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Lanthanum-Induced Mucosal Alterations in the Stomach (Lanthanum Gastropathy): a Comparative Study Using an Animal Model

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Abstract
Lanthanum (La) carbonate (LC) is one of the most potent phosphate binders that prevents the elevation of serum phosphate levels in patients with end-stage renal diseases undergoing dialysis. LC binds strongly to dietary phosphate and forms insoluble complexes that pass through the gastrointestinal tract. La deposition in patients treated with LC is a recently documented finding particularly observed in gastric mucosa. We herein describe the detailed gastric mucosal lesions in 45 LC-treated patients and address the potential underlying pathologic mechanism using oral LC administration in rats. Microscopically, La deposition, as shown by subepithelial collections of plump eosinophilic histiocytes or small foreign body granulomas containing coarse granular or amorphous inclusion bodies, was found in the gastric mucosa of 44 (97.8%) of the 45 dialysis patients in the study cohort, which was most frequently associated with foveolar hyperplasia (37.8%). Using oral administration of rats with 1000 mg/day LC for 2 or more weeks, La deposition was consistently detectable in the gastric mucosa but not in other organs examined. In addition, various histologic alterations such as glandular atrophy, stromal fibrosis, proliferation of mucous neck cells, intestinal metaplasia, squamous cell papilloma, erosion, and ulcer were demonstrated in the rat model. Thus, orally administered LC can induce mucosal injury, designated here as La gastropathy, which may alter the local environment and result in La deposition in the gastric mucosa, thereby potentially inducing abnormal cell proliferation or neoplastic lesions.

Keywords Dialysis · Lanthanum carbonate · Lanthanum deposition · Stomach · Histology · Rat

Introduction

Hyperphosphatemia is one of the most common complications of end-stage renal disease (ESRD). Elevated phosphorus (P) levels can lead to an increased risk of cardiovascular events and mortality [1–3]. Strict diet and/or oral phosphate binders are essential for patients with ESRD to maintain the serum phosphate levels within normal limits. Therefore, aluminum- or calcium-based phosphate binders were previously utilized in clinical settings; however, aluminum is no longer used for this purpose due to its severe side effects including osteodystrophy and encephalopathy [4, 5]. In addition, calcium overload due to administration of calcium-based phosphate binders can lead to ectopic calcification and increase the risk of cardiovascular events [6].

Lanthanum (La) is a rare earth element that is widely used in a variety of industrial and electronic products such as polishing materials and light emitting diodes. La carbonate (LC) has gained recent attention as an effective and safe treatment for hyperphosphatemia. LC is a noncalcium- and nonaluminum-based phosphate binder. LC, which has been adopted in the USA and Japan since 2005 and 2009, respectively, is widely available. Hutchison et al. reported that the total global LC exposure in dialysis patients by April 2015

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was estimated to be 851,634 person-years [7]. LC binds efficiently to dietary phosphate and forms insoluble complexes that pass through the gastrointestinal (GI) tract, where LC is predicted to be poorly absorbed with low bioavailability (approximately 0.00127%) in humans [8]. Most of the absorbed La is presumed to be transported to the liver and secreted into bile [9], and La was found in the liver and bone [9, 10]. It is controversial whether La can cross the blood brain barrier and transfer to the brain [10–14]. The safety of LC was assessed by a few comprehensive long-term preclinical and clinical studies [7, 8, 15].

The most common adverse gastrointestinal effects such as nonspecific gastrointestinal symptoms including nausea, vomiting, diarrhea, and abdominal pain have been described in LC-treated dialysis patients [7, 15, 16]; moreover, La deposition, particularly in the gastroduodenal mucosa of dialysis patients, has been recently documented mostly by Japanese researchers [17–21]. In our previous study of three dialysis patients, wide La deposition was observed in the gastric mucosa and regional lymph nodes [21]. La deposition is suggested to be associated with various gastric mucosal changes such as intestinal metaplasia and foveolar hyperplasia [19, 21, 22]. It remains to be clarified whether LC administration directly affects the gastric mucosa because these gastric mucosal lesions are commonly observed in patients untreated with LC. Furthermore, La deposition was detected in association with gastric cancer in several cases [18, 19, 21]. Therefore, assessment of the potential oncogenic role of longstanding mucosal La deposition in the gastrointestinal system is necessary. In this study, we analyzed the clinicopathological features of mucosal La deposition in detail using a large series of LC-treated patients and LC-administered rodents.

Materials and Methods

Clinicopathological Analysis of Human Tissue

This study included 90 dialysis patients, 45 of whom were administered with LC at doses ranging from 500 mg to 2250 mg per day, who underwent gastroscopy followed by biopsy as part of routine work-ups for complications in the upper GI tract and/or endoscopic or surgical resection for gastric cancers between 2009 and 2017 at the University Hospital of Occupational and Environmental Health, Saiseikai Yahata General Hospital, and Japanese Red Cross Kochi Hospital. The dose of the administered LC varied according to a serum phosphate level in each patient. The following gastric materials were examined: 39 biopsy specimens, 5 surgically excised specimens (including 3 described in our previous [21]), and 1 endoscopic submucosal resection specimen. The study was approved by Ethics Committee of Medical Research, University of Occupational and Environmental Health, Japan (approval number H27-104).

All specimens were formalin-fixed and paraffin-embedded and processed using the routine protocols for histological examination. Gastric mucosa was evaluated, with a focus on various alterations including chronic inflammation, neutrophilic infiltration, and glandular atrophy, which were categorized as minimal to mild and moderate to marked. The presence of *Helicobacter pylori* (*H. pylori*) and intestinal metaplasia were also assessed.

Animal Model of Lanthanum Carbonate Administration

All procedures performed in the studies involving animals were in accordance with the Guidelines for Proper Conduct of Animal Experiments by Science Council of Japan and approved by the Ethics Committee of Animal Care and Experimentation of the University of Occupational and Environmental Health, Japan (approval number AE15-011). Forty male Wistar rats (Japan SLC, Shizuoka, Japan), that were 8 weeks of age and weighed about 180 g, were divided into experimental ($n = 25$) and control ($n = 15$) groups. All rats were housed in stainless steel cages at 23–24 °C under normal laboratory conditions (12-h light–dark cycle, lights on between 07:00 and 19:00), with free access to food and water. The general condition of the rats was monitored every day, and their body weight was measured every week. Rats of the experimental group received a daily dose of 1000 mg/kg LC suspended in 0.5% carboxymethyl cellulose by oral gavage, as described previously [10, 23, 24], which was administered in the morning for 5 days a week (assumed as three times higher than the maximum weekly human dose based on the FDA's surface area conversion table [25]). LC [$La_2(CO_3)_3nH_2O$] was purchased from Wako Pure Chemical Industries (Osaka, Japan). The LC dose was adjusted according to the most recently recorded body weight of each rat. Control rats were administrated 0.5% carboxymethyl cellulose by oral gavage. The rats were fasted overnight prior to the experiments. At 2, 4, 12, 24, and 40 weeks of administration, 5 LC-administered rats were sacrificed by exsanguination from the abdominal aorta under deep anesthesia; blood sample (6 ml) was collected and centrifuged for 15 min at 3000 rpm to obtain serum. Serum gastrin level was measured using radioimmunoassay at 2, 12, and 40 weeks of administration. At each time point, three control rats were sacrificed simultaneously as the LC-administered rats, as aforementioned. Stomach, esophagus, small and large intestines, liver, kidney, and spleen were removed. The tissue samples were fixed with 4% paraformaldehyde in 0.1 M sodium phosphate buffer solution (pH 7.4, Wako Pure Chemicals Industries). At 2 and 24 weeks of administration, the small gastric tissue samples (50 mg) were obtained from the antrum and subjected to a quantitative analysis of La and a measurement of rat tumor necrosis factor (TNF-α) using enzyme immunoassay

(Quantikine ELISA, R&D systems, MN). At 40 weeks of administration, approximately 20 μl of the gastric juice was collected after pylorus ligation, and its pH was measured immediately using digital pH meter.

Histological sections of 4-μm thickness were stained with hematoxylin and eosin or alcian blue-periodic acid-Schiff. Immunohistochemical examination was performed using mouse monoclonal antibodies against CD68 (ab31630; Abcam, Cambridge, UK) and Ki-67 (M7248; DAKO, Glostrup, Denmark) after antigen retrieval in citrate buffer (pH 6.0). Immunoreaction was achieved using a labeled polymer prepared by combining amino acid polymers with peroxidase and an anti-mouse immunoglobulin G antibody (Histofine Simple Stain Rat MAX PO; Nichirei, Tokyo, Japan). Diaminobenzidine solution was used for visualization, followed by nuclear counterstaining with hematoxylin. Cell proliferation was evaluated by counting positively labeled cells per 1000 epithelial cells in the antral mucosa that was approximately 1000 μm distant from the gastroduodenal junction, as described previously [26, 27].

For quantitative analysis of La deposition, approximately 100 mg tissue of gastric antral mucosa of rats administered for 2, 12, or 40 weeks were digested in nitric acid at 60 °C and were adjusted to a final volume of 15 ml. La concentrations were measured with inductively coupled plasma mass spectrometry (ICP-MS 7700×, Agilent Technologies, Santa Clara, CA). The lower limit of La quantification in the tissue samples was 9.0 mg/kg.

Electron Microscopy and Energy Dispersive X-ray Spectroscopy

Small rat gastric mucosa samples were examined by transmission electron microscopy (TEM; JEM-1400 Plus, JEOL, Tokyo, Japan) and energy dispersive X-ray spectroscopy (EDS; JED-2300T, JEOL), according to our previously described methods [28]. Histological sections of human and rat stomachs were stained with hematoxylin and eosin, followed by immersion with lead citrate to enhance the contrast of nuclei in the backscattered electron imaging, and were analyzed by scanning electron microscopy (SEM; Miniscope TM3000, Hitachi, Tokyo, Japan) and EDS (Quantax 70, Bruker Nano, Berlin, Germany), as previously described [21].

Statistical Analysis

All statistical analyses were performed using the EZR software package (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R foundation for Statistical Computing, Vienna, Austria) [29]. Categorical variables were analyzed using Fisher's exact probability test. Ki-67 labeling index in rat gastric tissue samples and serum gastrin levels in rat blood samples were evaluated by Student's *t* test. The extent of La deposition in rat gastric tissue samples was evaluated by Welch test. Correlation between the extent of La deposition in rat gastric tissue sample and total dose of LC administration in rats was evaluated using Pearson's correlation coefficients. A P value of less than 0.05 was considered as statistically significant.

Results

Clinicopathological Features of Gastric Lesions in Dialysis Patients

Clinicopathologic characteristics of 90 dialysis patients with or without LC administration are summarized in Table 1. No significant differences were observed in age, gender, endoscopic findings including erosion, ulcer, redness, neoplasms, sites of evaluated specimens, or the histopathological conditions including chronic inflammation, neutrophilic infiltration, glandular atrophy, intestinal metaplasia, and the presence of *H. pylori* between the two groups. Hyperplastic polyps tended to be more frequently observed in the LC-treated group than in the nontreated group, although the incidence was not significantly different (Fig. 1a). White granular deposits, suggesting mucosal La deposition, were more often detected endoscopically and were histologically confirmed in hyperplastic gastric foveoles in the LC-treated group ($P = 0.01$ and $P < 0.01$, respectively, Fig. 1a, b).

La Deposition in Human Gastric Mucosa

La deposition was observed in the gastric mucosa of 44 (97.8%) dialysis patients treated with LC using SEM and EDS. The histologic changes due to La deposition included subepithelial collections of plump eosinophilic histiocytes or small foreign body granulomas comprising coarse granular, amorphous, or ferruginous inclusion bodies in the gastric mucosa. Four patients were followed up for longer periods ranging from 19 to 29 months after withdrawal of LC administration; in these patients, the La deposition in the gastric mucosa was present during the follow-up (Fig. 1c, d).

Backscattered images of SEM showed bright materials in the lamina propria of the gastric mucosa (Fig. 1e). In EDS images, La and P were almost equally distributed in the mucosal histiocytes examined (Fig. 1f, g). The spectrum revealed La, P and calcium (Ca) peaks in the granular and amorphous substances within the histiocytes (Fig. 1h). The high peaks of silicon (Si), chlorine (Cl), and Ca probably derived from a glass slide and lead (Pb) from lead citrate staining were considered background EDS signals (Fig. 1h).

Table 1 Clinicopathologic characteristics of dialysis patients with or without lanthanum carbonate (LC) treatment

	LC-treated ($n = 45$)	Nontreated ($n = 45$)	P value
Age (years)	67 ± 9.0 (mean ± SD)	67 ± 12.4 (mean ± SD)	0.64
Gender (M/F)	23/22	27/18	0.53
Dose of LC (mg/day)	1207 ± 527 (mean ± SD)	None	
Duration of LC administration (months)	33.5 ± 25.4 (mean ± SD)	None	
Total dose of LC administration (g)	1207 ± 1212 (mean ± SD)		
Endoscopic findings			
Erosion	10 (22.2%)	14 (31.1%)	0.48
Ulcer, ulcer scar	10 (22.2%)	14 (31.1%)	0.48
Gastritis	9 (20%)	4 (8.9%)	0.23
Redness	2 (4.4%)	5 (11.1%)	0.43
Hyperplastic polyp	6 (13.3%)	3 (6.7%)	0.49
Fundic gland polyp	0 (0%)	1 (2.2%)	1.00
Carcinoma	5 (11.1%)	3 (6.7%)	0.71
Adenoma	0 (0%)	1 (2.2%)	1.00
White granular deposits	7 (15.6%)	0 (0%)	0.012
Site of specimen			0.61
Fundus	3 (6.7%)	2 (4.4%)	
Body	20 (44.4%)	25 (55.6%)	
Antrum	22 (48.9%)	18 (40%)	
Neutrophils			0.52
Minimal-to-mild	38 (84.4%)	41 (91.1%)	
Moderate-to-marked	7 (15.6%)	4 (8.9%)	
Mononuclear cells			0.67
Minimal-to-mild	25 (55.6%)	28 (62.2%)	
Moderate-to-marked	20 (44.4%)	17 (37.8%)	
Atrophy			0.14
Not evaluated	11 (24.4%)	9 (20%)	
Minimal-to-mild	17 (37.8%)	25 (55.6%)	
Moderate-to-marked	17 (37.8%)	11 (24.4%)	
Intestinal metaplasia (IM)	23 (51.1%)	17 (37.8%)	0.29
H. pylori	4 (8.9%)	4 (8.9%)	1.00
Foveolar hyperplasia	17 (37.8%)	4 (8.9%)	0.002
La deposition	44 (97.8%)	None	

Morphological Analysis of Rat Models

During the 40 weeks of observation, the body weights of rats increased in a time-dependent constant manner in both groups (Fig. 2). No significant change was observed in the body weight of experimental rats when compared with the control rats. No histopathologically identified lesions were observed in the organs other than the stomach of the LC-administered or the control rats during the 40-week observation period.

Table 2 summarizes the morphological changes in the gastric mucosa of both groups. No discernible histological changes were seen in the forestomach except for hyperplasia of the squamous epithelium during the 40-week observation period. Histological changes detected in the glandular stomach of the LC-administrated rats were as follows (Fig. 3): infiltration of chronic inflammatory cells including many eosinophils in the lamina propria mucosa, which appeared as early as 2 weeks after the treatment commencement, followed by mucous neck cell proliferation (approximately at 4 weeks); atrophy in fundic and pyloric glands (approximately at 12 weeks) and fibrosis in the lamina propria (approximately at 12 weeks); and regenerative foveolar epithelium (approximately at 4 weeks). Intestinal metaplasia was noted predominantly in the 40-week administration group. Erosion was observed approximately at 2 weeks, which was more frequent in the LC-administered groups than the control groups. Of the five rats treated for 40 weeks, 1 rat revealed an open ulcer in the glandular stomach. Squamous cell papillomas in the glandular stomach

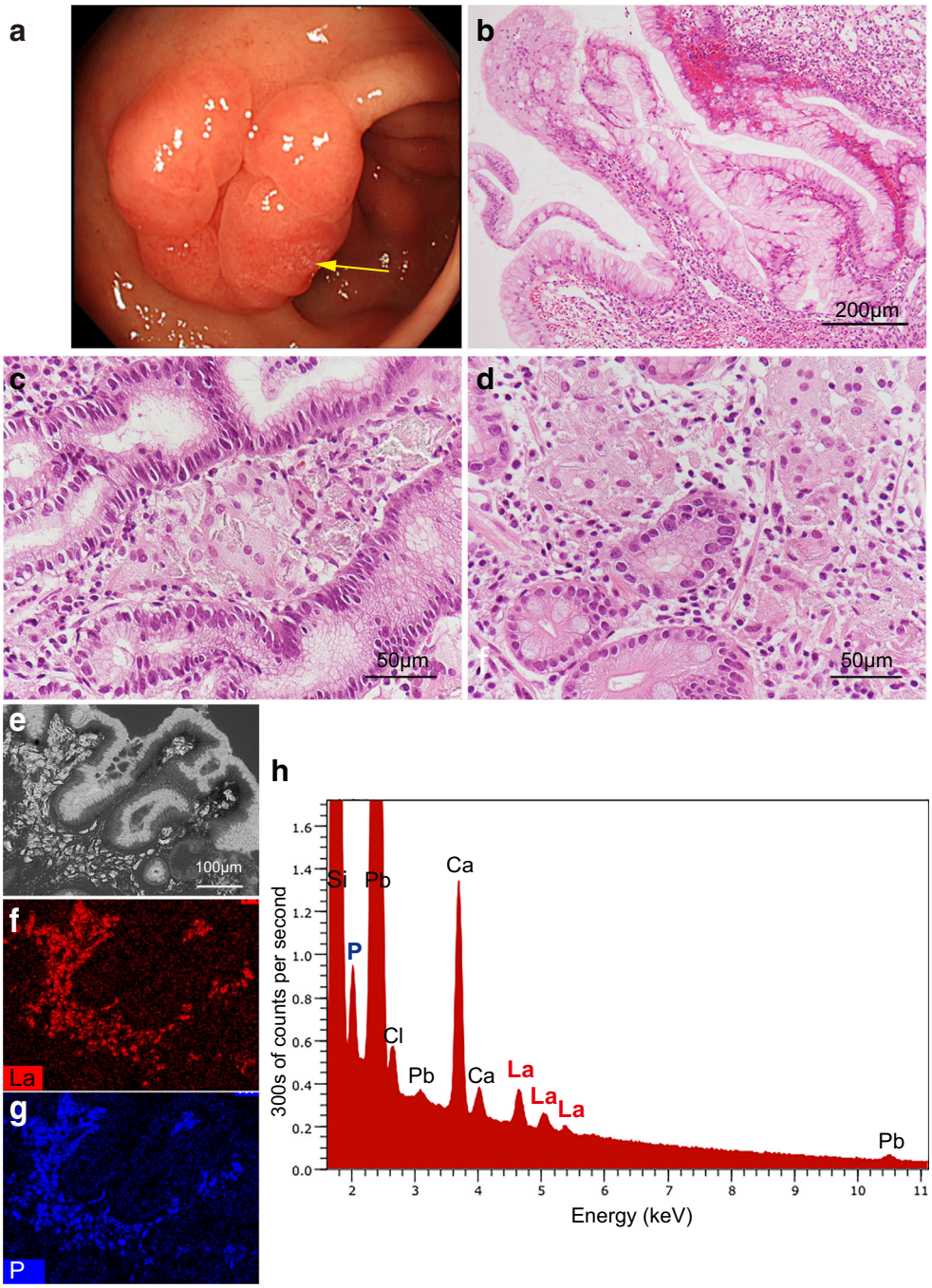

Fig. 1 Gastric mucosal changes in patients with end-stage renal disease who were treated with Lanthanum (La) carbonate (LC). **a** Endoscopic appearance of a hyperplastic polyp with white granular spots (*arrow*) arising in the antrum. **b** Microscopic view of foveolar epithelial hyperplasia. **c–d** The histiocytic reaction containing coarse granular, amorphous, or ferruginous bodies corresponding to La deposits, identified 24 months after LC administration (**c**) and 21 months after withdrawal of LC administration (**d**). **e** Backscattered images of scanning electron microscopy backscattered image showing deposits of bright materials in the subepithelial region of the gastric mucosa. **f**, **g** Energy dispersive X-ray spectroscopic image showing La and P colocalized with bright materials. **h** The spectrum (measurement time 300 s), showing peaks of La, P, and calcium (Ca). The peaks of silicon (Si) and chlorine (Cl) of a glass slide and lead (Pb) of lead citrate staining are also demonstrated

beneath the forestomach were observed in three and two rats in the 24- and 40-week administration groups, respectively. La deposition was observed as crystalloid materials or brown pigments and was frequently associated with accumulated histiocytes or small foreign body granulomas akin to those observed in gastric mucosa of humans administered LC (Fig. 3h). La was detectable in the lamina propria after 4 weeks of LC administration and was occasionally observed in the submucosa. No such LC deposition or granulomatous lesion was found in other organs examined microscopically. Immunohistochemically, CD68-positive histiocytes containing amorphous materials were observed in the subepithelial regions within the glandular stomach (Fig. 4a). The number of CD68-positive cells increased in a time-dependent manner. Furthermore, Ki-67-positive epithelial cells were frequently observed, predominantly in the antrum (Fig. 4b). Mean Ki-67 labeling index was significantly higher in all experimental groups that were administered LC for 12 weeks or longer than the control groups (Table 3).

By SEM, after at least 2 weeks of LC administration, backscattered electron images of the glandular stomach revealed brightly appearing amorphous materials within

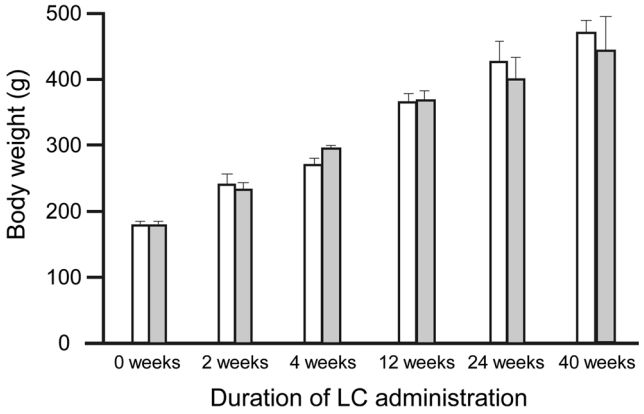

Fig. 2 During the 40 weeks of experimentation, there was a constant increase in the body weight of rats in both the LC-treated (gray columns, $n = 5$) and untreated (white columns, $n = 3$) groups. There was no significant difference in the body weight between the experimental and control rats

histiocytes, corresponding to the phagocytosed deposits containing La and P, which were confirmed by EDS as well as in humans. La deposition was observed not only in the lamina propria but also in the submucosa and was more predominantly seen in the mucosa of the antrum than in the body.

By TEM, the gastric mucosa exhibited electron-dense precipitates within secondary lysosomes in histiocytes and fibroblast-like cells just beneath the foveolar epithelium or in subepithelial areas and in some foveolar epithelial cells (Fig. 5a–c). The precipitates were observed as crystalloid or granular structures at high-power magnification (Fig. 5d). Similar particles were not observed in the control rats. TEM examination revealed an increase in the La deposition with an increase in the duration of LC administration.

Quantified La deposition in the antral mucosa also significantly elevated in a time-dependent manner (2 weeks: 12.2 ± 2.0 mg/kg; 12 weeks: 54 ± 20.3 mg/kg; 40 weeks: 284 ± 137 mg/kg, $P = 0.004$). The concentration of La deposited in rat stomach was significantly correlated with the total dose of LC administration ($r = 0.857$, $P < 0.001$, Fig. 6).

Gastric pH, Gastrin, and TNF-α

At 40 weeks of administration, the pH of the gastric juice was higher in the experimental group at 40 weeks of LC administration than the control group (5.2 ± 0.9 vs. 2.7 ± 0.5, respectively, $P = 0.04$). Although serum gastrin level of control rats was higher than the experimental group at 2 weeks of administration (470 ± 79.7 pg/mL vs. 850 ± 315 pg/mL, $P = 0.04$), no significant change was observed in serum gastrin level between groups in the remaining periods (12 weeks: 472 ± 45.5 pg/mL vs. 500 ± 34.6 pg/mL, $P = 0.40$, 40 weeks: 244 ± 59.4 pg/mL vs. 207 ± 15.3 pg/ml, $P = 0.34$). The concentration of TNF-α in mucosal tissue at 2 and 24 weeks of La administration was below 5 pg/mL.

Discussion

In Japan, LC as an oral P-binder was administered in dialysis patients since 2010, and the index case of gastric La

Table 2 Summary of histopathologic changes in the stomach of experimental and control rats

	Experimental rats ($n = 5$ per group)					Control rats ($n = 3$ per group)				
	2 weeks	4 weeks	12 weeks	24 weeks	40 weeks	2 weeks	4 weeks	12 weeks	24 weeks	40 weeks
Forestomach										
Hyperplasia of squamous epithelium	2	1	1	1	3	0	0	0	0	2
Glandular stomach										
Infiltration of inflammatory cells										
Minimal-to-mild	2	1	0	2	2	3	3	3	3	3
Moderate-to-marked	3	4	5	3	3	0	0	0	0	0
Glandular atrophy										
Minimal-to-mild	5	5	0	2	0	3	3	3	3	3
Moderate-to-marked	0	0	5	3	5	0	0	0	0	0
Stromal fibrosis										
Minimal-to-mild	5	5	0	0	0	3	3	3	3	3
Moderate-to-marked	0	0	5	5	5	0	0	0	0	0
Proliferation of mucous neck cells	0	5	5	5	5	0	0	0	0	0
Intestinal metaplasia	0	0	0	5	5	0	0	0	0	0
Erosion	2	3	3	3	5	0	1	0	1	1
Ulcer or ulcer scar	0	0	0	1	1	0	0	0	0	0
Squamous cell papilloma	0	0	0	3	2	0	0	0	0	0

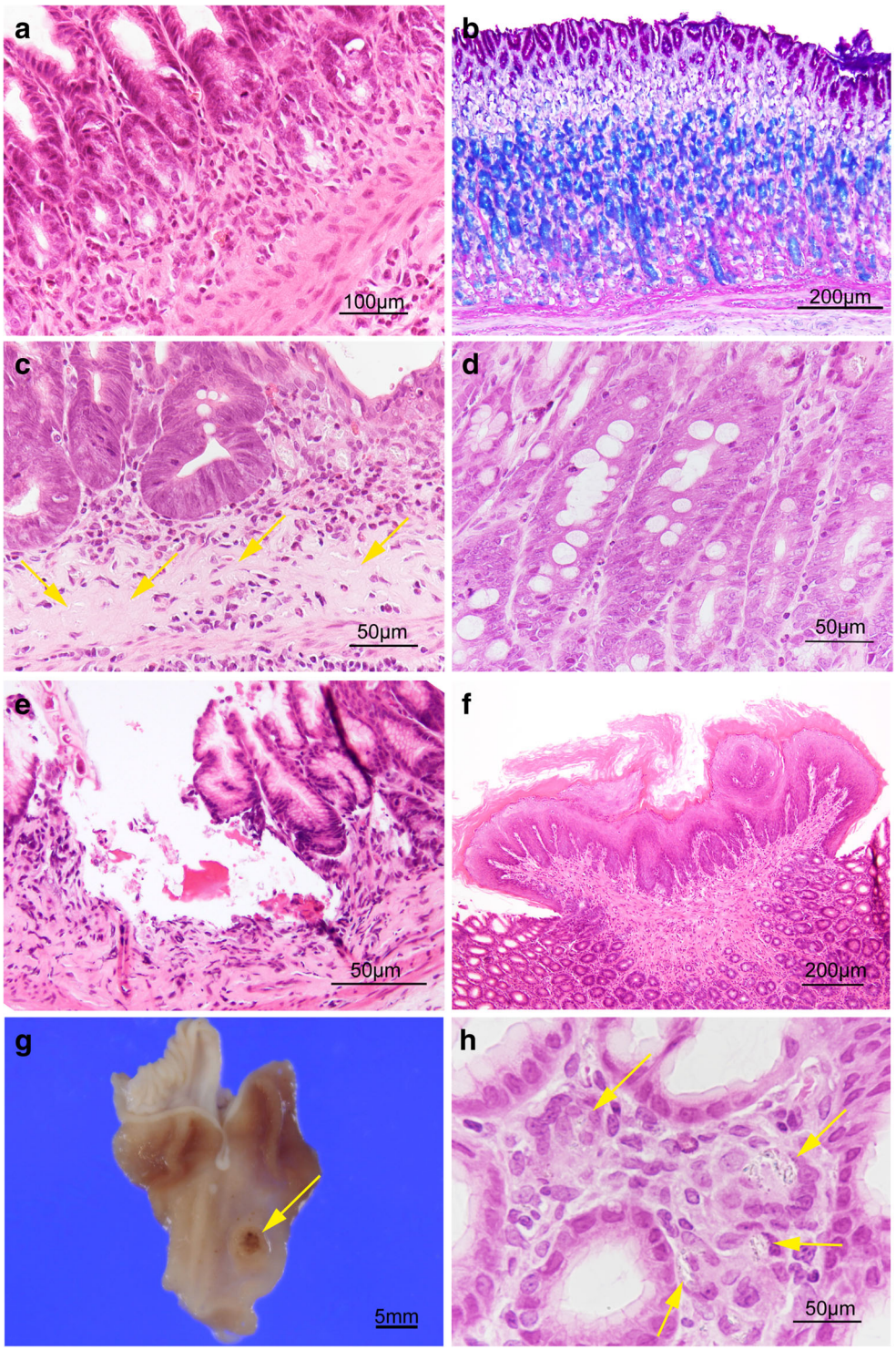

Fig. 3 **a** Gastric mucosal alterations in a rat model of LC treatment. Infiltration of chronic inflammatory cells are observed as early as 2 weeks of treatment. **b–e** Proliferation of mucous neck cells containing abundant and alcian blue-positive mucin (alcian blue-periodic acid-Schiff) (**b**), glandular atrophy with stromal fibrosis (*arrow*) (**c**), intestinal metaplasia (**d**), and erosion (**e**) are shown. **f–g** Squamous cell papilloma (**f**) and open ulcer (**g**) in the glandular stomach are also present. **h** La deposition in rat gastric mucosa is characterized by subepithelial collections of pump eosinophilic histiocytes or small foreign body granulomas containing coarse granular inclusion bodies (*arrows*)

deposition in the current series was in 2011. In recent years, similar cases have been increasingly reported. Although Goto et al. described that gastric La deposition was found in 85.7% of the patients treated with LC in their cohort [20], nearly all of the LC-administered patients in our series exhibited this phenomenon. In addition, the La deposition in the gastric mucosa might become evident sooner after the LC medication, which might remain in situ for a longer time than previously expected. Thus, this recently recognized finding should be taken into account in all patients treated with LC, and a detailed reassessment of its potential health damage is necessary.

In contrast, gadolinium (Gd) is another rare earth element that is clinically used as a contrast agent in magnetic resonance imaging. Nephrogenic systemic fibrosis (NSF) is a rare

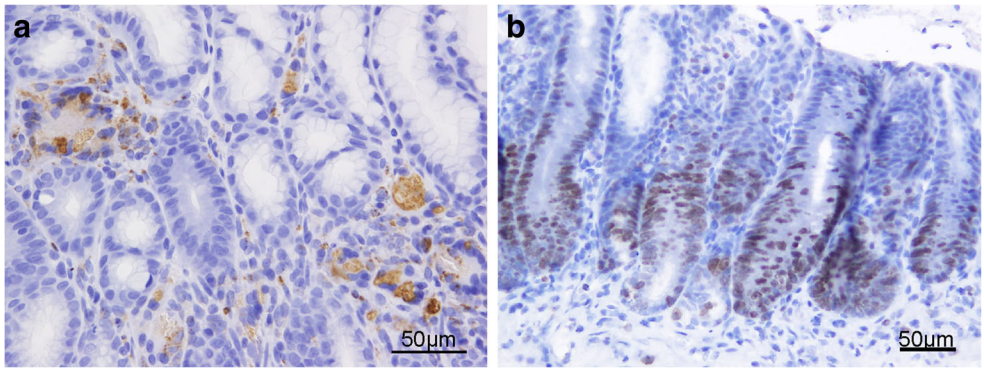

Fig. 4 **a**–**b** Immunohistochemistry of rat gastric mucosa. Histiocytes containing amorphous material are positive for CD68 (**a**), and epithelial cells are frequently labeled with Ki-67 after 40 weeks of LC administration (labeling index 30%, **b**)

systemic disorder in patients with ESRD that was first described in 2000 [30] and Gd-based imaging agents were suggested to cause NSF in patients with ESRD [31, 32]. Sanyal et al. reported that vascular and extracellular Gd deposition was observed in multiple organs such as skin, liver, lung, ileal wall, kidney, lymph node, skeletal muscle, dura matter, and cerebellum in an autopsy case with NSF [31]. In addition, Davis et al. reported that co-deposition of La and Gd was observed in the mesenteric lymph nodes of an NSF patient who was treated with LC [33]. However, no patient in our study was affected by NSF, and a systemic adverse effect of administrated LC like Gd-induced NSF has not been described to date. A peak of Ca colocalized with La and P in the EDS was detected in some of our patients, and the phenomenon is consistent with that previously reported [34, 35]. Furthermore, Greenson et al. showed that gastric mucosal calcinosis was obsereved in ESRD patients treated with a long-term therapy comprising aluminum-containing anatacids [36]. Thus, the administration of metal phosphate binders such as aluminum and LC may induce mucosal calcinosis. However, further investigation on a large number of patients may be needed to address additional complications associated with the La treatment.

Histological identification of mucosal La deposition is relatively straightforward in routine pathology practice due to its characteristic morphology repeatedly described in the literature [17–22, 34, 35]; however, fate of the deposited La in the mucosa remains poorly understood. Bervoets et al. noted electron-dense crystalline granular structures comprising La in lysosomes of rat hepatocytes after oral LC administration [10]. Our study using TEM also demonstrated that such electron-dense granular materials were present in lysosomes of tissue histiocytes or occasional multinucleated histiocytic cells in the stomach, indicating phagocytosed La; however, La is a rare earth metal that is likely resistant to intracellular digestion or metabolism after phagocytosis, which can potentially result in subsequent cellular damage and/or activation of tissue histiocytes that may induce various tissue reactions or alterations. Indeed, a variety of gastric lesions such as epithelial hyperplasia, hyperplastic polyps, intestinal metaplasia, and erosion or ulcers are almost invariably associated with La deposition in patients treated with LC. We would like to propose a designation of La gastropathy for such La-associated gastric lesions, although each lesion per se does not appear to be specific to La administration.

Table 3 Ki-67 labeling index in the stomach of experimental and control rats

Duration of LC administration	Ki-67 labeling index (mean ± SD)		*P* value
	Experimental rats ($n = 5$ per group)	Control rats ($n = 3$ per group)	
2 weeks	29 ± 5.4%	21.4 ± 4.3%	0.16
4 weeks	29.5 ± 4.4%	25.3 ± 1.8%	0.18
12 weeks	33.6 ± 5%	20.9 ± 1.3%	0.006
24 weeks	31.6 ± 3.1%	18.9 ± 0.5%	< 0.001
40 weeks	31.4 ± 3.8%	25.4 ± 1.7%	0.04

In our animal model, we were able to confirm the phenomenon that La could deposit in the gastric mucosa after oral LC administration and that the deposition was enhanced based on the length and total dose of LC administration that was reinforced by our observation of the clinical cases treated with LC. Specifically, foveolar epithelial hyperplasia was more prominent in LC-treated patients than untreated ones, which was in agreement with a previous study [22]. In addition, hyperplastic polyps and squamous cell papillomas tended to be frequent in LC-treated patients and rats, respectively. Thus, orally administrated LC might promote mucosal proliferative activity or increase the potential risk of tumor development in a time or dose-dependent manner, which has been poorly investigated in clinical or experimental settings.

Increased proliferative activity found in the gastric mucosal epithelium of our LC-treated rats was noteworthy and highlighted a possible adverse side effect of LC that has not been widely recognized. Due to the limited cases with neoplastic lesions associated with mucosal La deposition

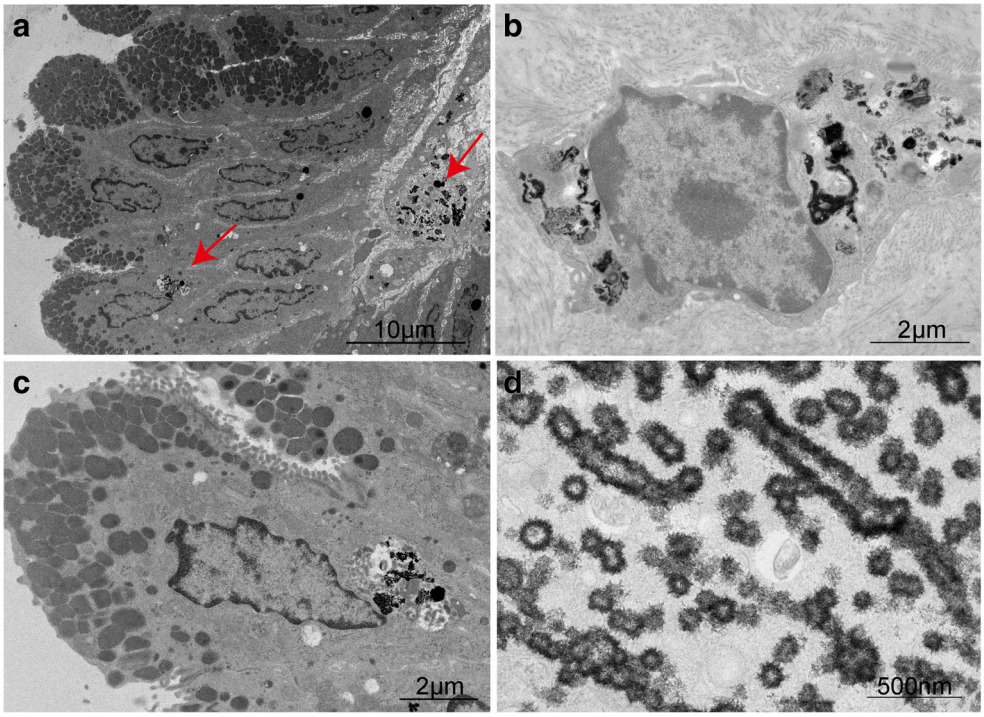

Fig. 5 Transmission electron microscopic image of the gastric mucosa of LC-administrated rats showing electron dense precipitates (**a**, red arrows), which are observed within secondary lysosomes in histiocytes (**b**) or some foveolar epithelial cells (**c**). The precipitates display crystalline or granular-like structures at high magnification (**d**)

[18, 19, 21, 22], this aspect of LC administration remains to be further addressed using a larger series of clinical specimens as well as experimental models. It is also notable that other gastric mucosal lesions such as chronic inflammation (gastritis), erosions/ulcers, and intestinal metaplasia were frequently associated with La deposition in human and rat gastric mucosa,

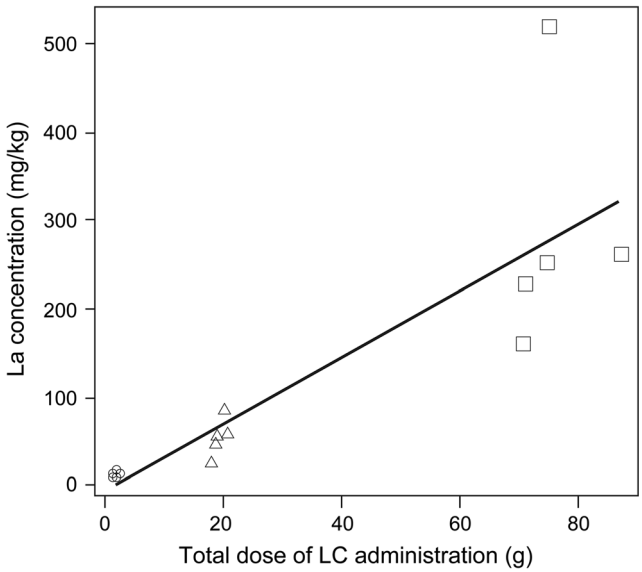

Fig. 6 Correlation between the extent of La deposition in rat gastric tissue sample and total dose of LC administration (circle: 2 weeks, triangle: 12 weeks, square: 40 weeks), showing significant increase in La deposition in the antrum according to the duration of LC administration ($n = 5$, $P = 0.004$). Pearson's correlation analysis indicated that the extent of La deposition in rat stomach and total dose of LC administration was significantly correlated ($r = 0.857$, $P < 0.001$)

which might function as an underlying pathologic process that might indirectly induce neoplastic changes. For example, intestinal metaplasia that can be induced by X-ray irradiation and chemicals such as polychlorinated biphenyl in the rat stomach is considered as a possible preneoplastic lesion [37]; however, such gastric lesions were commonly observed in patients not treated with LC.; therefore, they may be related to various factors associated with patient characteristics, such as ESRD, the elderly and *H. pylori* infection, other than LC administration. Gastroduodenal abnormalities such as gastritis, duodenitis, peptic ulcer, mucosal atrophy, and gastrointestinal angiodysplasia are common in patients with ESRD [38–41], and uremic rats also show adverse morphological changes such as gastric hypertrophy and hypergastrinemia [42, 43]. In the present study, we used normal Wistar rats to primarily focus on morphological alterations simply induced by orally administered LC. Although we considered that an ESRD animal model would be desirable in this regard, it was not feasible for this study. In addition, the dose of LC burden to experimental rats was relatively high in comparison with human in our study, and this may have exaggeratedly resulted in a variety of the mucosal lesions in rat stomachs. Therefore, a comparative study using an ESRD animal model as well as those with LC administration at various doses is necessary to address these points. However, gastric lesions such as gastritis and erosions/ulcers after LC administration may be induced by various other causes. For example, LC can inflict an injury on the gastric mucosa via minor but repeated physiological effects on mucosal epithelium. Subsequently, a part of the administrated and absorbed LC can be phagocytosed by tissue

histiocytes as histiocytic reaction. Generally, the macrophages or lymphocytes that infiltrate in the gastric mucosa stimulate the secretion of proinflammatory cytokines such as TNF-α, interleukin (IL)-1 beta, and IL-6. These cytokines are known to play an important role in the gastric injury via an inflammatory cascade and enhanced further recruitment of macrophages [44], although we could not observe elevated gastric mucosal concentration of TNF-α in our experimental rats at 2 and 24 weeks of LC administration potentially because of some technical problems such as the examination of insufficient mucosal tissue. Thus, the detailed molecular mechanism underlying La gastropathy is still unknown and needs further clarification.

The mechanism of La deposition in the gastric mucosa remains also elusive. Based on the observed dose-dependent deposition of La, we observed in the present study, passive diffusion by paracellular permeability or osmotic action is a plausible hypothesis as an initial step. The majority of orally administered LC in the gastric lumen can bind to dietary phosphate, thus producing an insoluble LaP complex that passes through the GI tract. Under an acidic environment, however, uncoupled or dissolved LC may precipitate with phosphate after the penetration into the gastric mucosa. Indeed, almost equal amounts of La and P were colocalized in the mucosal deposits by EDS, indicating La coupling with P. It is difficult to consider that LC or LaP complex directly penetrates into the gastric mucosa because La deposition is rare or unremarkable in the intestinal mucosa despite LC or LaP complex going through the intestinal lumen [20]; however, this finding is in contrast with the finding that La deposition was associated with intestinal metaplasia [19, 21, 22], which can reduce the acidity by attenuation of oxyntic cells. In our study, administered LC leads to reducing the acidity of gastric juice in experimental rats compared to that in control rats. Therefore, factors other than pH may underlie La deposition. Several studies proposed that intestinal metaplasia could lead to altered expression of tight junction proteins including claudin [19, 45] and revealed that the paracellular permeability of human gastric mucosa with intestinal metaplasia was significantly increased compared to that of mucosa without intestinal metaplasia, irrespective of the *H. pylori* infection [46]. Regarding gastric secretion parameters, no significant change was observed in serum gastrin level of experimental rats at 12 and 40 weeks of administration. Investigation of other parameters such as H^+ concentration, total acidity, and gastrin concentration of gastric juice are necessary to further address the mechanism of La deposition in the gastric mucosa.

Conclusion

In this study, we investigated the gastric alterations in a clinical cohort, the largest of its kind to date, and a rat model. La deposition was detected in the gastric mucosa of nearly all LC-administered patients in whom a variety of mucosal lesions such as chronic inflammation, intestinal metaplasia, epithelial hyperplasia, erosion, and ulcer were frequently observed in association with LC deposition. This study is also the first demonstration of La deposition in the rat gastric mucosa after oral LC administration, akin to clinical specimens, collectively with various gastric alterations, which we designate as La gastropathy. In addition to several preneoplastic conditions such as intestinal metaplasia and epithelial hyperplasia, enhanced mucosal proliferative activity observed after LC administration suggests a potential risk for tumorigenesis due to long-standing mucosal La deposition, which warrants careful follow-up studies in LC-treated patients.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Research Involving Human Participants and/or Animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study design involving humans was approved by the Ethics Committee of Medical Research, University of Occupational and Environmental Health, Japan (approval number H27-104). Informed opt-in/opt-out consent was obtained from all individual participants included in the study. All procedures performed in studies involving animals were in accordance with Guidelines for Proper Conduct of Animal Experiments by Science Council of Japan and approved by the Ethics Committee of Animal Care and Experimentation of the University of Occupational and Environmental Health, Japan (approval number AE15-011).

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