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Insight into the colonic disposition of celecoxib in humans

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ABSTRACT

Although the effect of NSAIDs such as celecoxib on the progression of colorectal polyps has been established, it is currently unknown how sufficiently high concentrations of celecoxib are reached in colonic tissue. Indeed, the lipophilic and poorly soluble celecoxib is orally dosed as an immediate release capsule without any colon-targeting delivery strategy. In the present study, we aimed to distinguish between plasma and gut driven caecal tissue accumulation of celecoxib in healthy volunteers. After developing a protocol to reliably collect colonic biopsies and contents, the disposition of celecoxib was assessed in plasma, caecal tissue and caecal contents collected after intake of a celecoxib capsule (200 mg; Celebrex®) with 240 mL of tap water. During a first colonoscopy (1.0–2.5 h after drug intake), plasma concentrations of celecoxib and its carboxy metabolite were increasing, while caecal tissue concentrations were relatively low. As no celecoxib was present in caecal contents, tissue accumulation was clearly plasma driven. During a second colonoscopy (6.0–7.5 h after drug intake), tissue concentrations of the drug and its metabolite were substantially higher despite decreasing plasma concentrations. As a high amount of celecoxib was found in the caecal contents, the increased tissue accumulation most likely resulted from direct uptake of celecoxib from the gut. These data demonstrate that incomplete small intestinal absorption of the poorly soluble drug celecoxib enables gut driven drug accumulation in caecal tissue, which is, most likely, critical for the role of this NSAID in the prevention of colorectal cancer.

1. Introduction

Colorectal cancer is the third most abundant type of cancer, with an estimated 1.8 million new cases in 2018 (Bray et al., 2018). Inflammation plays a key role in the development and progression of the disease; therefore, non-steroidal anti-inflammatory drugs (NSAIDs) such as sulindac, aspirin, and celecoxib are eligible candidates for the treatment and/or prevention of precursor lesions of colorectal cancer; i.e. polyps. NSAIDs inhibit the cyclooxygenase (COX) pathway, in addition to many COX-independent pathways. In humans, two important isoforms of COX exist: COX-1 is expressed constitutively and is important for maintaining the physiological functions of the gastrointestinal tract, while COX-2 is an inducible isoform that becomes overexpressed in states of inflammation and carcinogenesis (Gong et al., 2012). The effect of NSAIDs in colorectal cancer treatment and prevention has been reported in several epidemiological studies. For instance, after taking 400 mg of the COX-2 inhibitor celecoxib twice a day for three years, the incidence of recurrent adenomas was reduced by 45% (Bertagnolli et al., 2006). Treatment of familial adenomatous polyposis patients with 150 mg sulindac twice a day for 9 months reduced the number of polyps with 44% and their size with 35% (Herendeen and Lindley, 2003). Also for the COX-1 inhibitor aspirin, the Melbourne Control Case Study showed a 42% reduction in the risk of colorectal cancer (Burn and Sheth, 2016). However, COX-2 inhibitors such as celecoxib are generally preferred over COX-1 inhibitors as up-regulation of COX-2 happens in the early stages of carcinogenesis, and as they are associated with less severe side effects (Fujimura et al., 2006).

To exert their effect on colorectal cancer progression, the NSAIDs need to be present at sufficiently high concentrations in the colon. To date, however, the disposition of these NSAIDs in the human colon has not been evaluated yet. In this respect, it is currently unknown how

Abbreviations: COX, cyclooxygenase; CXB, celecoxib; CXB-OH, hydroxy celecoxib; CXB-COOH, carboxy celecoxib; C_{max}, maximal drug concentration; IS, internal standard, LC: liquid chromatography; T_{max}, time to reach maximal drug concentration

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these compounds reach the colon. After oral administration, the NSAIDs are absorbed in the systemic circulation and, as such, distributed across the body, including the colon. However, relatively high concentrations are needed to demonstrate anti-carcinogenic effects in *in vitro* cell cultures. Indeed, Grösch et al. (2006) reported that concentrations of celecoxib between 10 μM and 100 μM are generally used in cell culture experiments to get anti-carcinogenic effects, which are not reached in plasma by administering a single 200 mg dose. Therefore, it can be questioned whether direct uptake from the gut lumen into the colonic tissue might be required, even though neither of the NSAIDs have been formulated as a colon-targeting delivery system. In the present study, we aim to shed light on the colonic disposition of the COX-2 inhibitor celecoxib after oral administration of its dosage form Celebrex® (immediate release capsule, 200 mg)

Celecoxib (CXB) is a lipophilic (LogP = 3.5), and weakly acidic drug (pKa = 11.1) (Pfizer, 2016). It has been reported as a high permeability drug (Paulson et al., 2001), explaining why the compound is rapidly absorbed (t_{max} = 1 h) in dogs when administered orally as a solution. When dosing celecoxib as a solid, however, absorption is delayed with 1–2 h, which indicates its low solubility in the aqueous gastrointestinal environment (Paulson et al., 2001). Increasing the orally administered dose in rats resulted in a higher fraction of celecoxib that could be retrieved in the faeces (Paulson et al., 2000). As celecoxib is extensively metabolized in the human liver by CYP2C9 to form hydroxy celecoxib (CXB–OH), which can then be oxidized to carboxy celecoxib (CXB-COOH) by cytosolic alcohol dehydrogenases ADH1 and ADH2 (Gong et al., 2012), the appearance of celecoxib in the faeces implies that this poorly soluble drug does not get completely absorbed in the upper gastrointestinal tract (Paulson et al., 2000). With respect to celecoxib's effect on colorectal polyps and cancer, this observation further implies that direct uptake of celecoxib from the colonic lumen into the colonic tissue is possible, even though it should be noted that the low and scattered water content in the colon hinders drug dissolution and that the comparably smaller surface area of the colonic mucosa makes it less optimal for drug uptake than the small intestinal mucosa.

To evaluate the pharmacokinetic disposition of celecoxib at the level of the colon, the present study focused on two main goals: (i) to develop a protocol through which biopsies and colonic contents can be reliably collected, and (ii) to simultaneously assess celecoxib and its major metabolites in the systemic circulation, in the colonic lumen and in colonic tissue, allowing an improved insight into the underlying mechanisms.

2. Materials and methods

2.1. Chemicals

Celecoxib was acquired via TCI Europe N.V. (Zwijndrecht, Belgium). Hydroxy celecoxib and carboxy celecoxib were provided to us by Toronto Research Chemicals (Toronto, Canada). Propyl-p-hydroxybenzoate was provided by Sigma-Aldrich (St. Louis, Missouri, USA). Chem-Lab (Zedelgem, Belgium) and Biosolve (Valkenswaard, The Netherlands) supplied acetic acid and formic acid, respectively. Methanol (HPLC grade) and glucose were ordered from Acros Organics (HPLC grade; Geel, Belgium). Purified water for analytical purposes was obtained using a Maxima system (Elga Ltd., High Wycombe Bucks, UK). Hanks' Balanced Salt Solution without phenol red (HBSS) and 4-(2-hydroxyetheyl) − 1-piperazineethanesulfonic acid (HEPES) were obtained from Lonza (Verviers, Belgium), while sodium hydroxide (NaOH) pellets were purchased from Merck (Darmstadt, Germany).

2.2. Media

Medium (HBSS +) was prepared by dissolving 25 mM glucose in 500 mL HBSS, buffered with 10 mM HEPES and adjusted to pH 7.4 with 2 M of NaOH.

2.3. Clinical studies

2.3.1. Study medication

Celebrex® capsules (200 mg celecoxib; Pfizer, Brussels, Belgium) were ordered via the hospital pharmacy of the University Hospitals Leuven (UZ Leuven, Belgium).

2.3.2. Ethics

Clinical trials followed the tenets of the Declaration of Helsinki and were approved by the Federal Agency for Medicines and Health Products (FAMHP; EudraCT reference numbers 2017-000131-13, 2017-001194-16) and the Ethics Committee Research UZ/KU Leuven (reference numbers S60240, S60302, S60322). All volunteers provided written informed consent prior to participation. Volunteers with hepatitis B/C and/or HIV infection were excluded to guarantee the safety of the study personnel. Illness at the time of the study, allergy for NSAIDs, medication use, a history of acute/chronic gastrointestinal disease(s), (possible) pregnancy, and/or frequent exposure to radiation during the previous year were criteria for exclusion.

2.3.3. Study protocol

In all studies, volunteers were asked to follow a three-day low-fiber diet (e.g. white bread or pasta, canned fruit in heavy syrup or juice, no vegetables, clear drinks…) and to eat a last meal at 8 pm on the day before the study. To ensure fasted state conditions, volunteers could only drink water 12–2 h prior to the colonoscopy. All studies were schematically summarized in Fig. 1.

Study 1: Optimizing enema application and collection of colonic contents

Upon arrival at the endoscopy unit, the volunteers (aged between 23 and 30 years; 2 men and 3 women) were positioned on their left side and a varying volume of enema (250 mL or 500 mL of tap water) was administered rectally. Next, a sigmoidoscopy was performed to validate whether the left hemicolon was cleansed without spill over to the right hemicolon. Once this condition was achieved, the volunteer was brought under conscious sedation (midazolam 1–5 mg and pethidine 25–50 mg i.v.) in order to perform a full colonoscopy and to reliably sample colonic contents from the right hemicolon. To collect colonic contents, several approaches were evaluated: (i) collection with a polyCatch Retrieval Device (Endo-Flex, Germany), (ii) aspiration with a Faucher catheter (PVC, Fr 27 × 150 cm; Vygon, Brussels, Belgium) or a weighted duodenal tube (PVC, Fr 16 × 125 cm; Vygon, Brussels, Belgium), which were attached during the colonoscopy to the mucosa of the ascending colon with hemostatic clips (Boston Scientific, US), (iii) sampling as much caecal contents as possible through the suction channel of the colonoscope (a stiff brush was pulled through the entirety of the suction channel to collect the contents of the suction channel into a falcon tube).

Study 2: Region- and time-dependent accumulation in colonic tissue

In a preliminary study, one female volunteer (aged 23 years) was asked to take a Celebrex® capsule (200 mg celecoxib) with 240 mL of tap water either 2 h, 4 h, 6 h or 8 h prior to the colonoscopy. The time factor was evaluated on different test days. Upon arrival at the endoscopy unit, the volunteer was positioned on her left side and 250 mL of tap water was administered rectally. Next, the volunteer was brought under conscious sedation (midazolam 1–5 mg and pethidine 10–50 mg i.v.). During the colonoscopy, four biopsies were taken with a standard biopsy forceps (Radial Jaw™3 with needle; outside diameter 2.2 mm; Boston Scientific) at each of the following regions: caecum, hepatic

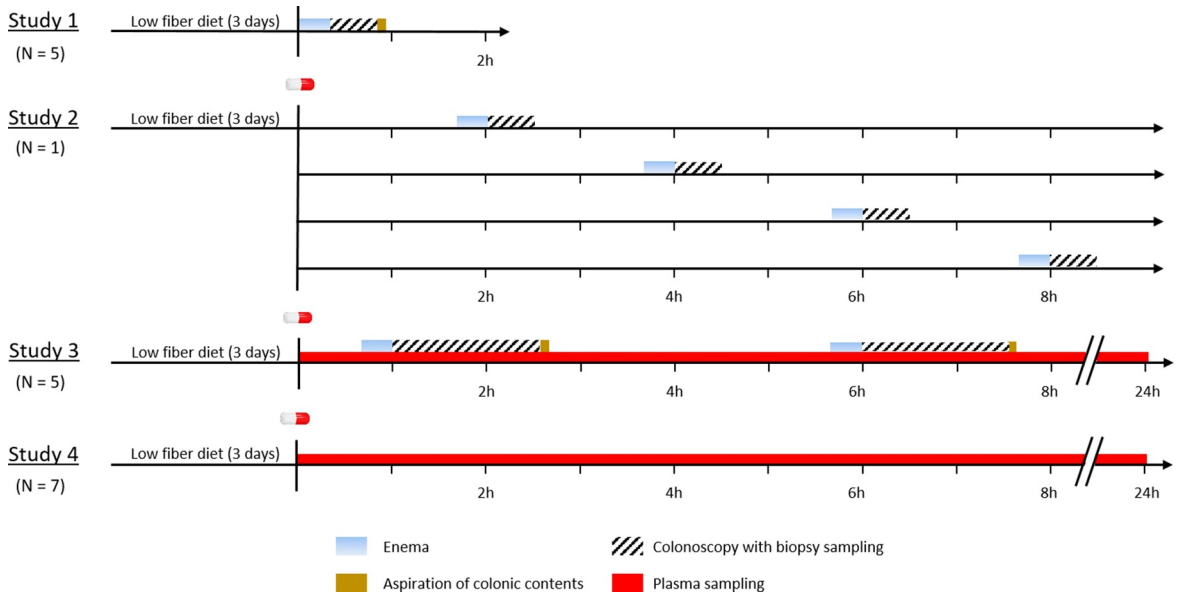

Fig. 1. Schematic representation of the clinical studies (study 1: optimizing enema application and collection of colonic contents; study 2: region- and time-dependent accumulation in colonic tissue; study 3: systemic versus gut-driven tissue accumulation; study 4: plasma concentration-time profile of celecoxib).

flexure, splenic flexure and sigmoid. The participant was not allowed to drink or consume food during the colonoscopy.

Study 3: Systemic versus gut-driven tissue accumulation

After colonic preparation with an enema as previously described, a colonoscopy was performed to obtain two blank biopsies. Next, a Celebrex® capsule (200 mg celecoxib) was orally administered with 240 mL of tap water to the volunteers (5 men, aged between 20 and 23 years). Between 1 and 2.5 h after drug intake, two caecal biopsies were taken every 15 min with a standard biopsy forceps (Radial Jaw™3 with needle; outside diameter 2.2 mm; Boston Scientific). After 2.5 h, as much colonic contents as possible (± 2 mL) were sampled through the suction channel of the colonoscope, and collected into a falcon tube by pulling a stiff brush through the entirety of the suction channel. A few hours later, the colonoscope was positioned again in order to sample caecal biopsies every 15 min between 6 and 7.5 h after drug intake. After 7.5 h, colonic contents were collected once again. During the colonoscopies, volunteers were not sedated, as sedation might influence gastrointestinal motility and thus the pharmacokinetic disposition of celecoxib. Venous blood samples were collected on ice in heparinized tubes (BD Vacutainer systems, Plymouth, U.K.) at predetermined time points, i.e., 0, 5, 15, 30, 60, 90, 120, 180, 240, 360, 480, and 1440 min after drug intake. Participants were not allowed to drink or consume food for the first 4 h after drug intake. After 4 h, participants were allowed to drink and/or eat ad libitum.

Study 4: Plasma concentration-time profile of celecoxib

Upon arrival at the endoscopy unit, volunteers (aged between 20 and 25 years; 3 men and 4 women) were asked to take a Celebrex® capsule (200 mg celecoxib) with 240 mL of tap water. Venous blood samples were collected in heparinized tubes (BD Vacutainer systems, Plymouth, U.K.) on ice at predetermined time points, i.e., 0, 5, 15, 30, 60, 90, 120, 180, 240, 360, 480, and 1440 min after drug intake. Participants were not allowed to drink or consume food for the first 4 h after drug intake. After 4 h, participants were allowed to drink and/or eat ad libitum.

2.4. Processing of biological samples

Biopsies

Upon sampling, biopsies were rinsed threefold with HBSS+, excess liquid was removed with a paper tissue, and biopsies were stored in 1 mL of methanol. Prior to further processing, samples were stored at 4°C. To quantify drug concentrations in biopsies, 10 µL of DMSO containing an internal standard (10 µM propyl-p-hydroxybenzoate) was added. Biopsies were then air dried for 1 h, weighed, placed back into the methanol and homogenized with a TissueLyser II (3 × 4 min, 1/30 s; Qiagen, Hilden, Germany). Subsequently, samples were centrifuged (20,817 x g, 1 min, 4 °C; Centrifuge 5417 R; Eppendorf, Hamburg, Germany) and the supernatant was evaporated under a continuous air flow before being reconstituted in 100 µL of methanol:water (50:50, v/v).

Colonic contents

Colonic contents were processed on the day of the experiment to determine the total and dissolved concentrations of celecoxib and its metabolites. After vortexing (Vortex Genie, Scientific industries, New York, USA) the contents for 1 min, the total drug concentration was measured by directly diluting 10 µL of the colonic contents with 990 µL methanol:acetate buffer pH 4.5 (45:55, v/v), which was then immediately centrifuged (20,817 x g, 5 min; Microcentrifuge 5424, VWR International); the supernatant was collected for liquid chromatography (LC) analysis. The dissolved drug concentration was measured by first centrifuging (20,817 x g, 5 min; Microcentrifuge 5424, VWR International) a fraction of the colonic contents after being vortexed for 1 min. Next, 10 µL of the supernatant was diluted in 990 µL methanol:acetate buffer pH 4.5 (45:55, v/v) and centrifuged (20,817 x g, 5 min; Microcentrifuge 5424, VWR International) once more; the supernatant was collected for LC analysis.

Venous blood samples

Immediately after collection, blood samples were stored on ice; at the end of the day of the experiment, they were centrifuged (1699 x g, 15 min, 4 °C; Centrifuge 5804 R; Eppendorf, Hamburg, Germany) to collect plasma samples, which were stored at −26 °C until analysis. To quantify drug concentrations in plasma, 10 µL of DMSO containing an internal standard (500 µM propyl-p-hydroxybenzoate) was added to 1 mL plasma. After adding 4 mL methanol, the mixture was thoroughly vortexed and subsequently shaken for 1 min. The samples were then centrifuged (1699 x g, 15 min, 4 °C; Centrifuge 5804 R; Eppendorf,

Hamburg, Germany) and the supernatant was collected for LC analysis.

2.5. LC analysis

Processed biopsy samples were analysed with LC–MS/MS, using an Acquity H-class UPLC system coupled to a Xevo TQ-S micro triplequadrupole analyser equipped with an ESI source (Waters, Milford, MA, USA). Separation was achieved using a Kinetex C18 column (2.6 μm, 50 × 2.1 mm, Phenomenex, Utrecht, The Netherlands) with methanol (solvent A) and water acidified with 0.1% formic acid (solvent B). The elution gradient was as follows: 0–0.8 min, 50% A; 0.81–1.8 min, 80% A; 1.81–3.0 min, 90% A; 3.01–4.50 min, 50%. The flow rate was 0.5 ml/min for the first 4 min, and 2 mL/min for the last 30 s of the run. Column temperature was held at 35°C, and the injection volume was 1 μL. The mass spectrometer was set to positive ionization MS/MS mode for celecoxib and its metabolites, and to negative ionization MS/MS mode for the internal standard with the following parameters: source temperature 150°C, capillary voltage 0.80 kV, cone voltage 30 V, cone gas flow 40 L/h, desolvation gas flow 1000 L/h and desolvation temperature 600°C. The mass transitions (m/z) followed were 381.8–361.9 (celecoxib), 397.8–377.9 (hydroxy celecoxib), 411.8–391.9 (carboxy celecoxib) and 178.8–91.8 (propyl-p-hydroxybenzoate). Stock solutions of celecoxib, hydroxy celecoxib, carboxy celecoxib, and the internal standard were prepared in DMSO at a concentration of 10 mM, and calibration curves were made by serial dilution in methanol/water (50:50, v/v), containing 1 μM internal standard. Linearity was observed between 4000 nM and 0.15, 0.07, 0.15 nM for celecoxib, hydroxy celecoxib and carboxy celecoxib, respectively. For validation purposes, control samples were prepared containing 10 μM, 1 μM and 100 nM of celecoxib and metabolites. Concentrations of celecoxib and metabolites could be precisely (intraday variability RSD ≤ 15.07% and interday variability RSD ≤ 11.81%) and accurately (range: 81.93–99.91%) determined in samples.

Processed plasma and colonic fluid samples were analyzed using HPLC with UV detection (252 nm). Separations were performed on a Novapak C18 column under radial compression (pore size 60 Å, particle size 4 μm, 8 mm i.d. x 100 mm, Waters, Milford, MA, USA). 100 μL sample was injected and eluted with a gradient of 25 mM sodium acetate buffer pH 4.5 (solvent A) and methanol (solvent B) at a flow rate of 1.0 mL/min for 18.5 min. The elution gradient was as follows: 0.0–1.5 min, 55% A; 2.5–7.0 min, 30% A; 8.0–13.5 min, 22% A; and 14.5–18.5 min, 55% A for reconditioning. Carboxy celecoxib, hydroxy celecoxib, the internal standard and celecoxib eluted at 6.6 min, 7.9 min, 8.6 min, and 12.5 min, respectively. Linearity was observed in a range from 20 μM to 10 nM. For validation purposes, control samples were prepared containing 10 μM, 1 μM and 100 nM. Concentrations of celecoxib and metabolites could be precisely (intraday variability RSD ≤ 4.04% and interday variability RSD ≤ 7.00%) and accurately (range: 89.95–99.89%) determined in samples. Chromatograms were integrated with Waters Empower 2 software (Milford, MA, USA).

2.6. Data presentation

This mechanistic study was designed as exploratory, i.e. without the intention to formally test hypothesis. As such, the data obtained are descriptive in nature and do not allow statistical comparison.

Plasma concentrations are represented as the amount of celecoxib or metabolite per volume of plasma (μg/mL). Concentrations in colonic contents are represented in amount of celecoxib or metabolite per volume of colonic content (μg/mL). The accumulation in colonic biopsies was normalized for the weight of the tissue, and represented as the amount of celecoxib or metabolite per amount of tissue (ng/mg). Data in text are presented as mean + SD, unless stated otherwise.

3. Results and discussion

Documentation regarding the sampling of colonic contents and the gastrointestinal behavior of low solubility compounds at the level of the colon is limited. The present study pursued to evaluate the colonic disposition of celecoxib in human volunteers, and to explore whether the accumulation into colonic tissue is either plasma or gut driven. To this end, we first needed to develop a protocol that allowed us to collect relevant luminal and tissue samples from the colon.

3.1. Optimizing enema administration and collection of colonic contents

To sample colonic tissue and contents in a reliable way, i.e. without affecting the disposition of celecoxib, the colonoscopy and the required preparation procedure should disturb the normal physiology of the colon as little as possible (e.g. by diluting the colonic contents). To our knowledge, only one group has attempted to sample proximal colonic fluid in the framework of drug disposition studies. Diakidou et al. (2009) collected the contents from the ileum and caecum following the administration of 10 mg of bisacodyl, a stimulant laxative, 50 h and 44 h prior to the colonoscopy in combination with a strict dietary regimen of liquefied food (e.g. fish/chicken/rice soups, fruit juices) to allow visual control. In the present study, however, we preferred not to use a laxative in order to avoid the risk of disturbing the physiological state of the proximal colon at the time of sampling. After the volunteers followed a three-day low-fiber diet, enemas were administered to cleanse only the left hemicolon and enable the safe passage of the colonoscope. Midazolam was used as sedation while performing the colonoscopy and could be a potential disturbing factor. By varying the frequency and volume of the enemas, we observed that a one-time

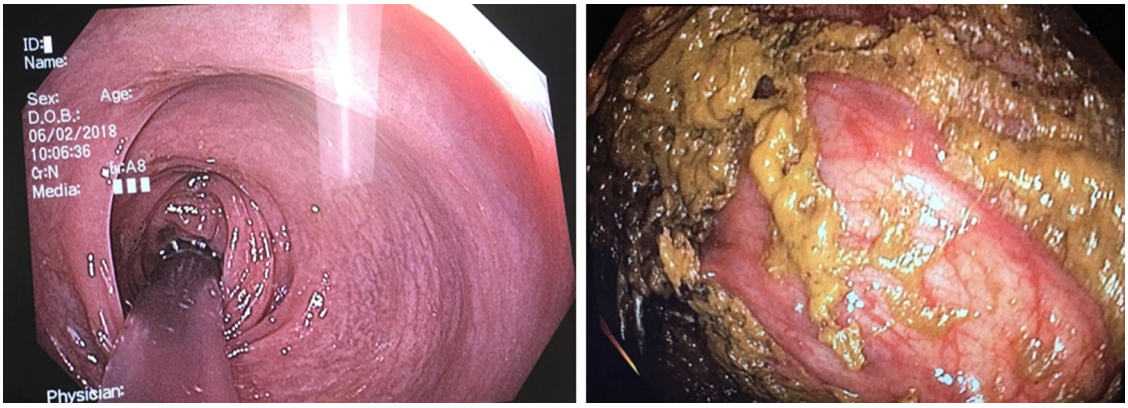

Fig. 2. Visualization of the cleansed left hemicolon (left) and the maintained physiological state of the right hemicolon (right) after a 250 mL enema.

enema of 250 mL of water appeared sufficient to cleanse the left hemicolon while avoiding spill over to the right hemicolon where then physiologically relevant samples could be taken (Fig. 2).

Next, multiple sample techniques were evaluated to collect proximal colonic contents. First, a polyCatch Retrieval Device (Endo-Flex, Germany) was inserted through the colonoscope into the caecum, where the retrieval pouch was used to collect colonic contents. This method was not optimal as fluids slipped out of the PolyCatch Retrieval Device after retraction. A second option was the use of aspiration catheters: a 27 Fr Faucher tube or a 16 Fr Gastro-duodenal feeding tube (Vygon, Belgium). The tubes were inserted through the colonoscope and attached to the mucosa of the ascending colon with hemostatic clips (Boston Scientific, US), after which we attempted to aspirate colonic contents regularly over a period of 4 h. Due to the high viscosity of the contents, however, the volume of the aspirated samples was limited and it appeared not possible to collect samples in a time-dependent way. Finally, using the suction canal of the colonoscope turned out to be the most reliable way to collect the contents from the proximal colon. Immediately after collecting colonic contents, a hard brush was inserted into the colonoscope and contents (± 2 mL) were pulled out and collected in a falcon tube. While this method allowed us to collect physiologically relevant samples, it is limited to one sample per colonoscopy.

3.2. Optimizing time and region to evaluate colonic celecoxib disposition

To assess the optimal time and colonic region to sample tissue for the evaluation of the disposition of celecoxib, we determined, in a preliminary experiment, the concentration-time profile of celecoxib in colonic tissue collected from four regions. Following a low-fiber diet for 3 days, one volunteer took one tablet of Celebrex® (200 mg of celecoxib) with 240 mL of tap water. After cleansing the left hemicolon with the above-mentioned enema, a colonoscopy was performed under conscious sedation, and four biopsies were taken from each of the following regions: the caecum, the hepatic flexure, the splenic flexure, and the sigmoid. The sampling of the biopsies was performed either 2, 4, 6, or 8 h after intake of Celebrex® on different test days. In Fig. 3, we see that the concentration (ng/mg) of celecoxib in the tissue decreased from 2 to 4 h and increased from 4 to 8 h after intake of the drug in all sampled regions except for the hepatic flexure where the highest accumulation was seen at 6 h. Similar to celecoxib, accumulation of the metabolite carboxy celecoxib was the highest 8 h after intake, but concentrations were about 30-fold lower in comparison with celecoxib. Tissue concentrations of celecoxib were the highest in the caecum in comparison with other regions. Based on these data, it was decided to further evaluate the disposition of celecoxib and its main metabolite only at the level of the caecum.

3.3. Time-dependent systemic and caecal disposition of celecoxib

First, we determined the concentration-time profile of celecoxib in the systemic circulation, without performing a colonoscopy. To match the colonic disposition studies, volunteers followed the same dietary regimen (a three-day low fiber diet), and were asked to take Celebrex (200 mg celecoxib) with 240 mL of tap water. The mean plasma T_{max} was 2.5 ± 0.9 h in healthy volunteers (Fig. 4), which is in agreement with the study performed by the group of Werner et al. (2002), who observed a mean T_{max} of 2.9 ± 1.2 h under fasted conditions (Werner et al., 2002). After reaching the T_{max}, the decrease in plasma concentrations is initially reflected in the decrease in colonic tissue concentrations of celecoxib from 2 to 4 h in Fig. 3. However, there is a discrepancy between the plasma T_{max} (2.5 h) and the above-mentioned T_{max} in caecal tissue (8 h, Fig. 3). This suggests that colonic tissue concentrations are not driven solely by plasma levels, but also by direct uptake from the gut.

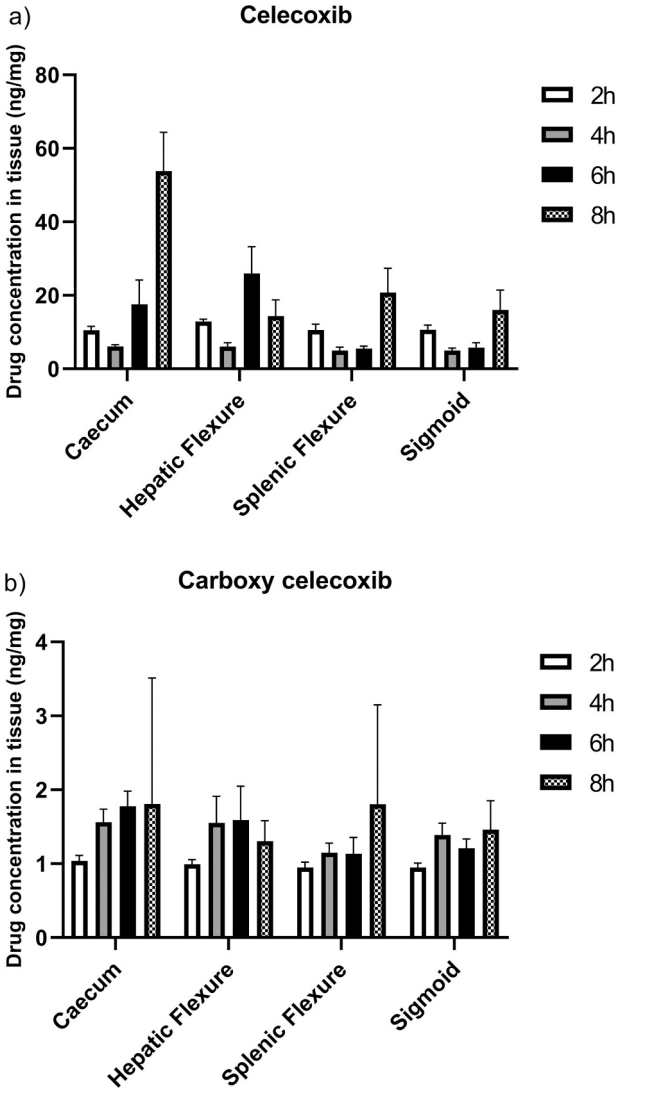

Fig. 3. Region- and time-dependent accumulation of celecoxib (a) and its metabolite carboxy celecoxib (b) in biopsies collected from one healthy volunteer after oral intake of one tablet of Celebrex (200 mg celecoxib) with 240 mL of tap water under fasted state conditions. Bars represent mean (+ SD) drug accumulation in biopsies ($n = 4$) from the caecum, hepatic flexure, splenic flexure and sigmoid, which were collected either 2 h (white bars), 4 h (gray bars), 6 h (black bars) or 8 h (speckled bars) after oral intake.

To further distinguish between plasma and possibly gut driven caecal tissue accumulation of celecoxib, a study was performed involving colonoscopies after intake of a Celebrex® tablet (200 mg celecoxib) with 240 mL of tap water. One hour after drug intake, biopsies were taken from the caecum every 15 min for 90 min, after which caecal contents were aspirated (first colonoscopy, phase 1). Based on the systemic concentration-time profile in Fig. 4, plasma levels are expected to increase in this first phase (between 1 and 2.5 h post drug intake) and drive caecal tissue accumulation. Six hours after drug intake, caecal biopsies were collected again every 15 min for 90 min, after which caecal contents were aspirated (second colonoscopy, phase 2). In this second phase (between 6 and 7.5 h post drug intake), plasma levels of celecoxib are expected to decrease. Tissue concentrations could be plasma driven (in which case they would decrease as well), but also gut driven if celecoxib arrived at the caecal lumen through transit.

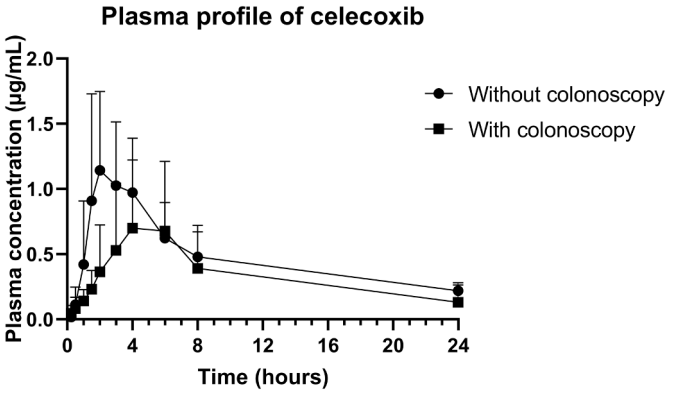

Fig. 4. Mean (+ SD) plasma concentration – time profiles in healthy volunteers after oral intake of one tablet of Celebrex (200 mg celecoxib) with 240 mL of water under fasted conditions. Studies were performed either without performing a colonoscopy (n = 7) (●) or with performing a colonoscopy (n = 5) (■).

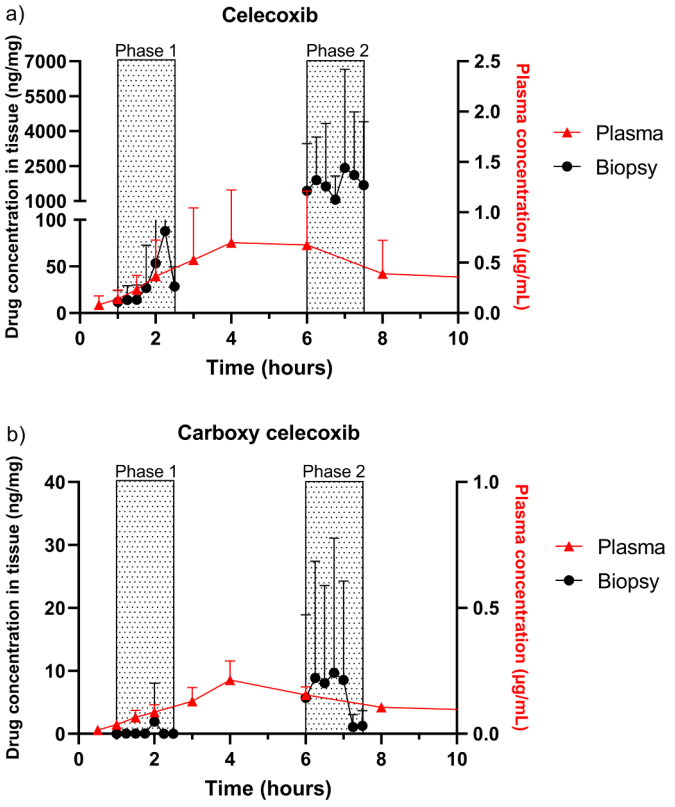

Fig. 5. Mean (+ SD) concentration–time profiles of celecoxib (a) and carboxy celecoxib (b) in plasma samples (▲) and caecal biopsies (●), collected from healthy volunteers (n = 5) after oral intake of one tablet of Celebrex (200 mg celecoxib) with 240 mL of water under fasted conditions.

The plasma concentration-time profile of celecoxib, shown in Fig. 4, indicates a substantially higher plasma T_{max} for celecoxib (4.4 h ± 0.9 h) in this colonic disposition study as compared to the T_{max} observed in the above-mentioned study without colonoscopy. We assume that the difference in T_{max} is due to rectal distention by the colonoscope, delaying gastric emptying (Youle and Read, 1984). Also, volunteers generally experience higher stress levels during the colonoscopy, which is known to modulate gastrointestinal motility (Greenwood-Van Meerveld et al., 2017; Mistiaen et al., 2002). Despite

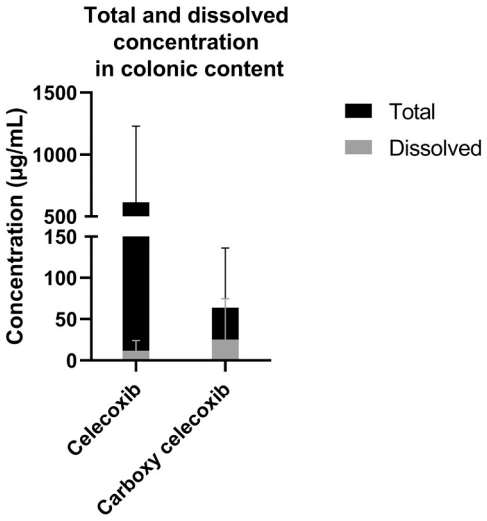

Fig. 6. Mean (+ SD) total and dissolved concentration of celecoxib and carboxy celecoxib in colonic contents sampled from healthy volunteers (N = 4) 7.5 h after oral intake of one tablet of Celebrex (200 mg celecoxib) with 240 mL of water under fasted conditions.

the longer T_{max}, it is still in-between the first and second colonoscopy, which is critical for our study design.

Fig. 5 depicts the mean concentrations of celecoxib and its main metabolite carboxy celecoxib in caecal tissue during the two colonoscopies (phase 1 and phase 2), in comparison with the systemic concentration-time profiles. As expected, the plasma concentration of celecoxib and its carboxy metabolite increased during phase 1. However, caecal tissue concentrations remained relatively low. No celecoxib could be detected in the luminal contents of the caecum, collected after 2.5 h (< 100 nM, limit of detection). The lack of celecoxib in the luminal contents is in line with the median gastric and small intestinal transit time of 56 and 255 min, respectively (Worsøe et al., 2011). Tissue accumulation is thus clearly plasma driven during phase 1.

During phase 2, tissue concentrations of the drug and its main metabolite were substantially higher than in phase 1, despite decreasing plasma concentrations. For instance, at 405 min after drug intake (phase 2), tissue concentrations of celecoxib and carboxy celecoxib were respectively 39- and 228-fold higher than after 105 min (phase 1). The level of carboxy celecoxib was 109-fold lower than the level of celecoxib. At the same time, relatively high levels of celecoxib and carboxy celecoxib were observed in the luminal contents, collected from the caecum after 7.5 h; total concentrations amounted to 614.4 ± 614.5 µg/mL and 63.8 ± 72.2 µg/mL, respectively (Fig. 6). These data clearly indicate that accumulation of celecoxib in caecal tissue is predominantly gut driven once the drug reaches the caecal lumen.

Our data in human volunteers corroborate earlier findings by the group of Paulson (2000) in rats: in the caecum or colon, the highest tissue concentration of celecoxib was found 8 h after oral administration, in contrast to the majority of other tissues, where maximum levels were seen after 1 h. Knowing that celecoxib is predominantly eliminated in feces after oral administration, the group of Paulson varied oral doses from 20 to 400 mg/kg in male rats, and observed that a higher dose leads to a higher recovery of celecoxib in faeces, which implies a higher fraction of unabsorbed drug. Paulson's group also reported that only 2% of intravenously administered celecoxib was recovered in urine or feces as the parent compound. Thus, the high level of celecoxib in colonic contents, observed in our study, most likely resulted from incomplete absorption in the upper gastrointestinal tract, and not from

biliary excretion. In contrast, extensive hepatic metabolization of absorbed celecoxib followed by biliary excretion likely contributes to the presence of carboxy celecoxib in colonic contents during phase 2 of the present study, even though intestinal secretion or colonic metabolization cannot be excluded.

Analysis of the luminal aspirates collected from the caecum in phase 2 of our study (7.5 h after drug intake) showed a difference between total (i.e., celecoxib as solid and solute; 614.4 ± 614.5 μg/mL) and dissolved (I.e., celecoxib as solute; 11.7 ± 12.5 μg/mL) drug concentrations, implying that celecoxib is mainly present in the solid state in the caecum. The mean solid fraction of celecoxib and carboxy celecoxib amounted to 0.91 ± 0.09 and 0.51 ± 0.32, respectively, which also indicates that the (probably) bile secreted carboxy celecoxib precipitates in the lumen. Although the total concentration of carboxy celecoxib (63.8 ± 72.2 μg/mL) was 8.9 times lower than celecoxib in colonic aspirates, the dissolved drug concentration of carboxy celecoxib is higher (25.4 ± 49.3 μg/mL). The large solid fraction of celecoxib reflects the low solubility of the compound in combination with the suboptimal dissolution conditions in the colonic lumen due to the scarce and scattered pockets (median 2 mL) of colonic fluid and the increase in viscosity from the proximal to distal lumen (Schiller et al., 2005). This may also explain why the highest tissue concentrations of celecoxib (Fig. 3) were observed in the caecum compared to the more distal regions, where dissolution probably becomes even harder. In addition to other factors, including gastrointestinal residence time, the poor dissolution conditions may also contribute to the highly variable drug concentrations that were observed in colonic aspirates and tissue.

When considering therapeutic efficacy, our *in vivo* studies reported a systemic C_{max} of 3.1 ± 2.0 μM in healthy volunteers, which is considerably higher than the IC_{50} (0.04 μM) of celecoxib for COX-2 (Penning et al., 1997). However, recent research indicates that the antineoplastic effect cannot be ascribed solely to COX-inhibition, but that many COX-independent pathways are involved, i.e. by repressing the PI3-K/PDPK1/Akt and Wnt/β-catenin oncogenic pathways (Vaish and Sanyal, 2012). The group of Egashira even reported that celecoxib can inhibit proliferation and induce apoptosis in HCT-116 cells independent of its action on COX-2 but by inducing TCF7L2 degradation (Egashira et al., 2017). It is noteworthy that the concentrations of celecoxib that are required to get an anti-carcinogenic effect in cell culture experiments are generally between 10 μM and 100 μM (Grösch et al., 2006), which are not reached in plasma by administering a single 200 mg dose. In this respect, the relatively high levels of celecoxib in the colonic lumen and the resulting accumulation in colonic tissue, which were demonstrated in the present study, seem essential to obtain the reported anti-carcinogenic effects.

4. Conclusion

By developing a protocol to reliably collect colonic tissue and contents, we were able to gain insight into the colonic disposition of the NSAID celecoxib. Intake of the oral dosage form Celebrex® initially results in limited plasma driven tissue accumulation in the colon. Several hours after drug intake, however, a substantial amount of celecoxib arrives at the colon due to incomplete absorption of this poorly soluble drug in the small intestine. Gut driven uptake then causes increased accumulation of celecoxib in colonic tissue, most likely enabling its inhibitory effect on the progression of colorectal cancer. As such, these data demonstrate the possible importance of colonic uptake of orally administered low solubility drugs, even without using a colon-targeted delivery strategy.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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References

Bertagnolli, M.M., Eagle, C.J., Zauber, A.G., Redston, M., Solomon, S.D., Kim, K., Tang, J., Rosenstein, R.B., Wittes, J., Corle, D., Hess, T.M., Woloj, G.M., Boisserie, F., Anderson, W.F., Viner, J.L., Bagheri, D., Burn, J., Chung, D.C., Dewar, T., Foley, T.R., Hoffman, N., Macrae, F., Pruitt, R.E., Saltzman, J.R., Salzberg, B., Sylwestrowicz, T., Gordon, G.B., Hawk, E.T., Study Investigators, A.P.C., 2006. Celecoxib for the prevention of sporadic colorectal adenomas. N. Engl. J. Med. 355, 873–884. https://doi.org/10.1056/NEJMoa061355.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., Jemal, A., 2018. Global cancer statistics 2018: GLOBOCON estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA. Cancer J. Clin. 68, 394–424. https://doi.org/10.3322/caac.21492.

Burn, J., Sheth, H., 2016. The role of aspirin in preventing colorectal cancer. Br. Med. Bull. https://doi.org/10.1093/bmb/ldw028.

Egashira, I., Takahashi-Yanaga, F., Nishida, R., Arioka, M., Igawa, K., Tomooka, K., Nakatsu, Y., Tsuzuki, T., Nakabeppu, Y., Kitazono, T., Sasaguri, T., 2017. Celecoxib and 2,5-dimethylcelecoxib inhibit intestinal cancer growth by suppressing the Wnt/β-catenin signaling pathway. Cancer Sci. 108, 108–115. https://doi.org/10.1111/cas.13106.

Fujimura, T., Ohta, T., Oyama, K., Miyashita, T., Miwa, K., Miyashita, T.-M., Surgery, G., 2006. gastrointestinal tract cancers: a review and report of personal experience development of selective COX-2 in-hibitors. China World J 12, 1336–1345.

Gong, L., Thorn, C.F., Bertagnolli, M.M., Grosser, T., Altman, R.B., Klein, T.E., 2012. Celecoxib pathways: pharmacokinetics and pharmacodynamics. Pharmacogenet. Genomics 22, 310–318. https://doi.org/10.1097/FPC.0b013e32834f94cb.

Greenwood-Van Meerveld, B., Johnson, A.C., Grundy, D., 2017. Gastrointestinal Physiology and Function. Springer, pp. 1–16. https://doi.org/10.1007/164_2016_118. Cham.

Grösch, S., Maier, T.J., Schiffmann, S., Geisslinger, G., 2006. Cyclooxygenase-2 (COX-2)–Independent anticarcinogenic effects of selective COX-2 inhibitors. JNCI J. Natl. Cancer Inst. 98, 736–747. https://doi.org/10.1093/jnci/djj206.

Herendeen, J.M., Lindley, C., 2003. Use of NSAIDs for the chemoprevention of colorectal cancer. Ann. Pharmacother. https://doi.org/10.1345/aph.1C489.

Mistiaen, W., Blockx, P., Van Hee, R., Bortier, H., Harrisson, F., 2002. The effect of stress on gastric emptying rate measured with a radionuclide tracer. Hepatogastroenterology 49, 1457–1460.

Paulson, S.K., Vaughn, M.B., Jessen, S.M., Lawal, Y., Gresk, C.J., Yan, B., Maziasz, T.J., Cook, C.S., Karim, A., 2001. Pharmacokinetics of celecoxib after oral administration in dogs and humans: effect of food and site of absorption. J. Pharmacol. Exp. Ther. 297.

Paulson, S.K., Zhang, J.Y., Breau, A.P., Hribar, J.D., Liu, N.W., Jessen, S.M., Lawal, Y.M., Cogburn, J.N., Gresk, C.J., Markos, C.S., Maziasz, T.J., Schoenhard, G.L., Burton, E.G., 2000. Pharmacokinetics, tissue distribution, metabolism, and excretion of celecoxib in rats. Drug Metab. Dispos. 28, 514–521.

Penning, T.D., Talley, J.J., Bertenshaw, S.R., Carter, J.S., Collins, P.W., Docter, S., Graneto, M.J., Lee, L.F., Malecha, J.W., Miyashiro, J.M., Rogers, R.S., Rogier, D.J., Yu, S.S., Anderson, G.D., Burton, E.G., Cogburn, J.N., Gregory, S.A., Koboldt, C.M., Perkins, W.E., Seibert, K., Veenhuizen, A.W., Zhang, Y.Y., Isakson, P.C., 1997. Synthesis and biological evaluation of the 1,5-Diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-Methylphenyl)-3- (trifluoromethyl)-1H -pyrazol-1-yl]benzenesulfonamide (SC-58635, Celecoxib). J. Med. Chem. 40, 1347–1365. https://doi.org/10.1021/jm960803q.

Pfizer, 2016. CELEBREX- celecoxib capsule [WWW Document]. URL http://labeling.pfizer.com/ShowLabeling.aspx?id=793 (accessed 1.10.20).

Schiller, C., FROHLICH, C.-P., GIESSMANN, T., SIEGMUND, W., MONNIKES, H., HOSTEN, N., WEITSCHIES, W., 2005. Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. Aliment. Pharmacol. Ther. 22, 971–979. https://doi.org/10.1111/j.1365-2036.2005.02683.x.

Vaish, V., Sanyal, S.N., 2012. Role of Sulindac and Celecoxib in the regulation of angiogenesis during the early neoplasm of colon: exploring PI3-K/PTEN/Akt pathway to the canonical Wnt/β-catenin signaling. Biomed. Pharmacother. 66, 354–367. https://doi.org/10.1016/J.BIOPHA.2012.01.004.

Werner, U., Werner, D., Pahl, A., Mundkowski, R., Gillich, M., Brune, K., 2002. Investigation of the pharmacokinetics of celecoxib by liquid chromatography-mass spectrometry. Biomed. Chromatogr. 16, 56–60. https://doi.org/10.1002/bmc.115.

Worsøe, J., Fynne, L., Gregersen, T., Schlageter, V., Christensen, L.A., Dahlerup, J.F., Rijkhoff, N.J.M., Laurberg, S., Krogh, K., 2011. Gastric transit and small intestinal transit time and motility assessed by a magnet tracking system. BMC Gastroenterol. 11, 145. https://doi.org/10.1186/1471-230X-11-145.

Youle, M.S., Read, N.W., 1984. Effect of painless rectal distension on gastrointestinal transit of solid meal. Dig. Dis. Sci. 29, 902–906. https://doi.org/10.1007/BF01312478.