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New mechanistic explanation for the localization of ulcers in the rat duodenum: Role of iron and selective uptake of cysteamine

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ABSTRACT

Cysteamine, a coenzyme A metabolite, induces duodenal ulcers in rodents. Our recent studies showed that ulcer formation was aggravated by iron overload and diminished in iron deficiency. We hypothesized that cysteamine is selectively taken up in the duodenal mucosa, where iron absorption primarily occurs, and is transported by a carrier-mediated process. Here we report that cysteamine administration in rats leads to cysteamine accumulation in the proximal duodenum, where the highest concentration of iron in the gastrointestinal tract is found. *In vitro*, iron loading of intestinal epithelial cells (IEC-6) accelerated reactive oxygen species (ROS) production and increased [^{14}C]cysteamine uptake. [^{14}C]Cysteamine uptake by isolated gastrointestinal mucosal cells and by IEC-6 was pH-dependent and inhibited by unlabeled cysteamine. The uptake of [^{14}C]cysteamine by IEC-6 was Na^+-independent, saturable, inhibited by structural analogs, H_2-histamine receptor antagonists, and organic cation transporter (OCT) inhibitors. OCT1 mRNA was markedly expressed in the rat duodenum and in IEC-6, and transfection of IEC-6 with OCT1 siRNA decreased OCT1 mRNA expression and inhibited [^{14}C]cysteamine uptake. Cysteamine-induced duodenal ulcers were decreased in OCT1/2 knockout mice. These studies provide new insights into the mechanism of cysteamine absorption and demonstrate that intracellular iron plays a critical role in cysteamine uptake and in experimental duodenal ulcerogenesis.

Introduction

Despite recent advances and better understanding of the etiology of duodenal ulcer disease, including the role of *Helicobacter pylori*, the pathogenesis of ulcer development remains poorly understood. During recent years, the proportion of *H. pylori*-negative duodenal ulcers has reached 20–30% [1,2], implicating other etiologic factors in the pathogenesis of duodenal ulceration. Animal models of duodenal ulcer disease have provided valuable insights into the early, pre-ulcerogenic events and subsequent cellular and biochemical changes in the pathogenesis of duodenal ulceration. Our laboratory found that administration of cysteamine (or structurally related chemicals including MPTP[2], 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induces perforating duodenal ulcers in the first 5–7 mm of the proximal duodenum within 24–48 h [3–5]. Based on functional and morphologic criteria, these animal models, which are similar to human duodenal ulcer disease [6], have been used for acute and chronic studies.

Cysteamine is a low-molecular weight aminothiol that is broadly distributed in organisms [7] and is a natural product of coenzyme A catabolism [8–10]. Levels of free cysteamine in rodent tissues and human plasma are generally very low [11,12]. We demonstrated elevations of cysteamine in different brain regions,

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[2] *Abbreviations used:* IEC-6, intestinal epithelial cells; OCT1–3, organic cation transporters 1–3; DMT-1, divalent metal transporter-1; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; ROS, reactive oxygen species; KO mice, knockout mice; HPLC, high-performance liquid chromatography; Real-time PCR, real-time polymerase chain reaction.

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plasma and red blood cells after cysteamine administration in adult rats [13]. However, the mechanism of cysteamine transport in the gastrointestinal tract has not previously been investigated.

Recently, we found that cysteamine-induced duodenal ulceration in rats was exacerbated by elevated levels of endogenous iron in the proximal duodenum, and cysteamine increased the expression of divalent metal transporter-1 [DMT-1] [14]. Most intestinal iron absorption takes place in the proximal duodenum, where cysteamine-induced duodenal ulcers occur. We showed that feeding rats an iron-deficient diet decreased cysteamine-induced duodenal ulcers with a concomitant decrease in the duodenal mucosal iron concentration. The H_2-histamine receptor antagonist, cimetidine, decreased duodenal iron concentration concomitant with reduced gastric acid secretion and decreased ulcer formation [14]. The mechanism whereby changes in duodenal iron concentration may modulate duodenal ulcer formation in response to cysteamine administration is unknown.

We hypothesized that cysteamine is taken up preferentially in the proximal duodenal mucosa, where iron absorption primarily occurs, and is transported by a carrier-mediated process, explaining the localization of organ-specific tissue injury induced by cysteamine administration. Specifically, we hypothesized (a) that cysteamine absorption is enhanced by iron in the proximal duodenum, and that iron loading accelerates and iron depletion slows [^{14}C]cysteamine uptake in IEC-6 cells; (b) that cysteamine uptake is a carrier-mediated process and is inhibited by cysteamine analogs; and lastly (c) that cysteamine uptake is modulated by inhibitors of the organic cation transporters, OCT1–3, and by suppression of OCT gene expression. The results of the current study provide new insights into the mechanism of cysteamine absorption in the proximal duodenum and demonstrate that intracellular iron plays a critical role in cysteamine uptake and chemically induced duodenal ulcerogenesis.

Materials and methods

Animal experiments

This study was approved by the Subcommittee for Animal Studies and the Research and Development Committee of VA Long Beach Healthcare System. Duodenal ulcers were induced in female Sprague–Dawley rats (Harlan Laboratories, Inc. Placentia, CA) by cysteamine administration (cysteamine-HCl, Sigma–Aldrich, St. Louis, MO) in three doses, 25 mg/100 g body weight, at 4 h intervals. In time course studies, rats were given water or cysteamine-HCl, 25 mg/100 g body weight, by intragastric (i.g.) gavage once and euthanized 0.5 or 2 h later. In other groups, cysteamine was given twice or three times at 4 h intervals and rats were euthanized 6 h, 12 or 24 h after the first dose of cysteamine. Groups of rats also received cysteamine-HCl 50 mg/100 g once by subcutaneous (s.c.) administration, and animals were euthanized 2 h later. At autopsy, mucosal scrapings of stomach, proximal duodenum, jejunum, ileum, colon, and samples of other organs (liver, kidney) were collected and frozen in liquid nitrogen and kept at –80 °C. Additional studies were done using 6 week old female FVB ($n = 5$) and OCT1/2 knockout mice (KO, $n = 5$) (Taconic, Hudson, NY). Mice were treated with 125 mg/100 g cysteamine by i.g. gavage once and euthanized 48 h later.

Determinations of redox-active sulfur-containing compounds by high-performance liquid chromatography (HPLC) and coulometric detection

Redox-active sulfur-containing compounds were measured without prior derivatization by using a liquid chromatograph equipped with an 8-channel coulometric array (CoulArray) detector (ESA, Inc., Chelmsford, MA) (11). In brief, the supernatant fractions from 5% metaphosphoric acid (MPA) homogenates of tissues were injected directly onto a Bio-Sil ODS-5S, 5-μm particle size, 4.0 × 250 mm, C18 column (Bio-Rad, Life Science Research Group, Hercules, CA) and eluted with a mobile phase consisting of 50 mM NaH_2PO_4, 0.05 mM octane sulfonic acid, and 2% (v/v) acetonitrile (pH 2.62) at a flow rate of 1 ml/min. A Rheodyne injection valve with a 5-μl sample loop was used to manually introduce samples. The 8-channel CoulArray detectors were set at 150, 250, 350, 450, 500, 550, 600, and 700 mV, respectively. Peak areas were analyzed using ESA software, and concentrations of each metabolite were obtained from appropriate standard curves and reported as nmol/mg protein.

Isolation of rat intestinal epithelial cells and uptake studies

Gastric and intestinal mucosal cells were isolated as described previously [15] with minor modification [16]. The glandular stomach, proximal duodenum, jejunum and colon were removed from nonfasted female Sprague–Dawley rats (150–200 g) and digested with 1.0 mg/ml pronase in an incubating solution (solution A) for 25 min in a shaking water bath at 37 °C with subsequent vortexing for 5 min. The composition of solution A was as follows (mM): 98.0 NaCl, 5.8 KC1, 2.5 NaH_2PO_4, 5.1 Na pyruvate, 6.9 Na fumarate, 2.0 glutamine, 24.5 HEPES Na, 1.0 Tris base, 11.1 D-glucose, 0.1 nonessential amino acids and 2.0 mg/ml (w/v) BSA with pH adjusted to 7.4. The cell suspension was then filtered through a series of nylon filters (151–74 μm) to remove coarse fragments. The cells were washed twice with incubation in medium (without pronase) and centrifuged at 500 g for 3 min to remove residual pronase. Finally, the pellets were resuspended in solution B (no pronase), pH 7.4 to a desired concentration (5×10^6 cells/ml). Cell viability was determined by trypan blue exclusion. Uptake of [^{14}C]cysteamine (specific activity 50 mCi/mmol; radiochemical purity 97%, American Radiolabeled Chemicals, Inc., St. Louis, MO) by isolated cells was measured as described previously [17] using an established rapid filtration technique [18] employing 0.45-μm nitrocellulose filters (Sartorius Filters, Hayward, CA) at 37 °C in Krebs–Ringer buffer at pH 5.0 and 8.2. Labeled (15 μM [^{14}C]cysteamine) and unlabeled cysteamine were added to the incubation medium at the onset of incubation. The uptake was stopped using 5 ml of ice-cold stop solution containing 280 mM mannitol and 20 mM Tris-HEPES, pH 7.5. The amount of radioactivity captured on the filters was measured in a Beckman 5801 LS, Beta Liquid Scintillation Counter (Haverhill, MA). Protein concentrations were measured using Bradford reagent (Bio-Rad, Hercules, CA).

Cell culture and uptake studies

Human-derived intestinal epithelial (Caco-2) cells and rat-derived IEC-6 cells (ATCC, Manassas, VA) were grown in Dulbecco's modified Eagle's medium (DMEM from Invitrogen, Carlsbad, CA) with 4.5 g/l glucose, supplemented with 1% nonessential amino acids, 10% fetal bovine serum (FBS), 100 U/ml penicillin, 50 U/ml streptomycin and were maintained at 37 °C in an atmosphere of 5% CO_2. Cells were plated at a density of 2×10^5 cells/well onto 12-well plates. Uptake studies were performed between passages 7 and 19 on confluent monolayers of Caco-2 cells and IEC-6. Cell viability was monitored using phase contrast microscopy and trypan blue staining. Uptake was measured at 37 °C in Krebs–Ringer buffer (in mM: 133 NaCl, 4.93 KCl, 1.23 MgSO4, 0.85 CaCl2, 5 glucose, 5 glutamine, 10 HEPES, and 10 MES, pH 7.4, unless otherwise specified). Labeled (3 μM [^{14}C]cysteamine) and unlabeled cysteamine were added to the incubation medium at the onset of the uptake experiment. In certain experiments, cells were pretreated with ferrous sulfate and L-sodium-ascorbate in a 1:100-fold molar