Article

The effect of esomeprazole on the upper GI tract release and systemic absorption of mesalazine from colon targeted formulations

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Abstract

The aim of the present study was to investigate the effect of coadministration of the proton pump inhibitor (PPI) esomeprazole on the upper GI tract behavior and systemic exposure of mesalazine from two mechanistically different colon targeted delivery systems: Claversal (pH-dependent release) and Pentasa (prolonged release). To this end, gastric, jejunal and systemic concentrations of mesalazine and its metabolite N-acetyl mesalazine were monitored in 5 healthy volunteers following oral intake of Pentasa or Claversal with or without PPI pre-treatment (cross-over study). Our exploratory study demonstrated that pre-treatment with a PPI may affect the release and absorption of mesalazine from formulations with different modified release mechanisms. Upon intake of Claversal, the onset of mesalazine absorption was accelerated substantially by PPI pre-treatment. While the PPI-induced increase in pH initiated the disintegration process already in the upper GI tract, the release of mesalazine started beyond the proximal jejunum. Upon intake of Pentasa, PPI pre-treatment seemed to increase the systemic exposure, even though the underlying mechanism could not be revealed yet. The faster release of mesalazine in the GI tract and/or the increased systemic absorption following PPI pre-treatment may reduce the ability of mesalazine to reach the colon. Future research assessing mesalazine disposition in the lower GI tract is warranted.

Keywords

PPI effect; drug absorption; clinical trial; gastrointestinal release; colon targeted drug delivery systems; mesalazine

1. Introduction

Oral administration of mesalazine is recommended as first line therapy to induce and maintain remission in patients with mild to moderate ulcerative colitis (Böhm and Kruis, 2014; Goyanes et al., 2015; Sandborn and Hanauer, 2003), acting topically from the colonic lumen to target inflammatory pathways (Goyanes et al., 2015). The need for high local intraluminal concentrations of mesalazine to exert its therapeutic effect has resulted in the development of colon targeted formulations. Such formulations aim to reduce absorption from the small intestine to maximize local concentrations at the site of action while limiting systemic exposure and possible adverse effects. For a recent in-depth review of colon-targeted formulations, we refer to Awad et al. (2022). For mesalazine, different delivery systems are available to reach the lower segments of the gut, based on prolonged release, pH- or microbiota-triggered release, or combined mechanisms (Lemmens et al., 2021).

Claversal is an example of a pH-dependent mesalazine formulation with Eudragit-L coating disintegrating at pH ≥ 6, targeting the mid to distal ileum and colon as the sites of delivery. Other pH-dependent formulations are manufactured with Eudragit-S coating (i.e., Asacol) releasing the drug at pH ≥ 7 in the terminal ileum and colon (Lemmens et al., 2021). Pentasa is a prolonged release formulation gradually releasing mesalazine from ethylcellulose-coated microgranules starting from the duodenum to the rectum (Lemmens et al., 2021). In comparison with pH-dependent release systems, prolonged release systems are less affected by interindividual variability in pH but more by differences in GI transit times. MultiMatrix systems (i.e. Mezavant) combine both pH-dependent and prolonged release mechanisms in order to overcome the disadvantages of one single mechanism (Lemmens et al., 2021). The recently marketed dosage form Asacol 1600 mg makes use of a dual pH- and microbiota-triggered coating (Varum et al., 2020a,b).

The effectiveness of these colon targeted systems heavily depends on the release profile of mesalazine, which should focus on the lower GI-tract but can be altered upon changes in the GI environment. In this respect, concomitant therapy with a proton pump inhibitor (PPI) may affect the performance of modified release systems susceptible to PPI-induced changes in GI physiology, including mesalazine formulations.

PPIs are widely prescribed to treat gastroesophageal reflux (GERD) and peptic ulcer bleeding by reducing gastric fluid secretion (Strand et al., 2017). The resulting increase in gastric pH, however, may also affect the dissolution and absorption of ionizable drug compounds (Zhang et al., 2014). In particular, the absorption of various poorly soluble weakly basic drugs, depending on adequate dissolution in the acidic stomach, significantly drops upon PPI intake. In addition, PPI treatment may reduce the available gastric and duodenal fluid volumes, again affecting dissolution and absorption of poorly soluble drugs (de Waal et al., 2020).

While the impact of concomitant PPI treatment on the performance of immediate release formulations for poorly soluble drugs has been widely recognized, the role of PPI-induced changes in the GI environment on the behavior of modified release systems is less clear (Patel et al., 2020). For instance, the increased gastric pH upon PPI intake could, in theory, cause premature release of mesalazine in the upper GI tract from pH-dependent colon targeted delivery systems, thereby lowering drug concentrations at the site of action. In addition, alterations in GI pH may affect the dissolution of mesalazine, considering its ionization behaviour with an acidic pK_a of 2.30 and a basic pK_a of 5.69 (French and Mauger, 1993). Therefore, the aim of the present study was to investigate the effect of coadministration of the PPI esomeprazole on the upper GI tract behavior and systemic uptake of mesalazine from two mechanistically different colon targeted delivery systems. To this end, an established GI aspiration approach (Augustijns et al., 2020) was employed to monitor gastric, jejunal and systemic concentrations of mesalazine in healthy volunteers following oral intake of the previously mentioned Pentasa or Claversal formulations with or without esomeprazole pre-treatment. In addition to mesalazine, concentrations of the metabolite N-acetyl mesalazine were monitored. Mesalazine can be acetylated by N-acetyl transferase in both the gut wall and the liver (Vree et al., 2000). N-acetyl mesalazine has some therapeutic activity, but can be secreted into the intestinal lumen by transporter mediated efflux (Shin et al., 2009).

2. Materials and methods

2.1 Chemicals

Mesalazine (5-aminosalicylic acid), N-acetyl mesalazine (N-acetyl-5-aminosalicylic acid), sodium phosphate monobasic monohydrate ($NaH_2PO_4.H_2O$), ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), sodium metabisulfite and sodium thiosulfate were bought from Sigma Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO, 99.9%, for spectroscopy), methanol (MeOH), acetonitrile HPLC grade (ACN) and ammonium formate were purchased from Acros Organics (Geel, Belgium). Sodium chloride (NaCl) and formic acid were supplied by VWR (Leuven, Belgium), deuterated mesalazine ($^2H_3$-mesalazine) and deuterated N-acetyl mesalazine ($^2H_3$-N-acetyl mesalazine) by Toronto Research Chemicals Inc. (North York, Canada), acetonitrile ULC/MS by Biosolve BV (Valkenswaard, The Netherlands), sodium hydroxide (NaOH) pellets by Merck (Darmstadt, Germany), hydrochloric acid (HCl, 1M) by Fisher Scientific (Leicestershire, U.K.) and Fasted State Simulated Intestinal Fluid (FaSSIF)/ Fed State Simulated Intestinal Fluid (FeSSIF)/ Fasted State Simulated Gastric Fluid (FaSSGF) powder by Biorelevant.com (Croydon, UK). Water was purified using a Maxima system (Elga Ltd., High Wycombe Bucks, UK).

2.2 Clinical study

2.2.1 Study Medication

Pentasa tablets for prolonged release (500 mg mesalazine, ethylcellulose-coated microgranules; Faes Farma S.A., Leioa, Spain), Claversal tablets for pH-dependent release (500 mg mesalazine, Eudragit L coating; Ferring N.V., Aalst, Belgium) and Nexiam tablets (40 mg esomeprazole; SA Grünenthal NV, St-Stevens-Woluwe, Belgium) were ordered via the hospital pharmacy of the University Hospitals Leuven (UZ Leuven, Belgium).

2.2.2 Study protocol

To study the effect of esomeprazole on the behavior of colon targeted formulations of mesalazine, an explorative crossover study was performed in 5 healthy volunteers (2 men, 3 women; age range 22-26 years old; BMI range 20.2-25.3 kg/m^2). Exclusion criteria for participation included illness at the time of the study, allergy for salicylic derivatives, medication use (excluding contraceptives), history of acute/chronic GI disease(s), and pregnancy. The study followed the tenets of the Declaration of Helsinki and was approved by the Federal Agency for Medicines and Health Products (FAMHP; EudraCT reference study number 2019-001009-26) and the Ethics Committee Research UZ/KU Leuven (reference number S62903). All volunteers provided written informed consent prior to participation.
In each volunteer, 4 conditions were tested at different days (non-blinded, non-randomized, washout period: at least 7 days):

1. oral intake of one Pentasa tablet (500 mg mesalazine)
2. oral intake of one Claversal tablet (500 mg mesalazine)
3. oral intake of one Pentasa tablet (500 mg mesalazine) following PPI pre-treatment
4. oral intake of one Claversal tablet (500 mg mesalazine) following PPI pre-treatment

Throughout this manuscript, conditions 1 and 2 are referred to as control conditions; conditions 3 and 4 are referred to as PPI conditions. All conditions were evaluated in the fasted state. For the PPI conditions, volunteers were pre-treated with a once-daily dose of Nexiam (40 mg esomeprazole, corresponding to the recommended daily dose) in the morning, starting two days prior to the test day; a third and final dose was taken in the morning of the test day.
Before the start of each test day at the University Hospitals Leuven (department of Gastroenterology), participants were asked to refrain from eating 12 h prior to the start of the study to ensure fasted state conditions. To collect GI fluids, two aspiration catheters were inserted via nasal intubation. A double-lumen aspiration catheter (Salem Sump PVC Gastroduodenal Tube, 14 Ch (4.7 mm) × 108 cm; Medtronic; Dublin, Ireland) was placed in the subject's stomach (antrum) and another, customized, catheter (Customized Reusable Aspiration 2 Channel Catheter, 14F8 (4.6 mm) × 200 cm; MUI Scientific;

Mississauga, Ontario, Canada) was placed in the proximal jejunum. Catheter position was verified using fluoroscopic imaging. In addition, an intravenous catheter was placed for repeated blood sample collection.

Afterwards, volunteers ingested one tablet of Pentasa or Claversal with 240 mL of tap water (t = 0) and sampling of GI fluids and blood was initiated. Considering the expected differences in the release and absorption profiles of mesalazine, different sampling schedules were applied for Pentasa and Claversal. Gastric and jejunal fluids were collected (< 3 mL) at predetermined time points (Pentasa: every 15 minutes for 5 h, Claversal: at 30, 60, 90, 120, 150, 180, 195, 210, 225, 240, 255, 270, 285 and 300 min after drug intake). Participants remained seated in a hospital bed (i.e., semi-supine position) for the entire aspiration period and were not allowed to eat or drink. After removal of both catheters (i.e., after 5 h), participants were allowed to drink and eat. In addition to the aspiration of GI fluids, venous blood samples were collected (5 mL) in heparinized tubes (BD Vacutainer systems, Plymouth, U.K.) for 24 h. For Pentasa, blood samples were collected at 30, 60, 90, 120, 140, 160, 180, 200, 220, 240, 270, 300, 330, 360, 390, 420, 450, 480, 510 and 540 min to end with a blood sample at 24 h after oral drug intake. For Claversal, blood samples were collected at 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 380, 400, 420, 440, 460, 480, 500, 520 and 540 min to end with a blood sample at 24 h after oral drug intake.

2.2.3 Processing of gastrointestinal samples

The pH of the collected gastric and jejunal samples was determined after aspiration using a BioTrode instrument (Hamilton, Bonaduz, Switzerland) calibrated prior to use. A part of the aspirate was centrifuged at 20,817g for 5 min (Microcentrifuge 5424, VWR International) to separate solid and dissolved compounds. Supernatant was then diluted 5 to 1000 times with sodium phosphate buffer (40 mM, pH 7.5) supplemented with sodium thiosulphate. Diluted samples were stored on ice pending quantification of mesalazine and N-acetyl mesalazine on the same day (see 2.4).

2.2.4 Processing of plasma samples

Blood samples were kept on ice during the study day and centrifuged at the end of the experiment (2880 g, 10 min, 4°C; Centrifuge 5804R, Eppendorf, Hamburg, Germany). Subsequently, plasma was stored at −20°C pending analysis. To quantify concentrations of mesalazine and N-acetyl mesalazine, 180 µL of acetonitrile containing internal standard (500 nM of [$^2H_3$]-mesalazine and [$^2H_3$]-N-acetyl mesalazine), 10 µM EDTA and 10 µM sodium metabisulfite was added to 20 µL of plasma and vortexed for 20 s to ensure protein precipitation. After centrifugation (20,817 g, 10 min, 4°C), 1 µL of the supernatant was injected into a UPLC system for quantification of mesalazine and N-acetyl mesalazine (see 2.4).

2.3 In vitro dissolution and solubility testing

To assess the dissolution kinetics of mesalazine from a Claversal tablet at different pH values encountered during GI transit, a three-stage dissolution experiment was performed. Biorelevant media were prepared according to instructions provided by Biorelevant.com. FaSSGF was prepared by adding 0.06 mg/mL FaSSIF/FeSSIF/FaSSGF powder to blank FaSSGF (1.99 mg/mL NaCl, adjusted to pH 1.6 with 1 M HCl). FaSSIF was obtained by dissolving 2.24 mg/mL FaSSIF/FeSSIF/FaSSGF powder in blank FaSSIF (0.42 mg/mL NaOH, 3.95 mg/mL $NaH_2PO_4.H_2O$ and 6.19 mg/mL NaCl, adjusted to pH 6.5 with 1 M HCl or 2 M NaOH). Double concentrated FaSSIF was prepared by dissolving double amounts of NaOH, $NaH_2PO_4.H_2O$, NaCl and FaSSIF/FeSSIF/FaSSGF powder compared to normal FaSSIF. The pH was adjusted to 7.5 with 2 M of NaOH, so that, after addition of 100 mL of double concentrated FaSSIF to 100 mL of FaSSGF, the final pH amounted to 6.5.

To simulate the control condition, the dissolution experiment was initiated by adding 1 tablet of Claversal (500 mg mesalazine) to 100 mL of FaSSGF in a beaker. After 30 min, 100 mL of double concentrated FaSSIF was added to the entire contents of the vessel. After 210 min, pH of the medium was adjusted to 7.5. To simulate the PPI condition, 1 tablet of Claversal was added to 100 mL of FaSSIF in a beaker. After 30 min, an extra amount of 100 mL of FaSSIF was added and followed by a pH change to 7.5 after 210 min. During the dissolution tests, the contents of the vessels were magnetically stirred (300 rpm, 37 °C). Samples were taken after 5, 10, 20, 30, 35, 40, 50, 60, 70, 80, 90, 110, 130, 150, 170, 190, 210, 215, 220, 230 and 240 min. Following centrifugation (20,817g, 5 min, room temperature), the supernatants were diluted 5 to 1000 times with a 40 mM sodium phosphate buffer pH 7.5 supplemented with 0.1 m/v% sodium thiosulphate as an antioxidant and analyzed immediately by HPLC analysis.

To explore the effect of increased pH upon PPI intake on the intestinal dissolution kinetics of mesalazine from Pentasa, a single-stage dissolution experiment without an initial gastric compartment was performed in FaSSIF-based media adjusted to different pH values (6.0, 6.5 or 7.0) by using 1 M HCl or 2 M NaOH.The experiment was initiated by adding 1 tablet of Pentasa (500 mg mesalazine) to 200 mL of FaSSIF in a beaker. During the dissolution test, the contents of the vessels were magnetically stirred (300 rpm, 37 °C). Samples were taken after 5, 10, 20, 30, 60, 90, 120, 150, and 180 min. The samples were immediately centrifuged, and the supernatants were diluted as mentioned above before being analyzed by HPLC.

The thermodynamic solubility of mesalazine was determined in FaSSIF using the shake flask method (Wuyts et al., 2013). Briefly, an excess of mesalazine powder (5 mg) was added to a microcentrifuge tube containing 300 µl of the above mentioned FaSSIF media at different pH-values. The tubes were placed in a prewarmed shaking incubator (KS4000i incubator, Ika, Staufen, Germany) at 37°C and 200

rpm. After 3 h, samples were centrifuged for 30 min at 20,817g and 37°C to separate the solid phase from the dissolved fraction. Subsequently, the supernatants were diluted 1000 times with sodium phosphate buffer (40 mM, pH 7.5) supplemented with sodium thiosulphate and analyzed by HPLC (see 2.4).

2.4 Quantification of mesalazine and N-acetyl mesalazine

2.4.1 Human and simulated GI fluids (clinical study & in vitro testing)

The concentrations of mesalazine and N-acetyl mesalazine in the diluted human and simulated GI fluids from the clinical study (see 2.2) and the in vitro dissolution testing (see 2.3) were determined using reversed-phase HPLC and fluorescence detection. A volume of 100 µL was injected into an Alliance Waters 2695 HPLC system (Waters). Compounds were separated using gradient elution on a Novapak C18 column (pore size 60 A, particle size 4 µm, 8 mm i.d. x 100 mm, Waters, Milford, MA, USA) under radial compression and at room temperature. Elution started with 5% methanol and 95% sodium phosphate buffer (40 mM, pH 7.5) at a flow rate of 1.0 mL/min. After 5.0 min, methanol was increased to 15% over 0.5 min. After 11.0 min, the column was rinsed with methanol:water (50:50 v/v) and reconditioned with the initial mobile phase for 6.0 min. Retention times of mesalazine and N-acetyl mesalazine amounted to 5.6 and 12.2 min, respectively. Both compounds were detected using fluorescence detection at 330 nm (excitation)/490 nm (emission) (FP-1520 fluorescence detector, Jasco).

Calibration curves were made in the initial mobile phase supplemented with blank gastrointestinal fluids and spiked with mesalazine and N-acetyl mesalazine. Linearity was demonstrated over the range of 10 µM to 2.5 nM for both compounds; all samples were diluted to fit in this range. Quality control samples were prepared in corresponding relevant media containing 1000, 10 and 5 nM of mesalazine and N-acetyl mesalazine. Concentrations of both compounds could be determined precisely (within-run RSD ≤ 10.1% and between-run RSD ≤ 14.5%) and accurately (relative bias <15%).

2.4.2 Plasma samples

The levels of mesalazine and N-acetyl mesalazine in the processed plasma samples from the clinical study (see 2.2) were determined by an LC-MS/MS method. Therefore, an Acquity H-class UPLC system coupled to a Xevo TQ-S micro triplequadrupole analyzer equipped with an HESI probe was used (Waters, Milford, MA, USA). Separation was achieved using an Acquity UPLC BEH Amide column (1.7 µm, 50 x 2.1 mm, Waters, Milford, MA, USA) and using acetonitril (solvent A), water (solvent B) and 200 mM ammonium formate adjusted to pH 3 with formic acid (solvent C) as eluents. The elution gradient was as follows: 0-2.0 min, 95% solvent A and 5% solvent C; 2.1-3.1 min, 60% solvent A and 40% solvent B; 3.2-14.0 min, 95% solvent A and 5% solvent C for reconditioning. The flow rate was 0.5 ml/min. Column temperature was held at 35 °C and the injection volume was 1 µL. A positive ionization

MS/MS mode was set for the mass spectrometer for all compounds with following parameters: source temperature 150 °C, capillary voltage 0.50 kV, cone voltage 41 V, cone gas flow 0 L/h, desolvation gas flow 1000 L/h and desolvation temperature 500 °C. The mass transitions (m/z) followed were 153.0-78.9 (mesalazine), 156.0-81.9 ($^2H_3$-mesalazine), 196.0-135.9 (N-acetyl mesalazine) and 199.1-137.1 ($^2H_3$-N-acetyl mesalazine). Mesalazine and $^2H_3$-mesalazine eluted at 1.73 min, whereas N-acetyl mesalazine and $^2H_3$-N-acetyl mesalazine eluted at 1.36 min. Stock solutions of mesalazine, $^2H_3$-mesalazine, N-acetyl mesalazine and $^2H_3$-N-acetyl mesalazine were prepared in DMSO at a concentration of 13 mM, 6.4 mM, 6.25 mM and 12.6 mM, respectively. Calibration curves were made by serial dilution in plasma. Linearity was demonstrated over the range of 50 µM to 25 nM for both compounds. Quality control samples were prepared according to the same sample preparation as previously discussed containing 10, 1 and 0.1 µM of mesalazine and N-acetyl mesalazine. Concentrations of both compounds could be determined precisely (within-run RSD ≤ 9.7% and between-run RSD ≤ 14.0%) and accurately (relative bias < 12%).

2.5 Data presentation

This study was designed as exploratory, i.e., without the intention to formally test hypotheses. As such, the data obtained are descriptive in nature and do not allow statistical comparison. pH is presented as mean + standard deviation (SD) over time and is also summarized in box-whisker plots. Plasma concentration-time profiles and intraluminal concentration-time profiles are presented as mean (+ SD). Systemic pharmacokinetic parameters (i.e., AUC, C_{max}, T_{max} and T_{lag}) are expressed as median values with minimum and maximum values between brackets. Dissolution-time profiles are presented as mean + SD whereas solubility is expressed as mean (± SD).

3. Results and discussion

3.1 Intraluminal pH profiles

The reduction of gastric acid secretion upon PPI intake is known to alter the gastrointestinal environment. In the present study, we first assessed the effect of esomeprazole pre-treatment on the pH of fluids aspirated from the stomach and the proximal jejunum after oral intake of the mesalazine formulations Pentasa and Claversal. Both gastric and jejunal pH were relatively stable over time in all conditions (Figure 1). Only in the first 30 min after administration of Pentasa with 240 mL of water without PPI pre-treatment, a temporary increase in the mean fasted state pH of the stomach was observed. This effect, which is not uncommon immediately after ingestion of a substantial amount of water (Litou et al., 2016; Van Den Abeele et al., 2020), could not be observed after intake of Claversal, in which case the first sample was aspirated only after 30 min instead of 15 min in the Pentasa condition.

When summarizing the pH-values of all aspirates in the control versus the PPI condition (Figure 2), it is clear that PPI pre-treatment resulted in a substantially elevated gastric pH: median pH amounted to 1.5 (IQR 1.4-1.8) in the control condition and 7.0 (IQR 6.7-7.3) in the PPI condition. This observation is in line with previous studies employing the same PPI regimen (i.e., 40 mg esomeprazole once-daily for three days) (Hens et al., 2020; Rubbens et al., 2016; Van Den Abeele et al., 2020) and is also similar to the effect of other PPIs, such as pantoprazole (Litou et al., 2016). Interestingly, not only the gastric pH was elevated by pre-treatment of esomeprazole; also in the proximal jejunum a substantial increase in median pH was observed from 6.1 (IQR 5.8-6.5) in the control condition to 6.8 (IQR 6.6-7.2) in the PPI condition (Figure 2B). Previous studies suggested that the PPI effect on pH would be fully attenuated in the distal duodenum or jejunum (Gan et al., 1997; Michalek et al., 2011). In those studies, however, food intake may have masked a possible effect, while the present study was performed in fasted state conditions.

3.2 Claversal

3.2.1 Systemic drug disposition

The observed PPI-induced increase in the upper GI pH could impact the release and absorption of mesalazine from Claversal, a pH-dependent delayed release formulation. Figure 3 depicts the mean plasma concentrations of mesalazine and its metabolite N-acetyl mesalazine as a function of time after intake of Claversal. PPI pre-treatment resulted in the earlier appearance of the drug in the systemic circulation: the median T_{lag} of mesalazine of 400 min (range 270 - 480 min) in the control condition decreased to 330 min (range 210 - 330 min) in the PPI condition. Similar results were observed for its metabolite: the median T_{lag} of N-acetyl mesalazine amounted to 420 min (range 270 - 480 min) in the control condition and 330 min (range 210 - 360 min) in the PPI condition. The earlier systemic appearance of mesalazine upon PPI pre-treatment also resulted in a reduced time to reach to maximal plasma concentrations. Upon PPI intake, median T_{max} -values of 420 min (range 300 - 480 min) and 460 min (range 360 - 540 min) were observed for mesalazine and acetyl mesalazine, respectively. In the control condition, however, the concentration-time profiles suggest T_{max}-values above 540 min (outside the sampling window).

The observed T_{lag} and T_{max} for mesalazine in the control condition appeared higher than the values reported in the summary of product characteristics of Claversal (T_{lag} between 3 and 4 h, T_{max} of 5 h). Also previously reported T_{max}-values for similar Eudragit-L coated tablets of mesalazine were lower: 5.1 h, 5.5 h and 6.5 h for Apriso, Mesasal and Salofalk, respectively (Sandborn and Hanauer, 2003; Yu et al., 2017). Wilding et al. did observe a higher T_{max} (8.3 ± 1.1 h) when administering 3 tablets of 500 mg Claversal (Wilding et al., 2003).

3.2.2 Intraluminal drug disposition

Overall, the observed earlier onset of absorption suggests faster release of mesalazine in the GI tract upon PPI pre-treatment. To explore a possible link between the observed PPI effect on mesalazine absorption and its release in the upper GI tract, we determined mesalazine concentrations in fluids aspirated from the stomach and proximal jejunum after oral intake of Claversal. In the control condition, no release of mesalazine was observed from the Claversal tablet within 5 h after intake (data not shown), most likely due to the acidic gastric pH (median 1.5) and the jejunal pH (median 6.1), which were below or only slightly above the disintegration threshold of the tablet's Eudragit L coating (pH ≥ 6 (Lemmens et al., 2021)). In the PPI condition, however, the observed increase in the pH of the GI fluids (median pH in stomach and proximal jejunum amounted to 7.0 and 6.8, respectively, see Figure 2) implies that the Claversal tablet was exposed to pH-values above its disintegration threshold immediately after intake. Still, also upon PPI pre-treatment, no or only minimal release could be observed in the stomach and proximal jejunum during 5 h after drug intake (data not shown). Only in a single subject (HV03), higher mesalazine concentrations were measured in jejunal fluids within the 5 h sampling window, but these could not be linked to systemic absorption (T_{lag} 5.5 h). The overall absence of dissolved mesalazine in the fluids aspirated from the proximal jejunum indicates that the earlier release of mesalazine from Claversal in the PPI condition, as evidenced by the systemic appearance after 4 hours (Figure 3), most likely occurred further down the GI tract.

Simple three-stage in vitro dissolution experiments, in which Claversal tablets were exposed to a series of pH values (1.6 – 6.5 – 7.5 versus 6.5 – 6.5 – 7.5 to simulate control versus PPI condition) confirmed that mesalazine release does not start immediately after exceeding the disintegration threshold pH. Dissolved mesalazine was observed only after dissolution times of 130 and 170 min in simulated PPI and control condition, respectively (Figure 4). These observations were in line with a previous study (Karkossa and Klein, 2018). The reduction in dissolution lag time in the simulated PPI condition (40 min) almost corresponded to the time (30 min) of the first, 'gastric' dissolution stage at either pH 1.6 (below the disintegration threshold) or pH 6.5 (above the disintegration threshold). Either way, it took at least 2 h of disintegration time before mesalazine release could be observed, even though Claversal was tested in optimal in vitro dissolution conditions. In vivo, however, less optimal dissolution conditions (i.e., scattered small intestinal fluid pockets (Schiller et al., 2005)) might have delayed drug release from Claversal tablets even more, resulting in the substantial lag in mesalazine absorption (Figure 3). In addition, Karkossa et al. observed a more pronounced delay in the in vitro dissolution of mesalazine when using physiologically relevant bicarbonate-based intestinal media (Karkossa and Klein, 2018) as compared to phosphate-buffered media that were also used in the current study.

3.3 Pentasa

3.3.1 Systemic drug disposition

Figure 5 depicts the mean plasma concentrations of mesalazine and its metabolite N-acetyl mesalazine as a function of time after intake of a Pentasa tablet containing ethylcellulose-coated microgranules with or without PPI pre-treatment. A summary of the pharmacokinetic data is provided in Table 1. In both conditions, mesalazine and acetyl mesalazine already appeared in the first or second plasma sample (30 – 60 min), followed by gradual absorption which is in accordance with literature (Sandborn and Hanauer, 2003; Yu et al., 2017). The almost immediate onset of mesalazine absorption from Pentasa sharply contrasts with absorption from Claversal (median $T_{lag\ control}$ 400 min), and is linked to the different release mechanisms, with Pentasa gradually releasing mesalazine from ethylcellulose-coated microgranules, and Claversal releasing mesalazine only after disintegration of the Eudragit-L coating (Lemmens et al., 2021).

In contrast to the pH-dependent release system of Claversal, the prolonged release formulation Pentasa is less likely to be affected by the increased upper GI pH, observed upon PPI pre-treatment. While the median values of both C_{max} and T_{max} were higher in the PPI condition (Table 1), it should be noted that both parameters exhibit large intersubject variability in line with the absence of a distinct absorption peak. The high variation observed for T_{max} was in accordance with the summary of product characteristics of Pentasa (reported T_{max} between 1 and 6 h). However, the mean plasma concentration-time profiles (Figure 5) suggest a slight PPI-induced increase in systemic exposure to mesalazine and N-acetyl mesalazine. Indeed, $AUC_{0\text{-}24h}$ increased for all volunteers upon PPI pre-treatment (increase between 22% and 101%), as can be seen in Figure 6.

3.3.2 Intraluminal drug disposition

Upon oral administration, a Pentasa tablet is expected to release its ethylcellulose-coated microgranules in the stomach. From the gastric concentration-time profiles in the present study (Figure 7), it is clear that mesalazine started to dissolve immediately, followed by a gradual release. Substantial but highly variable concentrations of mesalazine could be measured for a long period of time, indicating variable retention of the microgranules in the stomach. Median C_{max} amounted to 602.4 ng/mL (range 9.3 - 1087.3 ng/mL) in the control condition and 232.9 ng/mL (84.4 - 465.9 ng/mL) in the PPI condition. Only minimal concentrations of acetylated mesalazine were observed in the stomach (C_{max}: 1.2 ± 1.9 ng/mL, data not shown).

Based on the mean profiles depicted in Figure 8, mesalazine concentrations in the proximal jejunum seemed to increase upon PPI pre-treatment: median C_{max} amounted to 795.0 ng/mL (range 384.9 -

1768.6 ng/mL) in the PPI condition and 259.3 ng/mL (range 30.3 - 757.9 ng/mL) in the control condition. Even though Pentasa does not contain a pH-dependent release mechanism, the PPI-induced pH increase in the upper GI tract, observed in our study (Figure 2), could possibly affect the solubility and dissolution of mesalazine. Indeed, in vitro dissolution tests in FaSSIF-based media, set at pH-values relevant for the jejunal fluids collected in the PPI condition, demonstrated an increasing dissolution rate of mesalazine from Pentasa as a function of pH (Figure 9). After 3 h, the extent of dissolution amounted to 28.1 ± 2.3%, 23.4 ± 1.3% and 17.3 ± 1.4% in FaSSIF at pH 7.0, 6.5 and 6.0, respectively. A similar trend was observed for the solubility of mesalazine which increased upon increasing pH (FaSSIF $_{pH\ 7.0}$: 3.50 ± 0.07 mg/mL; FaSSIF $_{pH\ 6.5}$: 2.26 ± 0.28 mg/mL;FaSSIF $_{pH\ 6.0}$: 1.73 ± 0.05). The observed effect on the dissolution and solubility of mesalazine is consistent with the ionization behavior of mesalazine (acidic pK_a 2.30, basic pK_a 5.69) (French and Mauger, 1993), with conversion of zwitterionic species into negatively charged species at increasing intestinal pH.

Although the increasing dissolution rate of mesalazine with increasing intestinal pH may have contributed to the higher mean jejunal mesalazine concentrations in the PPI condition (Figure 8), no link between intraluminal pH, jejunal and systemic mesalazine concentrations could be revealed from the individual data of our clinical study. In this respect, it should be noted that such correlations can be hard to identify, considering the inhomogeneity of intestinal contents and the sampling at only one site in the intestine.

In the control condition, N-acetyl mesalazine concentrations (median $C_{max\ control}$: 260.0 ng/mL, range: 126.1 - 892.5 ng/mL) were nearly equivalent to mesalazine concentrations, indicating an extensive metabolism in the proximal jejunum due to N-acetyl transferase activity in the gut wall (Vree et al., 2000). In the PPI condition, however, a relative decrease in the intraluminal concentration of metabolite versus the parent molecule was observed for each subject (Figure 10). This observation suggests that the PPI pre-treatment may have affected the acetylation of mesalazine in the enterocytes and/or the efflux of acetylated mesalazine into the intestinal lumen. Further research on the possible interaction of esomeprazole with these processes is warranted, since literature does not provide a clear explanation (Kamishikiryo et al., 2013; Pauli-Magnus et al., 2001; Shin et al., 2009; Zhou et al., 1999). The reduced intestinal formation and/or efflux of N-acetyl mesalazine in the PPI condition (Figure 10) may have contributed to the increase in systemic exposure (Figure 6). It should be noted, however, that our data did not show a clear link between both observations.

4. Conclusions

Our exploratory study demonstrated that pre-treatment with the PPI esomeprazole may affect the release and absorption of mesalazine from colon targeted formulations with different modified release mechanisms. Upon intake of Claversal, a pH-dependent formulation, the onset of mesalazine

absorption was accelerated substantially by PPI pre-treatment. While the PPI-induced increase in intraluminal pH initiated the disintegration process already in the upper GI tract, the actual release of mesalazine still started beyond the proximal jejunum. Upon intake of Pentasa, a prolonged release formulation which starts to release mesalazine already in the stomach, PPI pre-treatment seemed to increase the systemic exposure to mesalazine, even though the underlying mechanism could not be revealed yet. The faster release of mesalazine in the GI tract and the increased systemic absorption following PPI pre-treatment may, in theory, reduce the ability of mesalazine to reach the colon while increasing the risk on adverse events. Future research assessing mesalazine disposition in the lower GI tract is therefore warranted to evaluate possible consequences on the effectiveness of modified release formulations to deliver therapeutic concentrations to the colon in the treatment of UC patients.

Credit authorship contribution statement

Arno Van Camp: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – Original Draft, Visualization, Project administration. **Tim Vanuytsel**: Conceptualization, Resources, Writing – Review & Editing, Supervision, Project Administration, Funding acquisition. **Joachim Brouwers**: Conceptualization, Methodology, Formal analysis, Data curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. **Patrick Augustijns**: Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Project Administration, Funding acquisition

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables

Table 1. Pharmacokinetic parameters for mesalasine and N-acetyl mesalazine after oral intake on on tablet of Pentasa (500 mg mesalazine) in control vs. proton pump inhibitor (PPI) condition. Data are presented as median values with minimum and maximum values presented between brackets (n = 5).

Mesalazine	**Control**	**PPI**
$AUC_{0\text{-}24h}$ (ng/mL*min)	50251 (25127 - 70728)	72001 (57767 - 223044)
C_{max} (ng/mL)	178 (141 - 434)	443 (149 - 525)
T_{max} (min)	180 (30 - 330)	300 (60 - 360)
T_{lag} (min)	60 (30 - 60)	60 (30 - 60)
Acetyl Mesalazine	**Control**	**PPI**
$AUC_{0\text{-}24h}$ (ng/mL*min)	243386 (173729 - 336450)	334140 (253675 - 572895)
C_{max} (ng/mL)	562 (297 - 782)	696 (435 - 906)
T_{max} (min)	220 (30 - 330)	330 (90 - 480)
T_{lag} (min)	30 (30 - 60)	30 (30 - 60)

Figure legends

Figure 1. Mean (+ S.D., n = 5) pH of aspirates from stomach and jejunum as a function of time after oral intake of one tablet of Pentasa (500 mg mesalazine; Figure 1A) or Claversal (500 mg mesalazine; Figure 1B) in control condition vs. proton pump inhibitor (PPI) condition.

Figure 2. Box-whisker plots summarizing the pH of all GI fluids aspirated after intake of Pentasa or Claversal. Figure 2A depicts gastric fluids aspirated in control condition (n = 169) and proton pump inhibitor (PPI) condition (n = 168). Figure 2B depicts jejunal fluids aspirated in control condition (n = 164) and PPI condition (n = 135). Boxes represent median and upper and lower quartiles; whiskers represent 5^{th} and 95^{th} percentiles.

Figure 3. Mean (+ S.D., n = 5) plasma concentrations of mesalazine (Figure 3A) and N-acetyl mesalazine (Figure 3B) as a function of time after oral intake of one tablet of Claversal (500 mg mesalazine) in control condition (dots) vs. proton pump inhibitor (PPI) condition (squares).

Figure 4. In vitro dissolution of mesalazine from a Claversal tablet in a three-stage dissolution test using Fasted State Simulated Gastric Fluid (FaSSGF) and Fasted State Simulated Intestinal Fluid (FaSSIF). Dissolution is expressed as the mean (+ S.D., n = 3) percentage dissolved mesalazine as a function of time. After 30 min at pH 1.6 in FaSSGF (control condition) or pH 6.5 in FaSSIF (proton pump inhibitor (PPI) condition), the pH value was maintained at 6.5 in FaSSIF for 180 min to end at pH 7.5 for 30 min for both conditions.

Figure 5. Mean (+ S.D., n = 5) plasma concentrations of mesalazine (Figure 5A) and N-acetyl mesalazine (Figure 5B) as a function of time after oral intake of one tablet of Pentasa (500 mg mesalazine) in control condition (dots) vs. proton pump inhibitor (PPI) condition (squares).

Figure 6. Individual systemic exposure to mesalazine and N-acetyl mesalazine (summed), expressed as $AUC_{0\text{-}24h}$, after oral intake of one Pentasa tablet (500 mg mesalazine) in control condition vs. proton pump inhibitor (PPI) condition.

Figure 7. Mean (+ S.D., n = 5) concentrations of mesalazine in the stomach as a function of time after oral intake of one tablet of Pentasa (500 mg mesalazine) in control condition (dots) vs. proton pump inhibitor (PPI) condition (squares).

Figure 8. Mean (+ S.D., n = 5) concentrations of mesalazine (Figure 8A) and acetyl mesalazine (Figure 8B) in jejunum as a function of time after oral intake of one tablet of Pentasa (500 mg mesalazine) in control condition (dots) vs. proton pump inhibitor (PPI) condition (squares).

Figure 9. Mean (+ S.D., n = 3) dissolution of Pentasa in the one-stage dissolution test using Fasted State Simulated Intestinal Fluid (FaSSIF) solutions with different pH values 7.0, 6.5 and 6.0.

Figure 10. Comparison of the individual proximal jejunal exposure to N-acetyl mesalazine (acetyl 5-ASA) and mesalazine (5-ASA), expressed as the ratio $AUC_{0\text{-}5h}$ Acetyl 5-ASA / $AUC_{0\text{-}5h}$ 5-ASA after oral intake of one Pentasa tablet (500 mg mesalazine) in control condition vs. proton pump inhibitor (PPI) condition.