Pharmacologic Properties, Metabolism, and Disposition of Linaclotide, a Novel Therapeutic Peptide Approved for the Treatment of Irritable Bowel Syndrome with Constipation and Chronic Idiopathic Constipation


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ABSTRACT

Linaclotide, a potent guanylate cyclase C agonist, is a therapeutic peptide approved in the United States for the treatment of irritable bowel syndrome with constipation and chronic idiopathic constipation. We present for the first time the metabolism, degradation, and disposition of linaclotide in animals and humans. We examined the metabolic stability of linaclotide in conditions that mimic the gastrointestinal tract and characterized the metabolite MM-419447 (CCEYCCNPACTGC), which contributes to the pharmacologic effects of linaclotide. Systemic exposure to these active peptides is low in rats and humans, and the low systemic and portal vein concentrations of linaclotide and MM-419447 observed in the rat confirmed both peptides are minimally absorbed after oral administration. Linaclotide is stable in the acidic environment of the stomach and is converted to MM-419447 in the small intestine. The disulfide bonds of both peptides are reduced in the small intestine, where they are subsequently proteolyzed and degraded. After oral administration of linaclotide, <1% of the dose was excreted as active peptide in rat feces and a mean of 3–5% in human feces; in both cases MM-419447 was the predominant peptide recovered. MM-419447 exhibits high-affinity binding in vitro to T84 cells, resulting in a significant, concentration-dependent accumulation of intracellular cyclic guanosine-3’,5’-monophosphate (cGMP). In rat models of gastrointestinal function, orally dosed MM-419447 significantly increased fluid secretion into small intestinal loops, increased intraluminal cGMP, and caused a dose-dependent acceleration in gastrointestinal transit. These results demonstrate the importance of the active metabolite in contributing to linaclotide’s pharmacology.

Introduction

Peptide therapeutics represent an emerging class of drugs with great pharmacologic potential because of their high specificity, affinity, and molecular target recognition (Yu et al., 2004). Linaclotide is a novel, orally administered 14-amino acid peptide approved for the treatment of irritable bowel syndrome with constipation (IBS-C) and chronic idiopathic constipation (CIC) (Wensel and Luthin, 2011; Lembo et al., 2011; Chey et al., 2012; Rao et al., 2012). Linaclotide is a potent guanylate cyclase C (GC-C) agonist and member of the guanylin family of peptides known to regulate cyclic guanosine-3’,5’-monophosphate (cGMP) (Currie et al., 1992; Forte, 2004; Hamra et al., 1993) through their activity at GC-C. The endogenous peptide hormones guanylin and uroguanylin act on GC-C expressed on the luminal surface of intestinal epithelial cells (Wiegand et al., 1992; Fan et al., 1996; Hamra et al., 1996, 1997; Schulz et al., 1990) to regulate intestinal fluid secretion in response to a meal. Linaclotide binds to GC-C with high affinity in a pH-independent manner, stimulates the intracellular production of cGMP, and elicits its potent pharmacologic response locally in the gastrointestinal (GI) tract with very low oral bioavailability (Busby et al., 2010). Experiments in genetically modified mice lacking the gene encoding GC-C (Gucy2−/−) have shown that this receptor is the molecular target of linaclotide (Bryant et al., 2010). In addition to increasing fluid secretion and accelerating GI transit, linaclotide has also...
been shown to have potent GC-C-mediated analgesic effects in several mechanistically different rodent models of visceral hypersensitivity (Eutamene et al., 2010).

These distinct pharmacologic effects on relieving pain and accelerating GI transit have been observed clinically (Andresen et al., 2007; Johnston et al., 2010). Phase 3 studies have shown that linaclotide treatment results in a significant reduction in abdominal pain, discomfort, bloating, and constipation symptoms in IBS-C patients (Roque and Camilleri, 2011; Chey et al., 2012; Rao et al., 2012) and improves bowel and abdominal symptoms in chronic constipation patients (Lembo et al., 2010). Thus, GC-C appears to be a promising therapeutic target for the treatment of functional GI disorders such as IBS-C and CIC.

Orally administered peptides typically have low absolute oral bioavailability, so achieving systemic plasma concentrations is often a challenge; however, this is an advantage with linaclotide, as the ligand-binding domain of the GC-C target is located on the luminal surface of intestinal epithelial cells and systemic exposure is not necessary for linaclotide to exert its pharmacologic effects.

Effective pharmacotherapy with linaclotide depends on the local concentrations present within the intestinal tract. This therapeutic goal is challenged by the varied and harsh environments along the GI tract, which decrease the amount of active peptide available. These environments include vastly different pH conditions ranging from pH 2.0 in the stomach up to pH 8.0 in the duodenum, as well as proteolytic enzymes such as gastric hydrolases (pepsins), pancreatic hydrolases (trypsin, chymotrypsin, elastase, aminopeptidases, and carboxypeptidases A and B), and intestinal brush-border membrane-bound enzymes (carboxypeptidases, endopeptidases, and aminopeptidases), which are also involved in peptide degradation (Johnson et al., 1994; Wilkinson, 1997; Sanderink et al., 1988; Woodley, 1994). This extensive system of presystemic metabolism and degradation has evolved to digest ingested proteins and large peptides into small, absorbable dipeptides and tripeptides and single amino acids (Daniel, 2004). In addition to pH instability and/or proteolytic degradation, peptide drugs containing disulfide bonds are inactivated by the reduction of these bonds in the GI tract. These reactions are performed by the glutaredoxin/glutathione reductase and thioredoxin/thioredoxin reductase systems, integral parts of the antioxidant defense system in the GI tract (Fernandes and Holmgren, 2004; Zahedi Arval and Holmgren, 2009).

In the studies described here, we have investigated the pharmacologic properties, metabolism, and disposition of linaclotide as well as the role the active metabolite plays in contributing to the pharmacologic effects of orally dosed linaclotide.

Materials and Methods

Linaclotide, MM-421202 (CCEY, MM-421522 (CCNPACTGCV), and [13C]Ala-linaclotide were obtained from Polypeptide Laboratories (Torrance, CA). MM-419447 (CCEYCCNPACTGCV) was synthesized at Polypeptide Laboratories (Wolfenbuettel, Germany). MM-420926 ([15N15C]linaclotide), MM-437150 ([15N13C9]linaclotide), and human heat-stable enterotoxin (hSTa) were synthesized at American Peptide Company (Sunnyvale, CA). Human guanylin was purchased from Promega (Madison, WI), and aminopeptidase was purchased from EMD Chemicals (Gibbstown, NJ).

Heat-stable enterotoxin from porcine origin (pSTa) was purchased from Sigma-Aldrich and Bachem Americas (Torrance, CA) and was radiodioltated at PerkinElmer Life and Analytical Sciences (North Billerica, MA). Iodination of pSTa produces two monoiiodinated molecules; the one labeled at Tyr14 (Thompson et al., 1985) was purified by high-performance liquid chromatography (HPLC) and used for radioligand binding competition studies, while the other, labeled at Tyr16, was used for radiommunoassay (specific activity 2,200 Ci/mmol). Monoclonal antibodies to pSTa were a gift from Dr. Ralph Giannella, University of Cincinnati (Brandwein et al., 1985). Bovine serum albumin (BSA) was obtained from Thermo Fisher Scientific (Waltham, MA). Sheep anti-mouse IgG beads were obtained from Invitrogen (Carlsbad, CA).

Animals. Male and female Sprague-Dawley (CD) rats were obtained from Charles River Laboratories (Wilmington, MA) and were housed in an environmentally controlled room. All animal studies were approved by the Ironwood Pharmaceuticals Institutional Animal Care and Use Committee.

Stability of Linaclotide In Vitro. The methods used to study the stability of linaclotide in simulated gastric fluid and in the presence of peptidase, trypsin, chymotrypsin, aminopeptidase, and carboxypeptidase A are described in Supplemental Methods.

Determining Systemic and Portal Vein Concentrations of Linaclotide and MM-419447 after Oral Administration of Linaclotide to Female Rats. Two cohorts consisting of four female Sprague-Dawley rats each were used in the study; one group (group 1) was equipped with portal vein cannulas and the second group (group 2) was equipped with portal vein cannulas. All animals received a single oral gavage of 10 mg/kg linaclotide, and systemic blood samples were collected from group 1 via the jugular vein cannulas before dosing and 0.08 (5 minutes), 0.33 (20 minutes), 0.67 (40 minutes), 1, 1.5, 3, 4.5, 6, and 24 hours after dosing. Portal vein blood samples were collected from the animals in group 2 at the same designated time points via the portal vein cannulas. Plasma was extracted from the blood samples and was analyzed for linaclotide and MM-419447 using liquid chromatography with tandem mass spectrometry (LC-MS/MS) as described in Supplemental Methods.

Determination of the Absolute Oral Bioavailability of MM-419447 in Female Rats. To determine the absolute oral bioavailability of MM-419447, four groups (n = 3) of female rats received MM-419447 (10 mg/kg) intravenously (i.v.), and four groups (n = 3) received MM-419447 (10 mg/kg) oral gavage. Blood was drawn by retro-orbital eye bleeding at specific time intervals and was frozen for subsequent LC-MS/MS analysis as described in Supplemental Methods. The limit of detection for MM-419447 in rat plasma was 1 ng/ml.

Oral bioavailability (F) was calculated using the following equation:

\[ F = \left( \frac{\text{AUC}_{p.v.}(0-6h) \times D_v}{\text{AUC}_{v.v.}(0-6h) \times D_{p.a.}} \right) \]

where \( D_v \) and \( D_{p.a.} \) equal the intravenous and oral dose, respectively.

Method for Quantitative Determination of Linaclotide and MM-419447 in Human Plasma. Linaclotide and MM-419447 were extracted from human plasma 2 hours after oral dosing of linaclotide (see Supplemental Methods). The plasma was analyzed for linaclotide and MM-419447 concentrations using LC-MS/MS as described in Supplemental Methods.

Recovery of Linaclotide and MM-419447 from Rat Feces after Oral Dosing. Three female CD rats weighing approximately 180 g were included in this study. Three hundred μl of a 6 mg/ml solution of linaclotide (in 20 mM Tris-HCl, pH 7.5) was administered orally to all rats using a disposable feeding needle. Animals were individually housed and placed on wire grids in lined cages to ensure that all feces were collected. Feces were collected every hour over 4 days.
55 hours after dosing. The total weight of feces collected at each time point was recorded. The feces were stored at −20°C until analysis. The concentrations of linaclotide and MM-419447 in the feces were determined by LC-MS/MS as described in Supplemental Methods.

**Extraction and Fractionation of Linaclotide and MM-419447 from Human Feces.** Linaclotide and MM-419447 were extracted from human feces using a Bond Elute Plexa solid-phase extraction (SPE) plate. Approximately 15 g of sample was homogenized in a blender with three volumes of deionized water (v/v) containing internal standard (MM-420026). The supernatant of a 2-g aliquot from each sample was filtered through a Whatman 0.2-μm polyvinylidene difluoride glass microfiber syringe filter (Whatman, Clifton, NJ) and loaded onto the SPE plate. The samples were washed with 5% methanol in water and eluted from the plate with 100% methanol. The samples were evaporated under nitrogen and reconstituted in 20% acetonitrile and 0.1% formic acid in water. A 40-μl injection of the extract was analyzed with an LC-MS/MS assay using a Hypersil Gold column (3-μm particle, 2.1 × 50 mm).

The HPLC method used a binary buffer system with 5% acetonitrile and 0.1% formic acid in water as the weak solvent (A) and 20% acetonitrile and 0.1% formic acid in water as the strong solvent (B). The HPLC gradient started with 50% B for 4 minutes, ramped down to 10% over the next 3.5 minutes and returned back to 50% B after 30 seconds. The analytes were detected by a multiple reaction monitoring method using an Applied Biosystems API 5000 mass spectrometer operating in positive ion electrospray ionization mode. The method monitored the transitions 764 > 182 for linaclotide, 682 > 136 for MM-419447, and 789 > 192 for MM-420026 (IS). The peak area ratios of linaclotide/IS and MM-419447/IS and the theoretical concentrations of calibration samples were fitted to the quadratic function with 1/x^2 weighting, excluding the origin.

**Metabolism of Linaclotide In Vivo and In Vitro.** The stability and metabolism of linaclotide were studied in vivo in surgically ligated intestinal loops and in vitro in intestinal fluid extracted from the small intestine of rodents and humans as described in Supplemental Methods.

**Competitive Radioligand Binding Assays Using Human T84 Cells.** Competitive radioligand binding assays using T84 cells and [125I]pSTa were conducted as described by Busby et al. (2010). Competitive radioligand binding assays using rat intestinal brush-border membranes were conducted as described in Supplemental Methods.

**Cyclic GMP Accumulation in T84 Cells and in Fluid of Ligated Intestinal Loops.** Accumulation of GMP in T84 cells was conducted as described by Busby et al. (2010). To assess the biologic activity of linaclotide and MM-419447 when fully reduced, both peptides were incubated in the presence of 5 mM dithiothreitol (DTT) for 30 minutes at 37°C followed by alkylation with 50 mM iodoacetamide for 30 minutes at room temperature. The fully reduced and alkylated samples were then purified using SPE to remove excess DTT and iodoacetamide, quantitated using UPLC-HRMS (described in Supplemental Methods) and subjected to T84 cells grown in 96-well plates (250,000 cells/well) and compared with fully oxidized parent peptides. Cyclic GMP was quantified using LC-MS/MS.

To measure the amount of cGMP in fluid obtained from ligated rat intestinal loops (n = 10), the samples were immediately added to 6% ice-cold trichloroacetic acid (TCA) at a ratio (v/v) of 300 μl of TCA to 100 μl of fluid sample and stored at −20°C until assayed. After thawing on ice, the samples were homogenized for 30 seconds, and the precipitate was removed by centrifugation (1550g, 10 minutes). TCA was extracted from each sample with water-saturated ether, and the amount of cGMP in intestinal fluid samples was determined with a competitive enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). All results are representative of several independent experiments.

**Intestinal Fluid Secretion.** Female CD rats (n = 10/group) were used to generate intestinal loops (1–3 cm in length) in the duodenum, jejunum, and ileum (prepared as described in Supplemental Methods). Loops were injected with either 200 μl of MM-419447 (5 μg), linaclotide (5 μg), or vehicle (KRGH), and the animals were allowed to recover for 90 minutes before sacrifice. The loops were excised, and the length and weight of each intestinal loop was recorded both before and after collection of the intestinal fluid content. A weight-to-length ratio (W/L) was calculated and expressed as a percentage of the control value, a commonly used surrogate to measure intestinal secretion (Thigajragaraj et al., 2004).

**Gastrointestinal Transit in Rats.** A well-established model was used to measure GI transit in the upper GI tract, since measurements of colonic transit in rats have proven difficult due to the reservoir function of the cecum during periods of intestinal hypersecretion (Fondacaro et al., 1990). Female CD rats 6 to 8 weeks of age were fasted for 18 to 24 hours before treatment, but had access to water ad libitum during this period. The rats (n = 10/group, except the 6.25 μg/kg group of n = 5) were administered oral doses (300 μl) of either MM-419447 (6.25, 12.5, 25, 50 μg/kg), linaclotide (6.25, 12.5, 25, 50 μg/kg), or vehicle (20 mM Tris-HCl, pH 7.5) immediately before an oral dose of 10% activated carbon/10% gum arabic powder suspension (500 μl in water). After 10 minutes, the animals were euthanized, and their intestines, from the pyloric junction to the cecum, were removed. The GI transit is expressed as the percentage of the distance traveled by the charcoal front compared with the total length of the intestine. Studies following the same protocol were also performed in male CD rats.

**Statistical Analysis.** The determination of statistical significance of data was performed using either a one-way or two-way analysis of variance (ANOVA), followed by unpaired two-tailed Student’s t test or Wilcoxon and Mann-Whitney tests. All values are expressed as the mean ± S.E.M. P < 0.05 is considered statistically significant.

**Results**

**Proteolytic and Acid Stability of Linaclotide In Vitro.** Linaclotide was stable in simulated gastric fluid for at least 3 hours at 37°C, indicating that it is not degraded under the acidic conditions present in the stomach. Linaclotide was not digested during 3 hours of in vitro incubation with trypsin or pepsin, while insulin, a known substrate of these proteases, was digested. Similarly, linaclotide was not digested by aminopeptidase or chymotrypsin, while the control peptides (hSTa for aminopeptidase and guanylin for chymotrypsin) were rapidly hydrolyzed.

Incubation of linaclotide with carboxypeptidase A resulted in the formation of a single reaction product designated as MM-419447 (Fig. 1). The rate of linaclotide loss under these conditions was equal to the rate of appearance of MM-419447 (Fig. 1), with almost complete conversion of linaclotide to MM-419447 after 6 hours of digestion with carboxypeptidase A. To confirm the structure of MM-419447, the peptide was synthesized and shown to have the same retention time in UPLC as that of the peptide produced from linaclotide after digestion with carboxypeptidase A. The observed m/z ([M + H]^+) value for the MM-419447 product of linaclotide digestion with carboxypeptidase A was 1363.3333, and the observed m/z ([M + H]^+) value for MM-419447 synthesized by solid phase (1363.3319) were both within 1.8 ppm of the theoretical value predicted for this peptide (1363.3344). These observations identified MM-419447 as a peptide with an exact mass of 1362.3266 Da (elemental composition of C50H70N14O19S6), corresponding to the metabolite of linaclotide that lacks the C-terminal Tyr^{14} residue. Carboxypeptidases digest proteins
by cleaving the peptide bonds at C-terminal amino acids, resulting in the removal of C-terminal amino acid residues. In the case of linaclotide, under nonreducing conditions the cleavage of amino acids by carboxypeptidase A stops after the removal of the first C-terminal amino acid, Tyr^14.

**Sensitivity of Linaclotide to Chymotrypsin and Pancreatin in the Absence and Presence of a Reducing Agent.** Linaclotide in its native configuration was resistant to digestion by chymotrypsin, even though the peptide contains a tyrosine residue at the fourth position, which is a site predicted to be recognized by this protease. In addition, incubation of linaclotide with pancreatin, a mixture of proteases isolated from pig pancreas that is known to contain carboxypeptidase A (Bauer and Miskinis, 1947), resulted only in the generation of MM-419447. However, in the presence of a reducing agent (5 mM DTT), linaclotide was digested by chymotrypsin to two peptides (with sequences CCEY and CCNPACTGCY after cleavage of the C-terminal side of the Tyr^14) and to small peptide fragments by pancreatin.

**Absolute Oral Bioavailability of MM-419447 in Rats.**
Previous pharmacokinetic studies showed that the oral bioavailability of linaclotide was 0.1% after the administration of a single 10 mg/kg dose (Busby et al., 2010). When MM-419447 was measured in rat plasma in the same experiment, very small amounts of this metabolite were found, demonstrating the generation of this metabolite in vivo. The combined oral bioavailability of linaclotide and MM-419447 was very low (0.13%), and the oral bioavailability of MM-419447 after administration of a single 10 mg/kg dose of MM-419447 was 0.1%, confirming that MM-419447 is a minimally absorbed peptide, similar to linaclotide (Table 1).

**Systemic and Portal Vein Concentrations of Linaclotide and MM-419447 after Oral Administration of Linaclotide to Female Rats.** After oral dosing to Sprague-Dawley rats, systemic exposure to linaclotide and MM-419447 was minimal, and the majority of the plasma, whether sampled from the jugular or portal vein, had concentrations of the test articles that were below the quantitation limits of 1 ng/ml (linaclotide) and 2 ng/ml (MM-419447) (Fig. 2).

**Stability of linaclotide in the presence of carboxypeptidase A.** Linaclotide was incubated in the presence of carboxypeptidase A under nonreducing conditions for up to 6 hours. Linaclotide and MM-419447 were monitored in these reactions by LC-MS/MS. The values shown represent the mean ± S.D. (n = 3 per time point).

**Fig. 1.** Stability of linaclotide in the presence of carboxypeptidase A. Linaclotide was incubated in the presence of carboxypeptidase A under nonreducing conditions for up to 6 hours. Linaclotide and MM-419447 were monitored in these reactions by LC-MS/MS. The values shown represent the mean ± S.D. (n = 3 per time point).

In systemic (jugular vein) circulation, the maximum observed linaclotide plasma concentration (C_max) in any animal was 2.97 ng/ml, and the time of the maximum observed plasma concentration (T_max) was 0.08 hours (5 minutes). The corresponding highest individual portal vein C_max was 5.95 ng/ml and the T_max was 0.08 hours (5 minutes). For the metabolite, the highest individual systemic C_max was 2.28 ng/ml, and the highest individual portal vein C_max was 5.09 ng/ml. The metabolite T_max in both cases was 0.08 hours (5 minutes). By 0.67 hours (40 minutes), both systemic and portal vein plasma concentrations were below the lower limits of quantitation (LLOQ) for linaclotide and the metabolite, with the exception of animal 4, which had a systemic MM-419447 concentration of 2.80 ng/ml at 3 hours. The systemic and portal vein pharmacokinetic profiles of linaclotide and MM-419447 after oral dosing are shown in Fig. 2. Despite the high oral dose, concentrations in the jugular and portal veins were consistently low (≤6 ng/ml), indicating that the low absolute oral bioavailability of both peptides is due to poor absorption.

**Recovery of Linaclotide and MM-419447 from Rat Feces after Oral Administration of Linaclotide.** The total linaclotide recovered from the feces of rats over 55 hours after oral dosing (10 mg/kg), as measured by LC-MS/MS, was 0.03% of the dose in the form of linaclotide and an additional 0.95% in the form of the metabolite MM-419447. In all rats tested, the majority of linaclotide and MM-419447 was recovered in the first 24 hours after oral dosing.

**Plasma Concentrations of Linaclotide and MM-419447 in Humans after Oral Administration of Linaclotide.** In a phase 1, food-effect U.S. study, plasma samples from fed and fasted subjects dosed with 290 μg of linaclotide once daily for 7 days showed no quantifiable concentrations of linaclotide or MM-419447 (LLOQ = 0.2 ng/ml for linaclotide and LLOQ = 2.0 ng/ml for MM-419447). When linaclotide was administered as a single 10× dose (2897 μg) on the eighth day, linaclotide concentrations were quantifiable in plasma from 2 of the 18 subjects (both fasted), and MM-419447 was not detectable in the plasma of any subject (Table 2). In the four pivotal phase 3 trials in patients with IBS-C and CIC, plasma was sampled on day 1 and day 29 in 465 of 1654 patients who received either 290 (n = 313) or 145 μg (n = 152) of linaclotide. Only two patients (both of whom received the 290-μg dose) had measurable plasma concentrations of linaclotide, each <0.5 ng/ml, and none of the 465 patients had measurable plasma concentrations of MM-419447.

**Recovery of Linaclotide and MM-419447 from Human Feces after Oral Administration.** In the phase 1 open-label, two-period, crossover, food-effect study in healthy volunteers described in the previous section, stool samples

**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MM-419447</th>
</tr>
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<tbody>
<tr>
<td>T_max (h)</td>
<td>0.33</td>
</tr>
<tr>
<td>C_max (ng/ml)</td>
<td>27.0</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-6 h&lt;/sub&gt; (ng·h/ml)</td>
<td>36,100</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-24 h&lt;/sub&gt; (ng·h/ml)</td>
<td>≥29.7</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>≥0.1</td>
</tr>
</tbody>
</table>

* The ± symbol indicates that the reported value is an upper estimate of the area under curve (AUC) because the concentration of the lowest standard was used as the concentration of any sample with a concentration lower than the quantitation limit.
were collected during the second period of dosing from fed and fasted subjects after oral administration of 290 μg of linaclotide once daily for 7 days, and then again after the administration of a single 10× dose (2897 μg) on the eighth day. Recovery of active peptide ranged from 0 to 20% of the dose with a mean of 3–5% of dosed linaclotide. Virtually all of the recovered active peptide was MM-419447, confirming that it is a human metabolite (Table 3). Linaclotide was found in the stool samples from subjects only after administration of the 2897-μg dose in the fed state; these three subjects had a median of 0.4% of dosed linaclotide in their stool. The amount of total recovered active peptide was similar in fed and fasted subjects (Table 3).

Metabolism of Linaclotide In Vivo in Surgically Ligated Rat Intestinal Loops. The metabolism of linaclotide in surgically ligated rat intestinal loops was studied as a function of time by injecting linaclotide in three locations (duodenum, mid-jejunum, and ileum). The fluid inside the loops was metabolized more slowly (t1/2 = 36 minutes) than when incubated in diluted intestinal fluid, stopping the metabolism by removing proteins in the loop fluid using ultrafiltration, and quantifying by radioimmunoassay. Linaclotide was not detectable within 5 minutes when incubated in duodenal and jejunal loops (t1/2 = 0.4 minutes); linaclotide injected in ileal loops was metabolized more slowly (t1/2 = 36 minutes).

Metabolism of Linaclotide In Vitro in Intestinal Fluid. To further explore the metabolism of linaclotide in rodent intestine in vitro, linaclotide was incubated in a solution obtained from ligated intestinal loops from rats, and concentrations of active peptide were determined by LC-MS/MS. A 30-minute incubation resulted in the complete degradation of linaclotide. MM-419447, the active metabolite, formed almost immediately and reached a maximum concentration 10 minutes after incubation, after which the rate of degradation exceeded the rate of formation. Both peptides were degraded within 60 minutes. A related pattern of metabolism and degradation was observed when linaclotide was incubated in intestinal fluid from a mouse (data not shown). Linaclotide is metabolized in a similar manner in intestinal fluid isolated from human cadavers, with immediate formation of MM-419447 and subsequent degradation of this metabolite. These experiments were performed using intestinal fluids obtained from a particular region of the rodent or human intestine (proximal jejunum) using different preparation methods, so the results do not necessarily reflect the in vivo kinetics of linaclotide metabolism/degradation after oral administration. These experiments were conducted to study only the process of linaclotide metabolism in the intestine.

Pathway of Linaclotide Metabolism in Rat Intestinal Fluid. Reaction conditions were chosen to trap intermediates of the metabolism of linaclotide in rat intestinal fluid. After incubation of linaclotide in diluted intestinal fluid (10% loop fluid and 90% protein-filtered loop fluid), the peptides detected included native linaclotide, native MM-419447, linaclotide with one reduced disulfide bond and two cysteines, and MM-419447 with one disulfide bond reduced and two cysteines. Similar results were obtained when [13C1] Ala-linaclotide was incubated under the conditions previously described. These results indicate that reduction of the disulfide bonds of linaclotide and MM-419447 is a necessary initial step in their degradation in intestinal fluid.

In a second experiment, proteolytic fragments of linaclotide and MM-419447 were trapped by slowing the reaction rate with diluted intestinal fluid, stopping the metabolism by removing proteins in the loop fluid using ultrafiltration, and reducing and alkylating the resulting sulphydryl groups with iodoacetamide. Accurate mass measurements from LC/MS and MS/MS peptide sequencing were used to determine the identities of the fragments. All incubations were performed with linaclotide and [13C1]-Ala-linaclotide to confirm the fragment identities. Figure 3 shows a list of the fragments of linaclotide that were detected in this experiment. The fragments are listed in three groups representing various digestion patterns (Fig. 3). In these experiments, the digestion reactions were deliberately stopped at 15, 60, and 120 minutes to trap the reduced forms and peptide fragments. After overnight incubation, the digestion products were indistinguishable from the background species present in a reaction conducted without linaclotide (not shown). Supplementary Fig. 1 shows the early appearance and then disappearance of selected peptide fragments of linaclotide resulting from digestion in rat intestinal fluid as a function of time. Similar results were obtained when two fragments of
**TABLE 2**

Summary of pharmacokinetic results from clinical studies performed with linaclotide

The clinical protocols and all clinical trial procedures were approved by an institutional review board, and the trial was designed, conducted, and reported in accordance with the principles of good clinical practice guidelines. All patients gave written informed consent before their participation in the trial.

<table>
<thead>
<tr>
<th>Study Number</th>
<th>Linaclotide Doses (Number of PK Subjects)</th>
<th>Linaclotide Plasma Concentrations</th>
<th>MM-419447 Plasma Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PK STUDIES IN HEALTHY VOLUNTEERS</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MCP-103-001 (phase 1, U.S.)</td>
<td>Single 29 µg (4); 97 µg (8); 290 µg (4); 966 µg (4); 2897 µg (4)</td>
<td>Not detectable (&lt;3.00 ng/ml)</td>
<td>Not detectable (&lt;3.00 ng/ml)</td>
</tr>
<tr>
<td>0456-CL-0011 (phase 1, Japan)</td>
<td>Single 109 µg (6); 327 µg (6); 1090 µg (6); 3270 µg (6)</td>
<td>Not detectable (&lt;0.200 ng/ml)</td>
<td>Not detectable (&lt;2.00 ng/ml)</td>
</tr>
<tr>
<td>MCP-103-002 (phase 1, U.S.)</td>
<td>QD × 7 days 29 µg (8); 97 µg (8); 290 µg (8); 966 µg (8)</td>
<td>Not detectable (&lt;3.00 ng/ml)</td>
<td>Not detectable (&lt;3.00 ng/ml)</td>
</tr>
<tr>
<td>MCP-103-013 (phase 1 food effect, U.S.)</td>
<td>QD × 7 days 290 µg (9 fed, 10 fasted) Single 2897 µg (9 fed, 9 fasted)</td>
<td>Not detectable (&lt;0.200 ng/ml)</td>
<td>Not detectable (&lt;2.00 ng/ml)</td>
</tr>
<tr>
<td><strong>SPARSE SAMPLING (DAY 1 AND 29) IN PATIENTS WITH CIC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIN-MD-01 (phase 3)</td>
<td>QD × 12 weeks 145 µg (51); 290 µg (53)</td>
<td>Not detectable (&lt;0.200 ng/ml)</td>
<td>Not detectable (&lt;2.00 ng/ml)</td>
</tr>
<tr>
<td>MCP-103-303 (phase 3)</td>
<td>QD × 12 weeks 145 µg (101); 290 µg (98)</td>
<td>Not detectable (&lt;0.200 ng/ml)</td>
<td>Not detectable (&lt;2.00 ng/ml)</td>
</tr>
<tr>
<td><strong>SPARSE SAMPLING (DAY 1 AND 29) IN PATIENTS WITH IBS-C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIN-MD-31 (phase 3)</td>
<td>QD × 12 weeks 290 µg (64)</td>
<td>Not detectable (&lt;0.200 ng/ml)</td>
<td>Not detectable (&lt;2.00 ng/ml)</td>
</tr>
<tr>
<td>MCP-103-302 (phase 3)</td>
<td>QD × 26 weeks 290 µg (98)</td>
<td>Not detectable (&lt;0.200 ng/ml) except in 2 fasted subjects</td>
<td>Not detectable (&lt;2.00 ng/ml)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subject 12 Cmax = 0.735 ng/ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subject 19 Cmax = 0.212 ng/ml</td>
<td></td>
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</tbody>
</table>

CIC, chronic idiopathic constipation; IBS-C, irritable bowel syndrome with constipation; QD, every day; PK, pharmacokinetics study.

Linaclotide, MM-421202 (sequence CCEY) and MM-421522 (sequence CCNPACTGCY), were incubated overnight under the same conditions, resulting in their complete degradation (data not shown).

When an analogous experiment was conducted using a preparation of human intestinal fluid obtained from cadavers, a subset of the proteolytic fragments of linaclotide found in the rat experiment was observed (shown in Fig. 3, bolded peptides), indicating a common linaclotide metabolism/degradation pathway in both rats and humans.

**Analysis of the Pharmacologic Activity of Reduced and Alkylated Linaclotide and Linaclotide Fragments.** Reduced and alkylated linaclotide and MM-419447 did not elicit detectable cGMP accumulation activity in T84 cells when tested at 1, 10, 100, and 1000 nM (Supplemental Fig. 3A), but linaclotide and MM-419447 in their oxidized forms did (Supplemental Fig. 3A).

The peptides MM-421202 (CCEY) and MM-421522 (CCNPACTGCY) are two fragments detected after digestion of linaclotide in rat intestinal fluid (Fig. 3) that together span the linear sequence of linaclotide. MM-421202 and MM-421522 were synthesized and tested for their ability to stimulate cGMP synthesis in T84 cells. MM-421202 and MM-421522 did not induce cGMP accumulation activity in T84 cells when tested at 1, 100, and 1000 nM, concentrations at which linaclotide is active (Supplemental Fig. 3B).

**Complete Digestion of Linaclotide and MM-419447 to Naturally Occurring Amino Acids.** To detect amino acids as products of linaclotide metabolism in rat intestinal fluid, the incubations were conducted for 23 hours at 37°C in reaction mixtures containing 10% concentrated ligated rat intestinal loop fluid, 90% phosphate-buffered saline (PBS) supplemented with 2 mM reduced glutathione, and 1 mM NADPH. Under these conditions, linaclotide, MM-42102, and MM-421522 were completely digested (data not shown). Supplemental Table 1 summarizes the concentrations of amino acids measured using UPLC after digestion of these peptides under the conditions described earlier and subsequent amino acid derivatization using the AcQ•Tag reagent. Overnight incubation resulted in complete digestion of the peptides, with quantitative recovery of internal amino acids. In this experiment, twice as much tyrosine was recovered from linaclotide as from MM-419447, as expected from the sequences. Also, there was no glutamic acid detected after complete digestion of MM-421522 because this peptide lacks this amino acid residue. Based on the amount of linaclotide incubated, UPLC analysis demonstrated nearly quantitative recovery of the tyrosine, alanine, proline, and asparagine amino acids as well as most of the threonine in the linaclotide sample.

**Linaclotide and MM-419447 Binding to Intestinal Guanylate Cyclase C Receptors.** We have previously shown that GC-C is the molecular target of linaclotide (Bryant et al., 2010), and that linaclotide binds to T84 cells, which
express high levels of GC-C, in a concentration-dependent and pH-independent manner (Busby et al., 2010). To compare the pharmacologic activity of MM-419447 to that of linaclotide, synthetic MM-419447 was tested for its affinity to cell surface receptors in T84 cells. When MM-419447 was tested in a competitive radioligand-binding assay with \[^{125}\text{I}]\text{-pSTa}, a known agonist of GC-C, the binding of MM-419447 to cell-surface receptors on T84 cells was very similar to that of linaclotide at pH 7.0 (Supplemental Fig. 2; Table 4). Likewise, MM-419447 binds cell-surface receptors in rat small intestine brush-border membranes (pH 7.0) with a relative affinity comparable to linaclotide (Table 4). The binding of MM-419447 to receptors on T84 cells at pH 5.0 and 8.0 is also comparable to that of linaclotide, indicating that the binding affinities of linaclotide and its metabolite are pH independent.

Comparison of the competitive binding curves of linaclotide and MM-419447 using the \(F\) test indicated that their IC\(_{50}\) and \(K_I\) values were not statistically different \((P > 0.05)\).

**Effect of Linaclotide and MM-419447 on cGMP Accumulation in Human T84 Cells.** Binding of linaclotide to GC-C receptors stimulates the intrinsic guanylate cyclase activity of the receptor, resulting in increased concentrations of intracellular cGMP (Busby et al., 2010). MM-419447 also stimulates the accumulation of cGMP in human T84 cells in a concentration-dependent manner with statistically equivalent potency. The concentration-response curves were used to calculate the EC\(_{50}\) values for linaclotide and MM-419447: 112 ± 21 and 103 ± 26 nM, respectively. Comparison of the concentration-response curves of linaclotide and MM-419447 using the \(F\) test indicated that the EC\(_{50}\) values were not statistically different \((P = 0.7555)\). These results indicate that MM-419447 acts as an agonist of GC-C in vitro, with potency similar to that of linaclotide.

**Effect of MM-419447 on Fluid Secretion and cGMP Accumulation in Rat Small Intestinal Loops.** We further investigated whether the in vitro pharmacologic properties of MM-419447 would translate in vivo to common rat models of GI function. The stimulation of intestinal fluid secretion was

### TABLE 3
Recovery of linaclotide and MM-419447 from human feces after oral administration of linaclotide

<table>
<thead>
<tr>
<th>Subject</th>
<th>% Active Peptide</th>
<th>Dose 290 µg</th>
<th>Dose 2897 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>002</td>
<td>5.97</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>004</td>
<td>0.39</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>005</td>
<td>2.53</td>
<td>2.94</td>
<td></td>
</tr>
<tr>
<td>008</td>
<td>NS</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>011</td>
<td>NS</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>012</td>
<td>0.49</td>
<td>5.73</td>
<td></td>
</tr>
<tr>
<td>015</td>
<td>0.37</td>
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</tr>
<tr>
<td>018</td>
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</tr>
<tr>
<td>019</td>
<td>17.75</td>
<td>6.57</td>
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</tr>
<tr>
<td>Median</td>
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</tr>
<tr>
<td>Mean</td>
<td>4.89</td>
<td>3.34</td>
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</tr>
<tr>
<td>001</td>
<td>NS</td>
<td>2.21</td>
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<tr>
<td>003</td>
<td>2.11</td>
<td>3.72</td>
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</tr>
<tr>
<td>006</td>
<td>0(^a)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>007</td>
<td>0.75</td>
<td>1.96(^b)</td>
<td></td>
</tr>
<tr>
<td>009</td>
<td>8.18</td>
<td>5.20</td>
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<tr>
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</tr>
<tr>
<td>016</td>
<td>9.37</td>
<td>20.84(^a)</td>
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</tr>
<tr>
<td>017</td>
<td>0(^a)</td>
<td>0</td>
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</tr>
<tr>
<td>020</td>
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<tr>
<td>SD</td>
<td>3.6</td>
<td>6.6</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Values are shown as percentage of oral dose. NS, no sample available.
\(^b\) Percentage of active peptide present as MM-419447, unless noted.
\(^c\) Below detection limits.
\(^d\) 0.88% MM-419447, 0.18% linaclotide.
\(^e\) 2.22% MM-419447, 0.35% linaclotide.
\(^f\) 19.97% MM-419447, 0.87% linaclotide.

Fig. 3. Trapping and identification of linaclotide digestion intermediates after incubation in rat intestinal loop fluid. Linaclotide was incubated in diluted intestinal fluid for 15, 60, and 120 minutes and overnight. At each time point, proteins were removed, and the disulfide bonds in peptides were reduced with DTT followed by alkylation with iodoacetamide. The resulting peptides were identified by LC/MS. Boldface peptides were detected in both rat and human intestinal fluids after incubation of linaclotide.
assessed by measuring the volume of the luminal fluid content 90 minutes after the exposure of ligated duodenal, jejunal, and ileal loops to either 5 μg of MM-419447 or vehicle. Treatment with MM-419447 stimulated a significant increase in fluid secretion compared with vehicle-treated animals in loops from all regions of the small intestine (Fig. 4). This increase in fluid secretion after treatment with MM-419447 was accompanied by a marked increase in the intraluminal concentration of cGMP in the duodenum and jejunum, and a much less pronounced increase in cGMP in ileal loops (Fig. 4B). Similar results for increased fluid secretion and concomitant cGMP secretion in the duodenum and jejunum were observed previously after treatment with linaclotide (Busby et al., 2010). Low concentrations of cGMP accumulation in ileal loops injected with STa have been reported by others (London et al., 1997; Qian et al., 2000).

**Effects of Linaclotide and MM-419447 on Gastrointestinal Transit in Female Rats.** We measured the effects of orally administered MM-419447 and linaclotide in a dose-dependent manner (6.25 to 50 μg/kg) in a rat GI transit model. GI transit was determined by measuring the distance a dose of activated charcoal, administered orally immediately after MM-419447 or linaclotide dosing, traveled in the small intestine after 10 minutes relative to the total length of the small intestine. Oral administration of MM-419447 or linaclotide resulted in a significant and dose-dependent acceleration of GI transit compared with vehicle-treated rats (except for the 6.25 μg/kg MM-419447 dose group). The traveled distances ranged from 57.8–66.4% and 59.1–65% for MM-419447 and linaclotide, respectively, compared with 50.3% in vehicle-treated rats (Fig. 5). A side-by-side comparison of each dose of MM-419447 and linaclotide using two-way analysis of variance (ANOVA) revealed that their dose-response curves were not significantly different (P = 0.6876), suggesting equal potency in this model. Linaclotide also significantly accelerated GI transit in male CD rats (data not shown).

**Discussion**

GC-C is a receptor for the endogenous peptide hormones guanylin and uroguanylin and is predominantly expressed on the apical surface of epithelial cells of the small intestine and colon (Krause et al., 1994). Linaclotide is an orally administered 14-amino acid peptide agonist of GC-C which acts locally within the lumen of the GI tract to exert its effects without a need for systemic exposure.

The amino acid sequence and tertiary molecular structure of linaclotide differ from those of guanylin and uroguanylin. These hormones contain four cysteines, as opposed to the six within linaclotide. These extra cysteines allow linaclotide to form an additional intramolecular disulfide bond. This third disulfide bond enhances the relative GC-C agonist potency of linaclotide by locking it into its active conformation, strengthening its binding affinity for GC-C and increasing its stability within the GI tract. However, the reinforced tertiary structure of linaclotide does not prevent the proteolytic removal of its C-terminal tyrosine, which is not protected by the disulfide bonds, resulting in the formation of a 13-amino acid metabolite, MM-419447 (Des-Tyr14), described here for the first time.

Linaclotide is resistant to the harsh, acidic conditions of the stomach, as shown by its stability in simulated gastric fluid. Under nonreducing conditions it is also resistant to proteolysis by GI hydrolases, with the exception of carboxypeptidase A. This enzyme removes the C-terminal Tyr14 residue...
of the peptide, leaving the three intramolecular disulfide bonds and the tertiary structure necessary for increased protease resistance, GC-C binding, and potent GC-C agonist activity intact. The resulting peptide has been confirmed as a circulating metabolite in high-dose pharmacokinetic experiments performed in mice, rats, dogs, and monkeys (data not shown). The detection of MM-419447 in stool samples from human subjects orally dosed with linaclotide confirms that MM-419447 is also a human metabolite, although it has not been found in systemic circulation.

To determine whether this metabolite contributes to the pharmacologic effects of linaclotide, we synthesized MM-419447, characterized its in vitro pharmacologic properties, and tested it in rat models of GI function. MM-419447 exhibited in vitro binding to T84 cell surface receptors similar to that of linaclotide. In addition, MM-419447 significantly increased intracellular cGMP concentrations in these cells in a concentration-dependent manner, with similar potency to that of linaclotide. These results are consistent with the hypothesis that MM-419447, like linaclotide, is a potent agonist of GC-C and provide further evidence that MM-419447 contributes to the in vivo pharmacologic effects observed with linaclotide.

The ability of MM-419447 to stimulate fluid secretion was measured in vivo after injection into surgically ligated duodenal, jejunal, and ileal loops in rats. Fluid volume was significantly increased in all three small intestinal regions. This increased fluid secretion was associated with increased luminal cGMP concentrations, suggesting that MM-419447 acts through stimulation of GC-C receptors. The same pattern and magnitude of biologic responses were observed with linaclotide (Busby et al., 2010). The pharmacologic effects of orally administered MM-419447 on the rate of GI transit in rats were also assessed, as altered intestinal transit correlates with clinical symptoms of IBS-C and CIC (Mayer et al., 2008). MM-419447 showed a significant and dose-dependent acceleration in the rate of GI transit in rats that was similar to that seen for linaclotide, further supporting the hypothesis that MM-419447 is a potent agonist in vivo of GC-C and contributes to the local pharmacology of linaclotide within the intestine.

Recent evidence suggests a role for cGMP in mediating linaclotide’s effects on visceral pain (Castro et al., 2012, submitted for publication). Like linaclotide, MM-419447 stimulates cGMP production in the intestinal epithelium, and therefore this active metabolite may contribute to the analgesic effects observed after oral administration of linaclotide. The disposition of linaclotide and MM-419447 was also assessed. Pharmacokinetic (PK) studies in rats using orally and intravenously dosed MM-419447 demonstrated that, as with linaclotide, MM-419447 is minimally absorbed and has an absolute oral bioavailability of ≤0.1% in rats. After a 10-mg/kg oral dose of linaclotide, jugular and portal vein plasma concentrations of both linaclotide and MM-419447 were low (≤6 ng/ml) in rats, indicating that both peptides are poorly absorbed and hepatic exposure to them is minimal. Minimal systemic exposure to linaclotide was also observed in humans in three phase 1 studies and was confirmed with sparse PK sampling in four phase 3 trials. Plasma concentrations of linaclotide were typically not measurable and were always below 1 ng/ml. MM-419447 was never quantifiable (LLOQ = 2 ng/ml) and was therefore not confirmed as a circulating metabolite in humans, although it was observed in human stool samples. The minimal systemic availability of linaclotide in humans is consistent with its low permeability coefficient in Caco-2 cells (data not shown) and the low absolute oral bioavailability found in animals.

Despite the fact that both active peptides are minimally absorbed, the majority of dosed linaclotide is not excreted in the feces. In rats, less than 1% of the orally delivered drug was recovered in feces as linaclotide or MM-419447. Of the recovered peptide, >95% was in the form of the active metabolite MM-419447. This confirms that the vast majority of the drug is broken down within the intestine before excretion. Moreover, the results of a phase 1 study in humans showed that a mean of 3–5% of the oral linaclotide dose was excreted in the feces as a pharmacologically active peptide. As was observed in rats, MM-419447 was the predominant peptide detected. Nevertheless, the presence of parent and active metabolite in the feces demonstrates that some active peptide is available to stimulate the GC-C receptor throughout the small intestine and colon.

Because linaclotide is minimally absorbed into systemic circulation and the majority of the drug is not excreted either as parent or active metabolite in the feces, the intestinal fate of linaclotide is proteolytic digestion. Using a series of in vitro and in vivo experiments in rodents, as well as in human biomaterials from cadavers, we have characterized this degradation pathway. We observed rapid proteolysis of linaclotide in the duodenum and jejunum of the rat and a slower rate of disappearance in the distal small intestine (ileum). The proteolytic metabolism was confirmed with subsequent in vitro studies in which linaclotide was incubated with intestinal fluid from mice, rats, and humans. In these
studies, linaclotide decreased immediately while MM-419447 began to form immediately.

The digestion of either linaclotide or MM-419447 requires a process that first reduces their disulfide bonds. The intestine is known to have the capacity to reduce disulfide bonds in proteins and peptides (Dahm and Jones, 2000; Fernandes and Holmgren, 2004; Zahedi Avval and Holmgren, 2009). Similar to our previous observations in rats, the luminal contents of the human intestine were found to contain high thiol concentrations (3–8 mM) and the components of the glutaredoxin system, which has been shown to reduce the three disulfide bonds of linaclotide (unpublished).

Disulfide bond reduction leaves both linaclotide and MM-419447 highly susceptible to proteolytic degradation, as demonstrated by the rapid in vitro cleavage of reduced linaclotide by proteolytic enzymes, including chymotrypsin. The degradation pathway of linaclotide was further characterized by incubating it with diluted rat intestinal fluid to slow down the digestion rate and allow intermediate peptide degradation products to be trapped and identified. These linearized peptide fragments contained three or more amino acids. Some of these peptide fragments were synthesized and were found to have no GC-C agonist activity. Overnight digestion of the synthesized fragments resulted in their complete digestion, with quantitative recovery of internal amino acids. Isolation and identification of similar peptide intermediates after human intestinal fluid incubation when compared with those from rat intestinal fluid incubations suggest that the metabolism and degradation pathway of linaclotide in humans is the same as that in the rat. Figure 3 shows the intestinal fate of linaclotide, beginning with its conversion to MM-419447, followed by disulfide bond reduction, which inactivates both peptides. Intestinal hydrolases are then able to proteolytically degrade the linearized peptides to smaller peptides and amino acids, which are subsequently recycled. Individual amino acids are absorbed through the intestine, as are peptides consisting of two or three amino acids (Daniel 2004; Silk et al., 1985), while hexa-, penta-, and tetrapeptides are further digested by brush-border proteases before absorption (Kim et al., 1972).

In conclusion, a more complete understanding of the pharmacology and drug disposition of linaclotide now exists. Linaclotide is readily metabolized to an active metabolite, MM-419447, which mirrors the parent in both pharmacologic and drug disposition of linaclotide now exists. Linaclotide decreased immediately while MM-419447 began to form immediately.

The references for this section are as follows:

**References**


