ± 100.20 μM, respectively, $p = 0.254$; power = 0.09) (Fig. 2). Likewise, concentrations of individual bile acids did not differ significantly between remission and relapse. Total bile acid levels measured in this study were also not different from those measured in the supernatant of colonic contents of healthy fasted volunteers (7), but again here, the power of the test was very low (0.06). There are some early data in fecal water confirming that bile acids levels in ulcerative colitis patients and in healthy subjects are similar (26,27). Based on individual bile acid levels measured in this study, the difference of cholic acid levels in relapse (8.8 ± 13.3 mM) and in healthy subjects (29.9 ± 35.7 mM (7)) was of borderline significance ($p = 0.070$, power = 0.325). Interestingly, conjugated bile acids are observed more frequently in patients with ulcerative colitis than in healthy subjects (7).

Long-chain fatty acids, phosphatidylcholine and cholesterol levels in relapse and remission (Fig. 3) were not statistically different, but, due to high data variability, the power of relevant tests was low. A similar statement applies

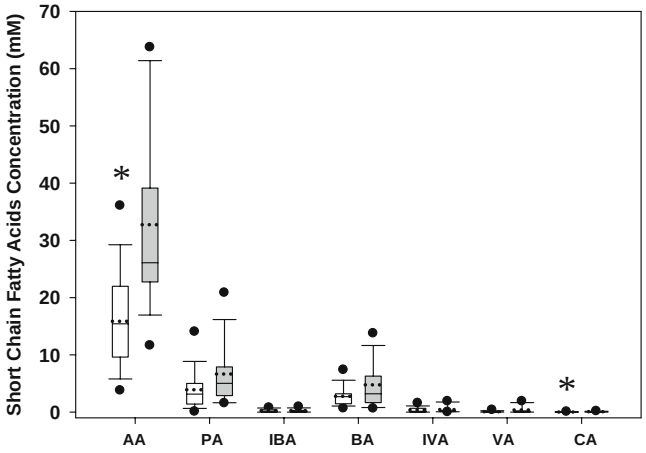

Fig. 1 Concentrations of individual short-chain fatty acids (SCFAs) in the supernatant after ultracentrifugation of contents of ascending colon of 11 patients with ulcerative colitis in relapse (*white boxes*) and in remission (*grey boxes*) on a sequential basis. Asterisks indicate that the difference between data in relapse and data in remission is significant for the particular SCFA. Key: AA, acetic acid; PA, propionic acid; IBA, iso-butyric acid; BA, n-butyric acid; IVA, iso-valeric acid; VA, valeric acid; CA, caproic acid.

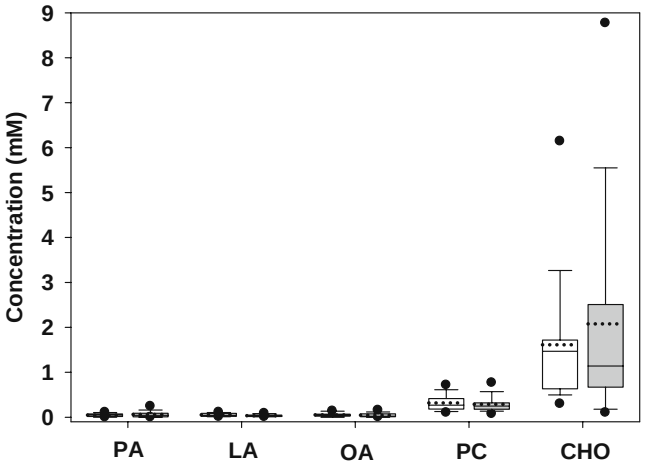

Fig. 3 Concentration of long-chain fatty acids, phosphatidylcholine, and cholesterol in the supernatant after ultracentrifugation of contents of ascending colon of 12 patients with ulcerative colitis in relapse (*white boxes*) and in remission (*grey boxes*) on a sequential basis. Key: PA, palmitic acid; LA, linoleic acid; OA, oleic acid; PC, phosphatidylcholine; CHO, cholesterol.

also for the comparison of the data of this study with data in healthy subjects (7). As in healthy subjects, triglycerides, diglycerides, monoglycerides and lysophosphatidylcholine were practically non-existent (detected in only a few samples) in patients with ulcerative colitis.

CONCLUDING REMARKS

To date, the environment of the ascending colon of patients with ulcerative colitis has been studied only in regard to pH.

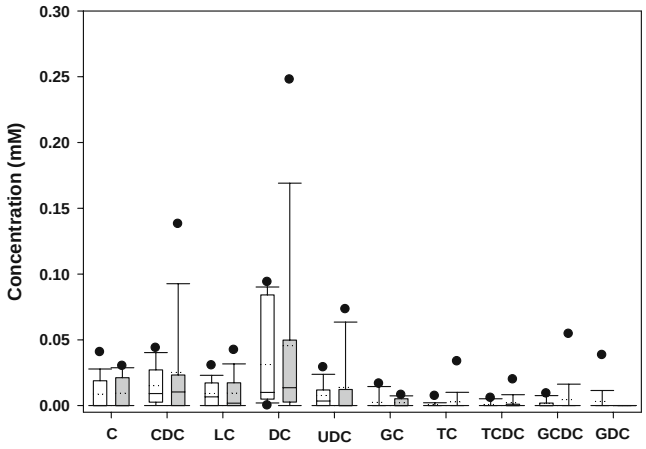

Fig. 2 Concentration of individual bile acids in the supernatant after ultracentrifugation of contents of ascending colon of 12 patients with ulcerative colitis in relapse (*white boxes*) and in remission (*grey boxes*) on a sequential basis. Key: C, cholic acid; CDC, chenodeoxycholic acid; LC, lithocholic acid; DC, deoxycholic acid; UDC, ursodeoxycholic acid; GC, glycocholic acid; TC, taurocholic acid.

Based on this study, total SCFA levels (primarily that of acetates) vary with the relapse/remission status, whereas treatment with prednisolone increases the volume of contents in the ascending colon. Even though the pH remains unaltered, alteration of SCFAs levels could affect drug solubility, for example, because of the formation of low-solubility salts with buffer species and salting in/out phenomena (28).

Further, the present study shows that, regardless of the relapse/remission status, the environment is different than that of healthy subjects (7) with regard to pH, buffer capacity, osmolality, soluble protein, isobutyric acid levels and, perhaps, valeric acid levels. Also, although the high data variability in this study precluded an unequivocal statistical result, levels of some bile acids might also be different in patients with ulcerative colitis. These differences, taken together, could have an impact on the release of drugs from orally administered dosage forms that are targeted to release the therapeutic agent in the colon (28). For example, pH and buffer capacity could affect the microclimate within the formulation. Very high concentrations of dissolved drug and excipients can be obtained locally within the formulation, and the buffer capacity will be a critical determinant of the internal pH and thereby solubility and release of (partially) ionized drugs. Osmolality (and ionic strength) could influence functionality of most polymers used for extended release formulations with potential consequences for drug dissolution (28), whereas total protein content can have an impact on drug's intralumenal solubility.

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REFERENCES

1. Wilson CG, Weitschies W, Butler J. Gastrointestinal transit and drug absorption. In: Dressman J, Reppas C, editors. Oral drug absorption, Informa Healthcare, New York, USA; 2010. pp. 41–65.
2. Milovic V, Stein J. Gastrointestinal disease and dosage form performance. In: Dressman J, Reppas C, editors. Oral drug absorption, Informa Healthcare, New York, USA; 2010. pp.127–139.
3. Sadfi AV, Cohen RD. Increasing the dose of oral mesalazine therapy for active unlcerative colitis does not improve remission rates. Aliment Pharmacol Ther. 2007;26:1179–86.
4. Metcalf AM, Phillips SF, Zinsmeister AR, MacCarty RL, Beart RW, Wolff BG. Simplified assessment of segmental colonic transit. Gastroenterology. 1987;92:40–7.
5. Schiller C, Froehlich C-P, Giessmann T, Siegmund W, Moennikes H, Hosten N *et al.* Intestinal fluid volumes and transit of dosage forms as assessed by numeric resonance imaging. Aliment Pharmacol Ther. 2005;22:971–9.
6. Nugent SG, Kumar D, Rampton DS, Evans DF. Intestinal luminal pH in inflammatory disease: possible determinants and implications for therapy with aminosalicylates and other drugs. Gut. 2001;48:571–7.
7. Diakidou A, Vertzoni M, Goumas K, Söderlind E, Abrahamsson B, Dressman J *et al.* Characterization of the contents of ascending colon to which drugs are exposed after oral administration to healthy adults. Pharm Res. 2009;26:2141–51.
8. Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) *versus* sulphasalazine in the treatment of active ulcerative colitis: a randomized trial. Br Med J. 1989;298:82–6.
9. Hernell O, Staggers JE, Carey MC. Physical-chemical behaviour of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. Biochemistry. 1990;29:2041–56.
10. MacFarlane GT, MacFarlane S, Gibson GR. Validation of a three-stage compound continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the human colon. Microb Ecol. 1998;35:180–7.
11. Apajalahti JH, Saerkilahti LK, Maeki BR, Heikkinen JP, Nurminen PH, Holben WE. Effective recovery of bacterial DNA and percent-guanine-plus-cytosine-based analysis of community structure in the gastrointestinal tract of broiler chickens. Applied Environ Microbiol. 1998;64:4084–8.
12. Chan GL, Erdmann GR, Gruber SA, Matas AJ, Canafax DM. Azathioprine metabolism: pharmacokinetics of 6-mercaptopurine, 6-thiouric acid and 6-thioguanine nucleotides in renal transplant patients. J Clin Pharmacol. 1990;30:358–63.
13. Maddocks JL. Assay of azathioprine, 6-mercaptopurine and a novel thiopurine metabolite in human plasma. Br J Clin Pharmacol. 1979;8:273–8.
14. Pue MA, Laroche J, Meineke I, de Mey C. Pharmacokinetics of pantoprazole following single intravenous and oral administration to healthy male subjects. Eur J Clin Pharmacol. 1993;44:575–8.
15. Backman JT, Karjalainen MJ, Neuvonen M, Laitila J, Neuvonen PJ. Rofecoxib is a potent inhibitor of cytochrome P450 1A2: studies with tizanidine and caffeine in healthy subjects. Br J Clin Pharmacol. 2006;62:345–57.
16. Martinez MN, Jackson AJ. Suitability of various noninfinity area under the plasma concentration-time curve (AUC) estimates for use in bioequivalence determinations: relationship to AUC from zero to time infinity (AUC0-inf). Pharm Res. 1991;8:512–7.
17. Carson SW, Letrent KJ, Kotlyar M, Foose G, Tancer ME. Lack of a fluoxetine effect on prednisolone disposition and cortisol suppression. Pharmacotherapy. 2004;24:482–7.
18. Vernia P, Gnaedinger A, Hauck W, Breuer RI. Organic anions and the diarhea of the inflammatory bowel disease. Dig Dis Sci. 1988;33:1353–8.
19. Grzybowska K, Gwoździński K, Zaleśna G, Kisiel A, Grzybowski P, Płaneta-Małecka I. Mucus physicochemical properties in children with ulcerative colitis. Pol Merkur Lekarski. 2004;17: S19–21.
20. Bryan RL, Newman J, Alexander-Williams J. Giant inflammatory polyposis in ulcerative colitis presenting with protein losing enteropathy. J Clin Pathol. 1990;43:346–7.
21. Anderson R, Kaariainen IT, Hanauer SB. Protein-losing enteropathy and massive pulmonary embolism in a patient with giant inflammatory polyposis and quiescent ulcerative colitis. The Am J Med. 1996;101:323–5.
22. Pullan RD, Thomas GAO, Rhodes M *et al.* Thickness of adherent mucus gel on colonic mucosa in humans and its relevance to colitis. Gut. 1994;35:353–9.
23. Yamamoto T, Maruyama Y, Umegae S, Matsumoto K, Saniabadi AR. Mucosal inflammation in the terminal ileum of ulcerative colitis patients: endoscopic findings and cytokine profiles. Dig Liver Dis. 2008;40:253–9.
24. Schulzke JD, Ploeger S, Amasheh M, Fromm A, Zeissig S, Troeger H *et al.* Ann NY Acad Sci. 2009;1165:294–300.
25. Vernia P, Caprilli R, Latella G, Berbetti F, Magliocca FM, Cittadini M. Fecal lactate and ulcerative colitis. Gastroenterology. 1988;95:1564–8.
26. Miettinen TA. The role of bile salts in diarrhoea of patients with ulcerative colitis. Gut. 1971;12:632–5.
27. Reddy B, Martin C, Wynder E. Feacal bile acids and cholesterol metabolites of patients with ulcerative colitis, a high-risk group for development of colon cancer. Cancer Res. 1977;37:1697–701.
28. Abrahamsson B, Söderlind E. Screening modified release oral formulations in pharmaceutical industry. In: Dressman J, Reppas C, editors. Oral drug absorption, Informa Healthcare, New York, USA; 2010. pp. 267–97.