RESEARCH COMMUNICATION

Lansoprazole Induces Collagenous Colitis in the Colon of Mongolian Gerbils

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Abstract

Collagenous colitis (CC) is an illness characterized by chronic diarrhea with possible effects on neoplastic development, but there have been no reports in animals. We therefore tried to establish CC development in a Mongolian gerbil (MG) model by long-term continuous lansoprazole (LPZ) administration and aimed to clarify the relationship between LPZ administration and CC occurrence. We divided 69 gerbils into 6 groups: *Helicobacter pylori* (Hp)-infected+high-dose LPZ, Hp-infected+low-dose-LPZ, Hp-infected, high-dose-LPZ, low-dose-LPZ, and control. The gerbils were sacrificed and entire colons were excised at experimental weeks 27, 54, and 108. We examined colonic lesions by staining of Swiss-roll intestines pathologically. A total of 3 gerbils had CC-like lesions in the proximal colon. All MGs with CC-like lesions were from LPZ treated groups (3 of 35; 8.6%). The thickened subepithelial collagen band detected in these lesions strongly resembled that of human CC lesions. Immunohistochemical analysis indicated a tendency for more chromogranin A-positive cells in the upper layer of colonic crypt following continuous LPZ administration. In conclusion, we successfully established development of CC-like lesions in an MG model by continuous LPZ administration and determined that the ectopic endocrine cells that were induced by LPZ administration may influence the occurrence of these lesions in the colon.

Keywords: Collagenous colitis - *Helicobacter pylori* - lansoprazole - Mongolian gerbils

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Introduction

The main symptom of collagenous colitis (CC), which was first reported by Lindstrom in 1976 (Lindstrom, 1976), is chronic diarrhea. Colonic lesions that result from this disease are characterized histologically by thickened subepithelial collagen bands (Bohr et al., 1996). Induction of CC by the drug lansoprazole (LPZ) has recently been reported (Thomson et al., 2002; Wilcox and Mattia, 2002; Hilmer et al., 2006; Chande and Driman, 2007). However, the clinicopathological features of LPZ-associated collagenous colitis have not been well described in humans (Umeno et al., 2008).

An animal model of CC would be helpful for analysis of this disease and possible effects on neoplastic development. However, to our knowledge, there have been no reports of CC in animals. In this paper we established a Mongolian gerbil (MG) model of CC using continuous long-term LPZ administration. A relationship between infection with *Helicobacter pylori* (Hp) and CC occurrence has been reported (Narayani et al., 2002). It is well-known that MGs can be easily infected with Hp, providing a good experimental animal in which to clarify the role of Hp in upper gastrointestinal tract disease (Hirayama et al., 1996). The lesions resulting from Hp infection in this model, such as chronic gastritis, peptic ulcers, and intestinal metaplasia, resemble the lesions induced by Hp in humans (Tatematsu et al., 2005; Tsukamoto et al., 2007). We have also previously analyzed the occurrence of stomach cancer in the Hp-infected MG model by analysis of gastric and intestinal phenotypes (Mizoshita et al., 2006). The lesions observed in the glandular stomach of Hp-infected MGs were similar to those observed in the human stomach. These data suggest that Hp-infected MGs may be a good model of human CC and that therefore CC-like lesions may occur in the colon of MGs following continuous, long-term LPZ administration.

In the present study, we found that CC-like lesions

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developed in the colon of MGs following continuous LPZ administration over 50 and 100 weeks. We also examined the pathology of these lesions by immunohistochemical analysis of chromogranin A (CgA) that was used as an endocrine cell marker.

Materials and Methods

Animals and Samples

A total of 69 13-week-old MGs (Meriones unguiculatus; MGS/Sea, Kyudo Co., Ltd., Fukuoka, Japan) were housed in plastic cages on hardwood chip bedding in an air-conditioned biohazard room with light/dark cycles of 12/12 hours. Thirty-four were the Hp-infected MGs, and 35 were uninfected ones. The gerbils were fed with CE2 (Cyubukagakushizai, Nagoya, Japan) containing LPZ. The experimental design was approved by the Animal Care Committee of Nagoya City University Animal Research Institute and the animals were cared for in accordance with institutional guidelines, which comply with the instructions of the Health Labour and Welfare Ministry regarding animal experiments (Tsukamoto et al., 2011).

Experimental design

The experimental design is illustrated in Figure 1. Sixty-nine gerbils were first divided into two major groups: Hp-infected and non-infected groups. Both of these groups were subsequently subclassified into three groups: a high-dose LPZ (1500 mg/day) group, a low-dose LPZ (300 mg/day) group, and a non-LPZ-treated group. The resulting six groups of gerbils were labeled groups A –F as follows: Hp-infected + high-dose LPZ (Group A, n=11), Hp-infected +low-dose LPZ (Group B, n=12), Hp-infected, no-LPZ (Group C, n=11), high-dose LPZ (Group D, n=12), low-dose LPZ (Group E, n=12), control (Group F, n=11). High-dose (Groups A and D) and low-dose (Groups B and E) LPZ groups were given LPZ mixed in the food from experimental week 4 (Tsukamoto et al., 2011). The gerbils were sacrificed humanely at experimental weeks 27, 54, and 108. All animals were subjected to deep ether anesthesia after 24 h fasting, and were then laparotomized and exsanguinated from the inferior vena cava, following which the entire colon was excised. The colon was washed with PBS to remove fecal materials.

Histopathological analyses

We used swiss-rolled intestines for histopathological analysis. The swiss-rolled intestines were stained with hematoxylin and eosin (H&E), and Masson's trichrome, using common methods. The degree of chronic active colitis was graded according to criteria modified from the Updated Sydney System (Dixon et al., 1996) by scoring the following parameters: inflammatory cell (mononuclear cell) infiltration (0-3; 0, normal; 1, mild infiltration into lamina propria; 2, moderate infiltration into lamina propria; 3, marked infiltration into lamina propria and multiple lymphoid follicle formation).

Immunohistochemistry

Immunohistochemical staining was carried out using antibodies against CgA and gastrin antigens (Yanaihara Institute Inc., Fujinomia, Japan). The precise procedures for immnohistochemical techniques were previously described (Mizoshita et al., 2006). Briefly, 4 μm-thick consecutive sections were deparaffinized and hydrated through a graded series of alcohols. After inhibition of endogenous peroxidase activity by immersion in 3% H2O2/methanol solution, antigen retrieval was achieved by heating in 10 mM citrate buffer (pH 6.0) in a microwave oven for 10 min at 98 °C. The sections were then incubated with primary antibodies. After thorough washing in phosphate-buffered saline (PBS), the sections were next incubated with biotinylated secondary antibodies, and then with avidin-biotin horseradish peroxidase complexes (Vectastain Elite ABC kit, Vector Laboratories, Inc., Burlingame, CA, USA). Finally, immune complexes were visualized by incubation with 0.01% H2O2 and 0.05% 3,3'-diaminobenzidine tetrachloride (DAB). Nuclei were counter-stained with Mayer's hematoxylin.

Statistical Analysis

Results are expressed as means ± SE. Data were analyzed using one-factor ANOVA and Tukey-Kramer's multiple comparison procedure, as appropriate. P-values <0.05 were considered as statistically significant.

Results

Development of CC-like lesions in the colon of MGs following continuous LPZ administration

Of the gerbil groups tested, a total of 3 gerbils had CC-like lesions in the colon. All three MGs with CC-like lesions had been given food containing LPZ. No lesions were observed in the colon of MGs that had been given normal food. The MGs with CC-like lesions represented 8.6% (3 out of 35) of the MGs that had been treated with LPZ. All of the CC-like lesions appeared in the proximal colon (the ascending colon in the human).

A thickened subepithelial collagen band was detected in the lesions of the colon (Figure 2), and the band had a strong resemblance to human cases. The collagen band was stained blue by the Masson's trichrome staining.

Immunohistochemical analysis of CgA

The colons of the MGs were further analyzed by

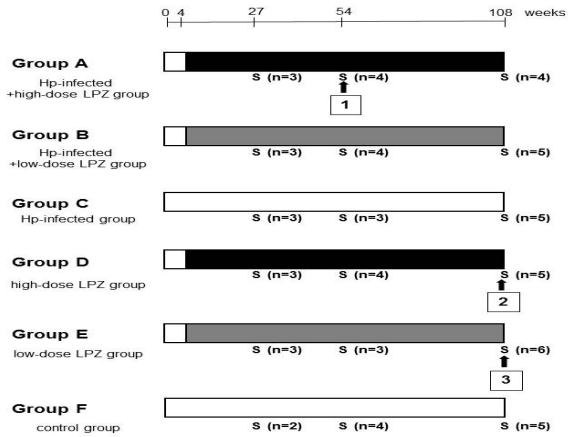

Figure 1. Experimental Design

Table 1. Analysis of the Colons of MGs with CC-like Lesions.

Group		upper layer	middle layer	bottom layer
A	Hp-infected +high-dose LPZ group	9.5± 1.0	4.0± 1.0	6.8± 2.8
B	Hp-infected +low-dose LPZ group	13.8± 3.1*	4.0± 0.6	6.8± 1.7
C	Hp-infected group	3.8±0.9	4.2±1.0	9.8±2.2 #,##
D	high-dose LPZ group	11±2.3 *,**	3.6±0.8	5.0±1.1
E	low-dose LPZ group	9.7±1.4 *	4.5±1.1	5.5±1.1
F	control group	4.8±0.7	3.2±0.9	8.6±0.8 #,##

Data are means ± SE, *p<0.05 compared with the middle layer. **p<0.05 compared with the bottom layer. #p<0.05 compared with the middle layer. ##p<0.05 compared with the upper layer.

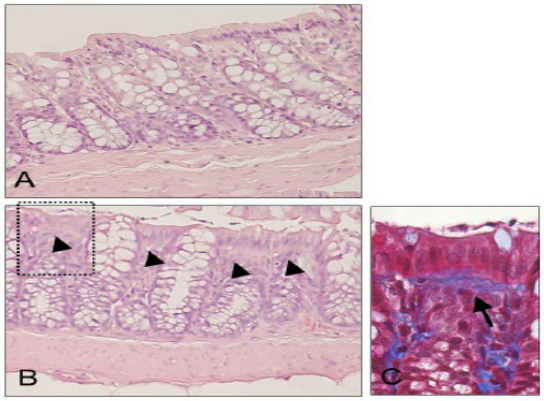

Figure 2. Histochemical Detection of a Collagen Band in MGs that Had Been Administered LPZ. A and B: H&E staining, C: Masson's trichrome staining. (A) No collagen band was detected in the non-LPZ Group (Groups C and F). A representative colon that was sampled at 108 weeks (Group F) is shown. (B) Increased inflammation within the lamina propria and a thickened subepithelial collagen band (arrow head) was observed in MG#1 in Figure 1 in the Hp + high-dose LPZ Group. (C) A higher magnification of the boxed area in (B) is shown. The thickened collagen band is stained blue (arrow)

immunohistochemical analysis of the expression of the endocrine marker CgA. In general, the CgA-positive cells were detected at the bottom of the colonic crypt. In the CC-like lesions, some CgA-positive cells were observed in the upper layer of the colonic crypt. However, the total number of CgA-positive cells in the colonic crypt was not significantly different between colons with and without CC-like lesions. CgA-positive cells were also observed in the upper layer of the colonic crypt in LPZ-treated MGs which did not have CC-like lesions. To determine if the localization of CgA-positive cells in the colonic crypt might be associated with LPZ treatment, we further quantified the number of CgA-positive cells in different regions of the colon of the MG groups. This assay indicated that there tended to be more CgA-positive cells in the upper layer than in the middle or the bottom layers of the colonic crypt in LPZ-treated groups compared to non-LPZ-treated groups. In contrast, in the groups that were not treated with LPZ, there were significantly more CgA-positive cells in the bottom layer than in the upper or middle layers of the colonic crypt (Table 1). However,

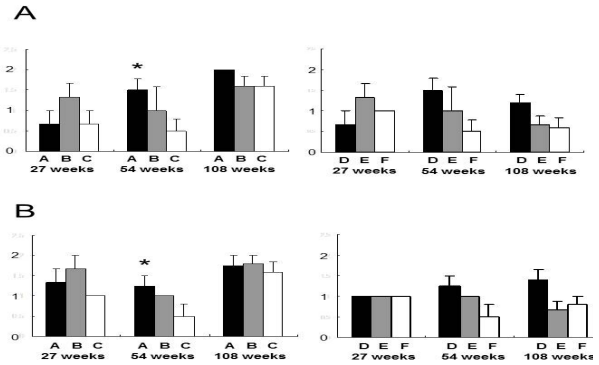

Figure 4. The Degree of Chronic Active Colitis in MG Colons. The degree of chronic active colitis in (A) the proximal colon and (B) the distal colon of the indicated MG groups was determined by analysis of the degree of infiltration of inflammatory cells. Black bars: high-dose LPZ, grey bars: low-dose LPZ, open bars: no-LPZ. The degree of infiltration of inflammatory cells tended to be higher in LPZ-treated groups than in non-LPZ treated groups. In particular, the degree of infiltration of inflammatory cells at 54 weeks in the Hp-infected + high-dose LPZ group (Group A) was significantly higher than in the Hp-infected + no-LPZ group (Group C). (*p<0.05)

there was no significant difference in the total number of CgA-positive cells between LPZ-treated and non-treated groups. In contrast to the expression of CgA-positive cells in the colons, no gastrin-positive cells were detected in the colons.

Inflammation Score

We also evaluated the degree of chronic active colitis at two points in the colon (proximal colon and distal colon) according to criteria modified from the Updated Sydney System as described previously (Figure 3). The degree of infiltration of inflammatory cells tended to be higher in the LPZ-treated groups than in the groups not treated with LPZ. In particular, the degree of infiltration of inflammatory cells in the Hp-infected + high-dose LPZ group (Group A) at 54 weeks was significantly higher than that in the Hp-infected + no-LPZ group (Group C).

Discussion

Our data provide clear evidence, for the first time, that CC-like lesions occur in the colon of MGs following continuous administration of LPZ. To our knowledge, this is the first report of the development of CC-like lesions in an animal model. All CC-like lesions appeared in the proximal colon, which is similar to CC-lesions in humans. This MG model has been shown to be a good animal model for analysis of Hp-associated upper gastrointestinal tract disease (Hirayama et al., 1996). By analysis of gastric and intestinal phenotypes and Hp eradication, we previously showed that cancer occurs in the stomach of Hp-infected MG (Mizoshita et al., 2006; Mizoshita et al., 2007; Cao et al., 2008). The data presented in the current paper indicate that the MG model may be useful for analysis of lower as well as upper gastrointestinal tract disease.

In the present study, CC lesions were observed in one Hp-infected MG and two uninfected MGs. It has been

reported that there is no statistically significant difference in the serum level of anti-Hp IgG between CC and control cases (Narayani et al., 2002). However, Hp eradication has occasionally been an effective therapy for CC (Narayani et al., 2002). Further studies are therefore needed to clarify the relationship between CC occurrence and Hp infection.

In this study, the degree of infiltration of inflammatory cells in LPZ-treated groups tended to be higher than that in groups not treated with LPZ. In particular, the degree of infiltration of inflammatory cells in the Hp-infected + high-dose LPZ group (Group A) was significantly higher at 54 weeks than in the Hp-infected + non-LPZ group (Group C). Further studies are also needed to clarify the relationship between LPZ administration and infiltration of inflammatory cells.

In the case of the upper gastrointestinal tract, hypergastrinemia is a physiological response to a reduction in gastric acid secretion, suggesting that LPZ, a proton pump inhibitor, influences endocrine cells, including G-cells, in the stomach (Thomson et al., 2010). LPZ is unique in its ability to inhibit colonic proton pumps by binding to cysteine residue 321 via 3 disulfhydryl bonds, suggesting that inhibition of colonic proton pumps might have an effect on colonic secretion and pH (Kaunitz et al., 1982). Taking into account the above-mentioned reports, we considered the hypothesis that LPZ might influence the function of endocrine cells in the colon, which might thereby induce CC-like lesions. In the present study, we observed some CgA-positive cells in the CC-like lesions in the upper layer of the colonic crypt of MGs that had been continuously administered LPZ. Moreover, there tended to be more CgA-positive cells in the upper layer than in the middle or bottom layers of the colonic crypt of MGs that had been continuously administered LPZ, compared with the animals to whom LPZ had not been administered. Consistent with this finding, it has been previously reported that the serum CgA level of a CC patient was elevated at the active stage and decreased at the inactive stage of colitis (Docherty et al., 2010), and that deposition of CgA was related to myocardial fibrosis in dilated cardiomyopathy patients (Xie et al., 2009). In the present study, there was no significant difference in the total number of CgA-positive cells between the LPZ-treated and the non-LPZ-treated groups. However, the distribution of CgA-positive cells was different between these groups. Thus, CgA-positive cells had moved to the upper layer of the colonic crypt as a result of LPZ administration. We therefore consider that ectopic localization of CgA-positive cells (i.e., CgA-positive cells in the upper layer of the colonic crypt) may be important for the histogenesis of CC, since, under these conditions, a collagen band is formed in the subepithelium.

In conclusion, we successfully established the development of CC-like lesions in an MG model by continuous LPZ administration. The ectopic localization of endocrine cells that was induced by LPZ administration may influence the occurrence of these lesions in the colon.

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