Acute Enteral Glutamine Infusion Enhances Heme Oxygenase-1 Expression in Human Duodenal Mucosa

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ABSTRACT The heat shock protein, heme oxygenase-1 (HO-1), contributes to the protection of the intestine. Some experimental models suggest that induction of HO-1 by glutamine may contribute to the preservation of intestinal mucosa. The effect of an enteral infusion of glutamine for 6 h on HO-1 expression in duodenal mucosa was studied in healthy men and women and compared with an isonitrogenous mixture of amino acids. After enteral infusion, endoscopic duodenal biopsies were performed and either fixed in formalin for immunohistochemistry or frozen for HO-1 mRNA analysis by reverse transcriptase-polymerase chain reaction. Histologic examination revealed that HO-1 was constitutively expressed in intestinal epithelial cells (IEC), and that glutamine increased the grade of HO-1 immunostaining (P ≤ 0.01). Glutamine also increased the percentage of HO-1 immunoreactive lamina propria cells (LPC, 10.5 vs. 7.5%, P ≤ 0.05). Glutamine significantly increased HO-1 mRNA expression compared with control amino acids: median (range) 156 (102–182) vs. 100 (68–179)% (P ≤ 0.05). The mRNA level for HO-1 was correlated with the percentage of immunoreactive LPC (r = 0.55, P = 0.017) and the grade of immunostaining in IEC (r = 0.51, P = 0.030). In conclusion, glutamine enhanced HO-1 mRNA and protein expression in human duodenal mucosa. These data support further evaluation of the effects of glutamine on intestinal HO-1 during states of intestinal inflammation. J. Nutr. 132: 2570–2573, 2002.

KEY WORDS: glutamine • heme oxygenase-1 • heat shock protein • intestine • humans

Heme oxygenase-1 (HO-1), the 32-kDa heat shock protein (hsp)32, is the inducible form of the enzyme converting heme to biliverdin, iron and carbon monoxide (1). The induction of HO-1 is usually considered as a protective response (2), probably through the degradation of toxic compound heme (3), the production of biliverdin, which has antioxidant properties (4), and the production of CO, which has anti-inflammatory and antiapoptotic properties (5,6). Moreover, the induction of HO-1 in experimental colitis in rats has been demonstrated to have beneficial effects on the remission of colitis (7,8). However, data on the expression of HO-1 in human duodenal mucosa have not been reported previously.

Glutamine (Gln), the major fuel for enterocytes (9), has been shown to promote intestinal growth and metabolism, and to maintain the structure and function of intestinal mucosa, especially in cases of gut injury (10,11) or after gut resection in animals (12). Gln also stimulated intestinal cell proliferation in vitro (13,14). In animal studies, Gln supply decreased the gut mucosal alterations and interleukin (IL)-8 production induced by experimental colitis (15). In rats, the protective effect of Gln on the intestine has been related to HO-1 induction in a model of ischemia-reperfusion injury (16). Indeed, Gln has been shown to induce protein expression from the hsp family, such as hsp70 in intestinal epithelial cell lines (17–19), or hsp25 and 72 in several organs in rats (20) and hsp72 in cardiomoyocytes in sheep (21), but the effects of Gln on hsp expression in human intestine are not known.

The aim of this study was therefore to evaluate the effects of enteral Gln on HO-1 expression in human intestinal mucosa. This paper documents for the first time that HO-1 is constitutively expressed in human duodenal mucosa and that Gln increases it in both epithelial and lamina propria cells.

SUBJECTS AND METHODS

Study protocol. Nine healthy volunteers (5 men and 4 women) participated in the study and were in good general health without any previous medical or surgical history. They were 22 y old (median, range 20–24 y) and had a normal body mass index (median 22.1 kg/m2, range 20.4–23.9 kg/m2). The study was approved by the local ethical committee and the subjects gave their written informed consent. Each subject was studied on two occasions in a random order. For 3 d, all subjects consumed a controlled diet providing 126 kJ and 1.2 g protein/(kg·d). After a 12-h overnight fast, subjects received over 6 h Gln [0.8 mmol/(kg·h)] or an isonitrogenous and iso-osmolar mixture of nonessential amino acids (Gly/Ala/Ser/Pro/Asp/Asn/His, 2:2:4:4:7:10:10), without other nutrients. Thirty min after the end of enteral infusion, an upper endoscopy was performed. Six mucosal biopsies were taken from the distal duodenum, three were fixed in formalin 10% and embedded in paraffin for histological examination and immunostaining for HO-1 and the other three were immediately frozen in liquid nitrogen in guanidinium isothiocyanate and stored at −80°C until HO-1 mRNA analysis.

Reverse transcriptase-polymerase chain reaction (RT-PCR). Mucosal RNAs were extracted from biopsies by a modified-extraction method as previously described (22). The quality and quantity of total RNA were determined by spectrophotometry using the A260/A280 nm ratio. The integrity was also controlled by visualization of 18S and 28S ribosomal bands. RNA expression of HO-1 was studied by RT-PCR as previously described (23). Briefly, 1 μg of total RNA was reverse transcribed into complementary DNA (cDNA) using 10 U/μL
of Moloney Murine Leukemia virus-reverse transcriptase (MMLV-RT; Promega, Charbonnières, France), 1X RT buffer (Promega), 8 μmol/L of hexanucleotides random primers (PN6; Pharmacia, Orsay, France) and 1 mmol/L of each of the deoxynucleotide triphosphates (dNTP, Promega) in a final reaction volume of 30 μL in the presence of 2 μL of ribonuclease inhibitor (Promega). Samples were incubated at 37°C for 60 min, followed by heating for 5 min at 95°C and stored at −20°C until use. The RT reaction mixture was amplified by PCR using sense and antisense primers (Eurogentec, Seraing, Belgium) specific for HO-1 and GAPDH, was used as an internal standard: HO-1, 5'-ACATCTATGTGGCCTGGAG-3' and 5'-GTTGACAGGAAGCAGTCT-3'; GAPDH, 5'-GTCATC-CATGACAACCTTGG-3' and 5'-GAGCTTGACAAAGTGGT-CGT-3'. The PCR reaction mixture (25 μL) consisted of sense and antisense primers (50 μmol/L each), 1 U of Thermoprime Plus ADN Polymerase (ABgene, Courtaboeuf, France), 200 mol/L of each of the four dNTP, 1X PCR buffer (Promega) supplemented with 2500 mol/L MgCl2 (Promega), 18.5 kBq of α-32P dATP (Amersham, Orsay, France) and 5 μL of RT samples. Amplification was performed by 24 cycles consisting of denaturation for 30 s at 94°C, primer annealing for 40 s at 60°C and primer extension for 40 s at 72°C using a thermal cycler MJ research (PTC 200). The PCR products were electrophoresed on a 6% polyacrylamide gel. After 2 d of exposure, the autoradiogram bands were analyzed by an image analyzer (Biocom Equipment, Lecphor software, Les Ulis, France). The levels of amplified product were normalized to constant amounts of GAPDH mRNA. The number of PCR cycles was chosen within the linear phase to use this assay as a relative measure of gene expression.

Immunohistochemistry. All samples were coded to allow an evaluation without knowledge of the treatment group. The indirect streptavidin-biotin peroxidase method was performed on paraffin sections of duodenal biopsies using a commercially available kit (Dako ChemMate, Dako A/S, Copenhagen, Denmark). In brief, formalin-fixed paraffin-embedded 5-μm tissue sections were deparaffinized by washes in xylene for 10 min and then dehydrated in ethanol. The sections were submerged in washing pH 6.0 citrate buffer and subjected to microwave treatment (2 × 5 min), then cooled for 20 min and rinsed with water wash. All samples were processed on a techmate 500 automated immunostainer (Dako). After pretreatment with hydrogen peroxide to quench endogenous peroxidase activity, the slides were incubated with primary polyclonal antibody against HO-1 (1:1500 dilution; HC-3001 Affiniti Res, Exeter, UK) for 25 min at room temperature. A streptavidin-biotin/ horseradish peroxidase detection system (K 5001, Dako) was used. Sections were counterstained with hematoxylin and then mounted with Entellan (Merck, Darmstadt, Germany). A negative control was performed by preadsorption of the antibody with the blocking peptide (HP-9301 Affiniti Res). A positive control was represented by Kupffer cells of the liver. Assessment of HO-1 immunostaining was performed by a single investigator (M.C.) who was unaware of the treatment, on 12 light microscopy high-power fields using a X40 lens. The percentage of immunoreactive cells in the lamina propria (LPC) was recorded in at least 500 cells on each slide. For epithelial cells (IEC), the grade of immunostaining was calculated by multiplying the percentage of immunoreactive cells by the semiquantitative score of the staining intensity (from 1 to 4, with 1 representing a focal staining, 2, 3 and 4 representing diffuse weak, to moderate and strong staining, respectively) as previously described (24).

Statistical analysis. Given the number of experiments, results were compared using nonparametric analysis with the Wilcoxon paired t test and the Spearman correlation test. For each RNA analysis, statistical tests were performed using the mean of triplicates. Differences were considered significant at P ≤ 0.05.

RESULTS

The immunostaining for HO-1 protein in human duodenal mucosa (Fig. 1 and Table 1) revealed that HO-1 was constitutively expressed in 99% of IEC from the villi, in 1% of IEC

FIGURE 1  Immunostaining for HO-1 protein in duodenal biopsies of healthy humans after a 6-h enteral infusion of glutamine (b and d) or a control isonitrogenous mixture of amino acids (a and c).
from the crypts and in 7.5% of total LPC. However, HO-1 was expressed more in the LPC of the villi core (10.2%), than in those of the crypt region (3.7%).

Gln infusion, compared with control amino acids, significantly increased the grade of the immunostaining in IEC (P ≤ 0.01) and the percentage of the immunoreactive LPC (P ≤ 0.05), but had no effect on HO-1 expression in crypt cells (Table 1). Gln affected HO-1 expression in the LPC of the villi core (P = 0.026), and tended to affect those of the crypt region (P = 0.085).

Gln infusion also increased (P ≤ 0.05) the mRNA level for HO-1 in whole duodenal biopsies relative to control infusions: median (range): 156 (102–182) vs. 100 (68–179)%. Gln increased HO-1 mRNA level in 7 of 9 subjects (Fig. 2). In addition, the mRNA level for HO-1 was correlated with the percentage of immunoreactive LPC (r = 0.55, P = 0.017) and with the grade of the immunostaining in IEC (r = 0.51, P = 0.030). Standard microscopic examination of duodenal biopsies revealed otherwise normal histologic features with no signs of mucosal lesion in any subject.

**DISCUSSION**

The present study provides, to our knowledge, the first reported data on HO-1 in human duodenal mucosa: HO-1 is constitutively expressed in this tissue and expression is increased by Gln in both epithelial and lamina propria cells. The specific induction of HO-1 by a pharmacologic or nutritional modulation may represent a potential therapeutic intervention during inflammation, including inflammatory bowel disease (1). Indeed, HO-1 induction has been shown to have a protective role in experimental colitis in rats through a reduction of free radical production and an inhibition of nitric oxide synthase (iNOS) activity (8). In contrast, we observed previously that HO-1 mRNA level was not increased in the inflamed intestinal mucosa of ileoanal reservoirs (i.e., during "pouchitis" after total colectomy for ulcerative colitis), whereas cyclooxygenase-2 and iNOS mRNA levels were increased (23). The insufficient induction of HO-1 during intestinal inflammation could thus play a role in the pathogenesis and perpetuation of inflammatory bowel diseases.

Recently, it was reported that short-chain fatty acids, which have anti-inflammatory properties in colitis (25), markedly induced hsp25 expression in rat intestinal villous cells (26).

Gln has well documented beneficial effects on gut mucosa (9–12). We reported previously that Gln may specifically improve protein metabolism in human gut by a stimulating mucosal protein synthesis (27,28) and inhibiting the ATP- ubiquitin–dependent proteolytic pathway (27). Gln also decreased proinflammatory (IL-6 and IL-8) cytokine production by human gut in vitro (29). Furthermore, Gln induced hsp expression in a wide variety of heat-shocked cells (30,31) and in intestinal epithelial cell lines (17–19). However, the number of in vivo studies of the modulation of hsp expression by Gln is limited. In stressed or unstressed rats, tissue protection induced by intravenous Gln was associated with an induction of hsp25 and hsp72 expressions in several organs, such as the heart, the lungs or the colon (20). In endotoxemic sheep, Gln increased hsp72 expression in cardiomyocytes without modification of hemodynamic variables (21). Gln also had a protective effect on intestine through HO-1 induction after ischemia-reperfusion injury (16). The present work is the first to report the effects of Gln on HO-1 mRNA and protein expression in human gut, as well as in any type of human tissue.

In duodenal mucosa, HO-1 was constitutively expressed in nearly all IEC and ~10% of LPC from the villi core, whereas its expression was minimal in deep mucosa. Gln increased intestinal HO-1 expression in both LPC and IEC, and this histological finding was correlated with an increase of mRNA levels for HO-1. In addition, the effect of Gln was observed mainly in IEC and LPC from the villi core, whereas HO-1

![FIGURE 2](https://i.imgur.com/3.png)

**FIGURE 2.** Representative experiment (A) and intraindividual comparisons (B) between HO-1 mRNA expression measured by reverse transcriptase-polymerase chain reaction in duodenal biopsies of healthy humans after a 6-h enteral infusion of glutamine or a control isonitrogenous mixture of amino acids. *P ≤ 0.05 vs. control amino acids.
expression in crypt cells was unaffected. Thus, Gln infused via the enteral route may have a local effect on HO-1 expression in villous cells. The ability of parenterally administered Gln to affect HO-1 expression in deep mucosa in humans was suggested from previous data in rats (20), but this requires further research. However, in the physiologic situation, provision of nutritional substrates to the mucosa appears to be a key factor in the maintenance of gut integrity. Moreover, glutamine oxidation via the tricarboxylic acid cycle, which is increased in the absence of glucose, has been suggested as a prerequisite for induction of HO-1 in hepatoma cells (32).

Whether Gln could affect HO-1 expression under conditions of intestinal injury should be investigated further, for example, during pouchitis, because it involves a defective expression of HO-1 (23). Early reports have suggested that Gln and butyrate-containing suppositories may improve the outcome of pouchitis, but HO-1 was not examined in this study (33). The modulation of HO-1 expression by Gln may contribute to its protective effect, together with the previously reported reduction of proinflammatory cytokines production (15,27), and preservation of intestinal protein metabolism (27,28).

In summary, HO-1 is constitutively expressed in epithelial cells and to a lesser extent in lamina propria cells of the human duodenum. Enteral Gln enhanced HO-1 mRNA and protein expression in human gut mucosa. These results provide an additional rationale for the evaluation of enteral Gln supplementation in various clinical situations, such as intestinal inflammation.

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LITERATURE CITED


