

# Chemoprevention of esophageal adenocarcinoma in a rat surgical model by a cysteinyl leukotriene receptor-1 antagonist

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**Abstract.** Reflux of gastroduodenal contents into the esophagus leads to the development of esophagitis and inflammation-associated pathologies, such as Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC). The role of the lipoxygenase (LOX) pathway in carcinogenesis has been recently reported; however, its involvement in esophageal carcinogenesis remains unclear. To address this, the present study investigated the potential of pranlukast, a cysteinyl leukotriene receptor-1 antagonist, to suppress the progression of BE and EAC in a rat duodeno-gastroesophageal reflux (DGER) model. Male Wistar rats that underwent DGER were divided into two groups. One group was fed commercial chow (control group), and the other was fed experimental chow containing pranlukast (pranlukast group). The rats were sacrificed at 10, 20, 30 and 40 weeks after surgery, and their esophagi were examined. Expression levels of 5-LOX, CD68, IL-8, VEGF and Ki-67 were investigated using immunohistochemistry, and apoptosis was analyzed using the TUNEL method. In the pranlukast group, esophagitis was milder, and the incidence of BE and EAC was significantly lower ( $P<0.05$ ) compared with that in the control group at 40 weeks after surgery. The number of cells positive for IL-8 and VEGF were significantly lower in the pranlukast group compared with the control group. Proliferative activity was also lower in the pranlukast group compared with the control group ( $P<0.05$ ). Pranlukast treatment increased apoptosis ( $P<0.05$ ). Overall, Pranlukast suppressed esophageal carcinogenesis in a rat DGER model, decreasing inflammatory cytokines such as IL-8 and VEGF.

## Introduction

In recent years, the incidence of esophageal adenocarcinoma (EAC) has been increasing (1,2). Given the extremely poor prognosis of EAC, it is crucial to focus on disease prevention. Barrett's esophagus (BE) is considered a precursor lesion of EAC and is believed to develop as a result of chronic esophageal reflux (3,4). The development of these diseases is influenced by the persistent inflammatory environment caused by the reflux of gastroduodenal contents (5-7). These process, gastro esophageal reflux disease (GERD) to EAC, is thought to be involved in the inflammation-metaplasia-adenocarcinoma (IMA) sequence (8,9). Chronic reflux of gastroduodenal contents into the esophagus leads to severe esophagitis and triggers cell proliferation. Prolonged proliferation progresses from hyperplasia to metaplasia, ultimately resulting in EAC. Clinical studies have highlighted the importance of chronic duodenogastroesophageal reflux (DGER) in the development of BE and EAC (10-12). Our rat models, which mimic human reflux esophagitis, have demonstrated that DGER can sequentially induce BE and EAC (13-15). Other researchers have also reported the impact of DGER in animal models (16,17).

The arachidonic acid (AA) cascade is extensively studied as a biological regulatory pathway. Metabolites of the AA cascade play a crucial role in the development and progression of inflammatory diseases and cancers. This metabolic pathway involves cyclooxygenases (COX) and lipoxygenases (LOX). While the administration of COX-2 inhibitors in rat models has been shown to suppress the inflammatory-metaplasia-adenocarcinoma (IMA) sequence (18), the role of the LOX pathway remains unclear.

Leukotrienes, which are potent pro-inflammatory lipid mediators, are synthesized from AA in various cell types, including mast cells, eosinophils, neutrophils, basophils, and macrophages (19). AA stimulates the production of two groups of leukotrienes under the action of 5-LOX, LTB<sub>4</sub> and cysteinyl leukotrienes (CysLTs). CysLTs bind to cysteinyl leukotriene receptors, primarily CysLT<sub>1</sub>R and CysLT<sub>2</sub>R. These receptors are expressed on various cells, such as mast cells, macrophages, and monocytes, and contribute to the production of various inflammatory cytokines (20).

The LOX pathway has been found to have a significant impact on various inflammatory conditions. It is released by cells present or recruited to the site of inflammation. LOX

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metabolites act as chemoattractants and activators of inflammatory cells, contributing to inflammation, immune responses, and host defense against infections (21). Prolonged production of leukotrienes leads to sterile inflammation in chronic inflammatory diseases (22). Leukotrienes are implicated in several inflammatory responses, including cell proliferation, angiogenesis, and cell survival, which are fundamental steps in oncogenesis (23).

Numerous studies have highlighted the crucial role of LOX-derived leukotrienes in carcinogenesis and cancer progression (24-29). CysLT1R, a receptor involved in the LOX pathway, is highly expressed in human colon and prostate cancers and is negatively associated with patient survival (25,26). It has been demonstrated that LOX-derived leukotrienes are more numerous in human esophageal adenocarcinoma compared with those in the normal esophagus (27-29). Additionally, the administration of a LOX pathway inhibitor has demonstrated chemopreventive effects in certain animal models (30,31). These findings provide evidence that the LOX pathway of AA metabolism plays a critical role in the development of cancer.

In clinical practice, medications targeting the LOX pathway have been implicated in the treatment of asthma and allergic rhinitis. Pranlukast, montelukast, zafirlukast, and zileuton are routinely used as primary therapeutic agents. The pharmacological mechanism of pranlukast, montelukast, and zafirlukast involves the antagonism of CysLT1R. Meanwhile, zileuton can be used to treat asthma by inhibiting 5-LOX activity. However, the clinical application of zileuton is limited due to its hepatotoxicity. Pranlukast, on the other hand, was introduced for clinical use in Japan in 1995 and has been marketed in several countries. Additionally, receptor antagonists have shown great potential in the treatment of asthma and other diseases. They may be promising candidates for chemopreventive interventions as their direct inhibition of these enzymes reduces the inflammatory response that contributes to carcinogenesis.

In this study, we investigated the chemopreventive effect of pranlukast, a CysLT1R antagonist, on inflammation-induced esophageal carcinogenesis using our established rat DGER model, which develops BE and EAC without the administration of any carcinogens (15).

## Materials and methods

**Animals and treatment procedures.** Eight-week-old male Wistar rats, weighing approximately 300 g (Charles River Laboratories Japan, Inc., Kanazawa, Japan), were used for the experiments. The rats were housed in cages with three rats per group and kept in a room maintained at a temperature of  $22\pm3^{\circ}\text{C}$  and a humidity of  $55\pm5\%$ , following a 12-h light-dark cycle. They were provided with standard solid chow (CRF-1, Charles River Laboratories Japan, Inc.) and tap water ad libitum. No carcinogens were administered throughout the study period. Prior to the surgical procedure, the rats underwent a 24-h fasting period and were anesthetized with isoflurane inhalation anesthesia. In isoflurane anesthesia, the initial dose is 3-5% and is maintained at approximately 1.5-2.5%. An upper abdominal incision was made, ensuring preservation of the stomach and vagal nerves. The esophagus was excised and ligated to the oral side of the stomach. A loop in the jejunum, located 4 cm from the ligament described

by Treitz, was identified. An end-to-side anastomosis was performed between the distal esophagus and jejunum using interrupted full-thickness stitches with a 7-0 monofilament suture. This surgical procedure allowed for the direct back-flow of gastric and duodenal juices into the esophagus (Fig. 1). After a 24-h recovery period, the rats were provided with free access to water and food. In the non-surgical group, five rats were bred under the same environmental conditions.

The operated rats were randomly divided into two groups: the control group (n=30) received commercial chow (CRF-1), while the pranlukast group (n=30) received experimental chow that was mixed with pranlukast (50 ppm, 3.3 mg/kg body wt/day). The dosage of pranlukast was determined based on a previous study that reported its inhibitory effect on tumor metastasis (32). Pranlukast was obtained from Cayman Chemical (USA). The body weight of the rats was measured before surgery and monthly thereafter.

**Pathological evaluation.** The rats were euthanized by exsanguination under isoflurane anesthesia at 10, 20, 30, and 40 weeks after surgery. The rats in the non-surgical group were euthanized after 40 weeks of breeding. Euthanasia was confirmed by observing that the rats were not breathing and by palpation for the absence of a heartbeat. Following euthanasia, the entire esophagus and jejunum (including the anastomosis) were surgically resected. The resected tissues were fixed in a 10% formalin solution for 24 h. Subsequently, the esophagus was sectioned at 3-mm intervals along its length and embedded in paraffin. Thin sections of 5  $\mu\text{m}$  thickness were prepared from each paraffin block for histological evaluation, which included hematoxylin and eosin staining as well as immunohistochemistry.

**Definition of pathological findings.** The pathological changes due to DGER were defined as follows: regenerative thickening (RT), epithelial thickening to more than double the normal thickness, together with acanthosis, an abnormal extension of papillae toward the mucosal surface, and parakeratosis; basal-cell hyperplasia (BCH), where the basal layer in the squamous epithelium thickened and occupied >15% of the epithelial layer; erosion, a lack of epithelium with cellular infiltration; BE, esophageal squamous epithelium replaced with columnar-lined epithelium comprising absorptive cells with brush borders and goblet cells; adenocarcinoma, an epithelial growth with atypical cells and structure and invasion of the submucosal layer.

**Immunohistochemistry and the TUNEL method.** Immunohistochemical staining and the TUNEL assay were performed to assess inflammatory cell infiltration and cytokine production in the esophageal epithelium. For immunohistochemical staining, the Envision System (Dako, Denmark) was utilized, with autoclave acceleration. 5- $\mu\text{m}$  sections of formalin-fixed, paraffin-embedded blocks were deparaffinized and then treated with absolute methanol containing 0.3% hydrogen peroxidase. Subsequently, the sections were incubated with normal goat serum (1:30) and left overnight at  $4^{\circ}\text{C}$  with the primary antibody. The primary antibodies used in this study were as follows: polyclonal rabbit anti-rat 5-LOX (Abbiotec, USA), monoclonal mouse anti-rat CD68 (Bio-Rad Laboratories, USA), polyclonal rabbit anti-rat IL8 (Abcam,

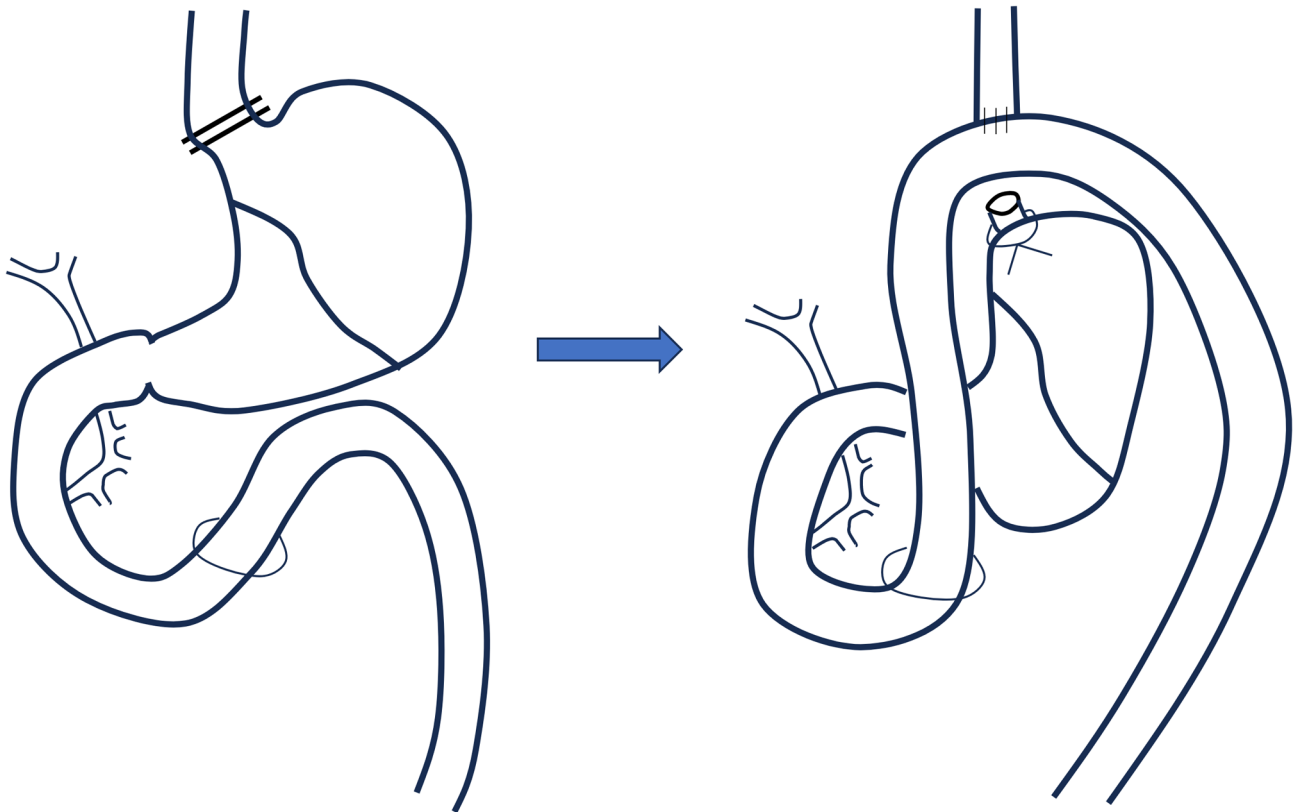


Figure 1. Diagram of the duodenogastroesophageal reflux model. The esophagus was excised and ligated to the oral side of the stomach. A loop in the jejunum, located 4 cm from the ligament described by Treitz, was identified. An end-to-side anastomosis was performed between the distal esophagus and jejunum using interrupted full-thickness stitches with a 7-0 monofilament suture. This surgical procedure allowed for the direct backflow of gastric and duodenal juices into the esophagus.

UK), and monoclonal anti-rat VEGF (Abcam, UK). Afterward, the sections were treated with a labeled polymer (Dako) for 2 h. The reaction products were developed by immersing the sections in 3,3-diaminobenzidine, and the slides were lightly counterstained with hematoxylin.

Cells that exhibited strong staining were considered immunohistochemically positive. The expression intensity of 5-LOX, CD68, IL-8, and VEGF was evaluated by counting labeled cells per high-power field (HPF) in the esophageal epithelium located 5 mm from the oral side of the anastomosis.

To assess the proliferative activity of the esophageal epithelium, immunohistochemical staining for the Ki-67 protein was performed. The primary antibody used for Ki-67 immunohistochemical staining was monoclonal mouse anti-rat Ki-67 antigen (Dako), following the procedure described earlier. The number of Ki-67-labeled cells was counted per 1000 epithelial basal cells (Ki-67 labeling index) in the esophageal epithelium, located 5 mm from the oral side of the anastomosis.

Apoptosis was evaluated using the TUNEL method (Apoptosis *In Situ* Detection Kit; Wako, Japan) according to the manufacturer's instructions. The number of apoptotic cells was expressed as the number of apoptotic cells per 1000 epithelial cells in the esophageal epithelium, located 5 mm from the oral side of the anastomosis.

**Statistical analysis.** The statistical analysis of the incidence of pathological findings was performed using Fisher's exact test. The expression of 5-LOX, CD68, IL-8, and VEGF, as

well as the proliferative activity and apoptosis, were presented as the mean value  $\pm$  SD. Comparisons between groups were conducted using the Mann-Whitney U test. Differences were considered statistically significant when the P-value was  $<0.05$ .

## Results

**General observations.** Fifty-six of the 60 rats were included in this study. Four rats (two from the control group and two from the pranlukast group) died due to complications, including malnutrition, pneumonia, and unknown causes. The effective number of rats examined in each group at different time points were as follows: 5 rats at weeks 10, 20, and 30, and 13 rats at week 40 after surgery (Table I). There was no significant difference in mortality observed between the two groups. Body weights were comparable between the control and pranlukast groups at 0, 10, 20, 30, and 40 weeks. Microscopic examination of the lungs, livers, and kidneys of the pranlukast group at 40 weeks did not reveal any pathological changes, suggesting no adverse effects of pranlukast.

**Histopathological findings.** In the control group, the distal portion of the esophagus exhibited macroscopic thickening and irregularity. Some areas of the rough epithelium showed small nodular elevations (Fig. 2A). Severe squamous esophagitis was observed in the distal portion of the esophagus (Fig. 3A). In the presence of severe esophagitis, BE and EAC developed in the surrounding areas (Figs. 4 and 5). BE was observed starting

Table I. Incidence of pathological findings.

Post-operative week	Group	n	Incidence of pathological findings (%)				
			RT	BCH	Erosion	BE	EAC
10	Control	5	5 (100)	5 (100)	5 (100)	0 (0)	0 (0)
	Pranlukast	5	5 (100)	5 (100)	4 (80)	0 (0)	0 (0)
20	Control	5	5 (100)	5 (100)	5 (100)	3 (60)	1 (20)
	Pranlukast	5	5 (100)	5 (100)	5 (100)	1 (20)	0 (0)
30	Control	5	5 (100)	5 (100)	5 (100)	4 (80)	2 (40)
	Pranlukast	5	5 (100)	4 (80)	4 (80)	2 (40)	0 (0)
40	Non-surgical	5	2 (40)	0 (0)	0 (0)	0 (0)	0 (0)
	Control	13	13 (100)	13 (100)	13 (100)	13 (100) <sup>a</sup>	9 (69) <sup>a</sup>
	Pranlukast	13	13 (100)	13 (100)	12 (92)	8 (62) <sup>a</sup>	2 (15) <sup>a</sup>

<sup>a</sup>P<0.05. RT, regenerative thickening; BCH, basal-cell hyperplasia; BE, Barrett's esophagus; EAC, esophageal adenocarcinoma.

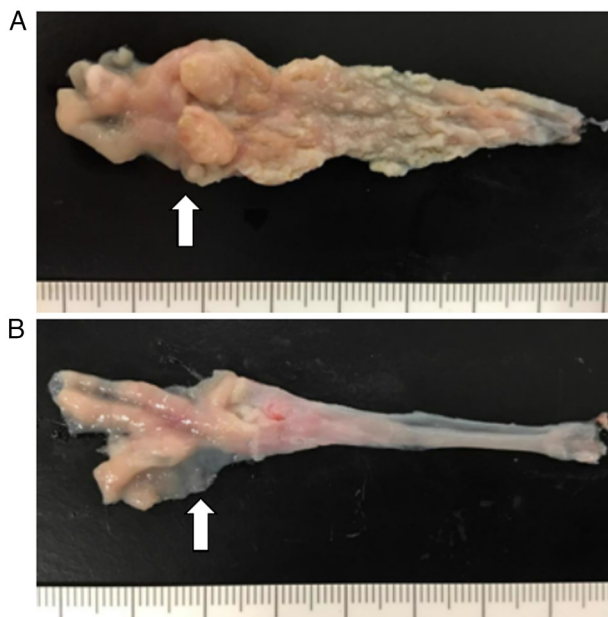


Figure 2. Macroscopic appearance of the esophagus in rats autopsied 40 weeks after surgery. (A) In the control group, the distal esophagus was dilated and thickened, and the epithelium was uneven and had small nodules. (B) In the pranlukast group, the esophagus was relatively smooth and thickening was mild. White arrows indicate the anastomosis line.

from the 20th week (60%) and progressively increased to 100% by the 40th week (Table I). EAC was observed starting from the 20th week (20%) and progressively increased to 69% by the 40th week (Table I).

In the pranlukast group, the distal portions of the esophagus appeared relatively smooth, and the degree of thickening was mild (Fig. 2B). Squamous esophagitis was milder compared to that observed in the control group (Fig. 3B). RT, BCH, and erosion were observed, but to a lesser extent. The incidences of BE and EAC at 40 weeks after surgery were significantly lower in the pranlukast group compared to the control group (P<0.05) (Table I). The length of Barrett's esophagus tended to be shorter in the pranlukast

Table II. Length of Barrett's esophagus.

Post-operative week	Group	n	Length of BE, mm
20	Control	3	9.6±4.7
	Pranlukast	1	3.0±0.0
30	Control	4	15.5±5.4
	Pranlukast	2	9.5±2.1
40	Control	13	16.2±8.4
	Pranlukast	8	10.8±4.8

Length of BE presented as  $\bar{x} \pm$  standard deviation. BE, BE, Barrett's esophagus.

group than in the control group, but there was no significant difference (Table II).

**Immunohistochemistry of 5-LOX, CD68, IL-8, and VEGF.** Both the control and pranlukast groups showed 5-LOX immunoreactivity throughout the study. The primary expression of 5-LOX was observed in infiltrating macrophages, with additional expression observed in other infiltrating leukocytes such as neutrophils, mast cells, and eosinophils. A few squamous epithelial cells also exhibited positive staining for the 5-LOX protein. There was no significant difference in 5-LOX expression in the submucosal tissue of the lower esophagus between the control and pranlukast groups (Fig. 6). In the lower esophagus of rats in the non-surgical group, 5-LOX immunoreactivity was barely observed.

CD68 immunoreactivity indicated the presence of macrophages in both groups throughout the study. There was no significant difference in CD68 expression in the submucosal tissue of the lower esophagus between the control and pranlukast groups (Fig. 7). In the lower esophagus of rats in the non-surgical group, CD68 immunoreactivity was barely observed.



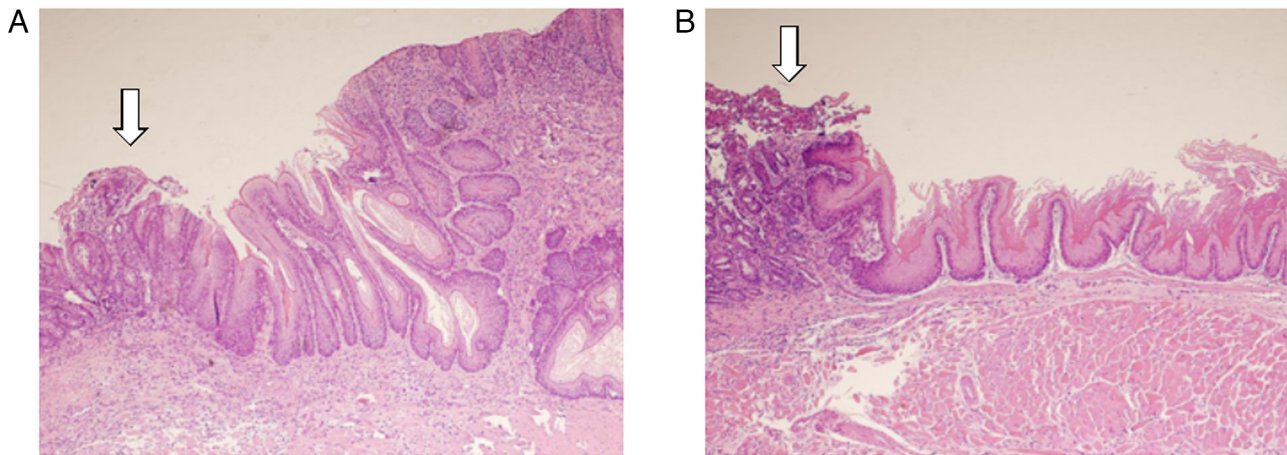


Figure 3. Microscopic findings in the distal portion of the esophagus. (A) In the control group, severe squamous esophagitis was observed. (B) In the pranlukast group, the degree of squamous esophagitis was milder. Magnification, x40. White arrows indicate anastomosis.

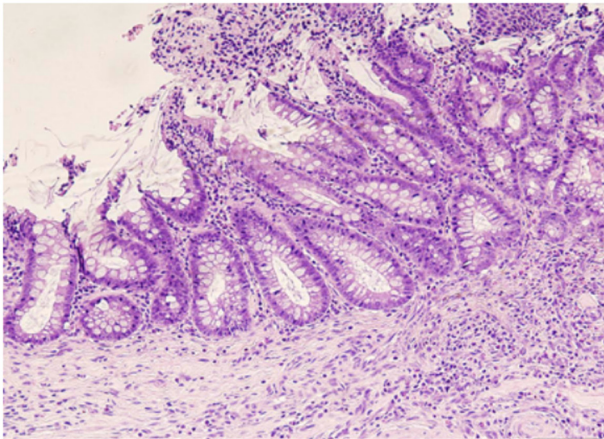


Figure 4. Barrett's esophagus in the lower esophagus of the control group. Magnification, x40.

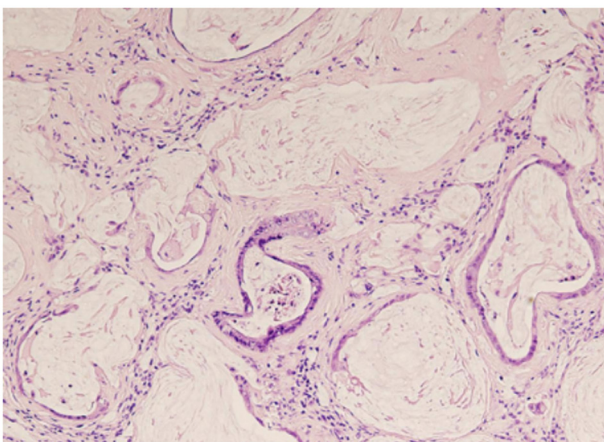


Figure 5. Esophageal adenocarcinoma in the lower esophagus of the control group. Magnification, x40.

Immunoreactivity for IL-8 and VEGF was observed in both groups throughout the study. IL-8 and VEGF are primarily expressed in macrophages. The expression of IL-8 and VEGF

increased in weeks 10 and 20, and then decreased sequentially in weeks 30 and 40 (Figs. 8 and 9). They were significantly suppressed in the pranlukast group compared to the control ( $P < 0.05$ ) (Figs. 8 and 9). In the lower esophagus of rats in the non-surgical group, IL-8 and VEGF immunoreactivity was hardly observed.

**Proliferative activity and apoptosis.** Ki-67 immunoreactivity was observed in the basal cells of the esophageal epithelium. The prevalence of Ki-67-positive cells was higher in the esophageal epithelium of rats with DGER compared to the epithelium of non-surgical rats ( $P < 0.01$ ). Proliferative activity increased at weeks 10 and 20 and then decreased sequentially at weeks 30 and 40, similar to IL-8 and VEGF expression. Pranlukast significantly suppressed the proportion of Ki-67 positive cells at weeks 10, 20, 30, and 40 ( $P < 0.05$ ) (Fig. 10).

Apoptosis, calculated using the TUNEL method, was more prevalent in the esophageal epithelium of rats with DGER compared to the epithelium of non-surgical rats ( $P < 0.05$ ). Pranlukast significantly increased the proportion of apoptotic cells at weeks 10, 20, 30, and 40 ( $P < 0.05$ ) (Fig. 11).

## Discussion

The present study provides evidence that chronic reflux of gastric and duodenal contents leads to the development of esophagitis, BE, and EAC in rats. This process is accompanied by the upregulation of IL-8 and VEGF, as well as the induction of 5-LOX by infiltrated macrophages. Consequently, increased cell proliferation and survival occur in the esophageal epithelium. The CysLT1R antagonist pranlukast effectively suppresses the upregulation of IL-8 and VEGF, resulting in decreased cell proliferation and a reduction in the development of BE and EAC.

The 5-LOX pathway plays a crucial role in inflammation, particularly in inflammatory diseases where it contributes to cellular damage by catalyzing the production of leukotrienes. Studies have shown a significant increase in leukotriene levels in human esophageal biopsy samples from patients with reflux esophagitis and BE (33). LOX metabolites are thought to act as important mediators of inflammation and

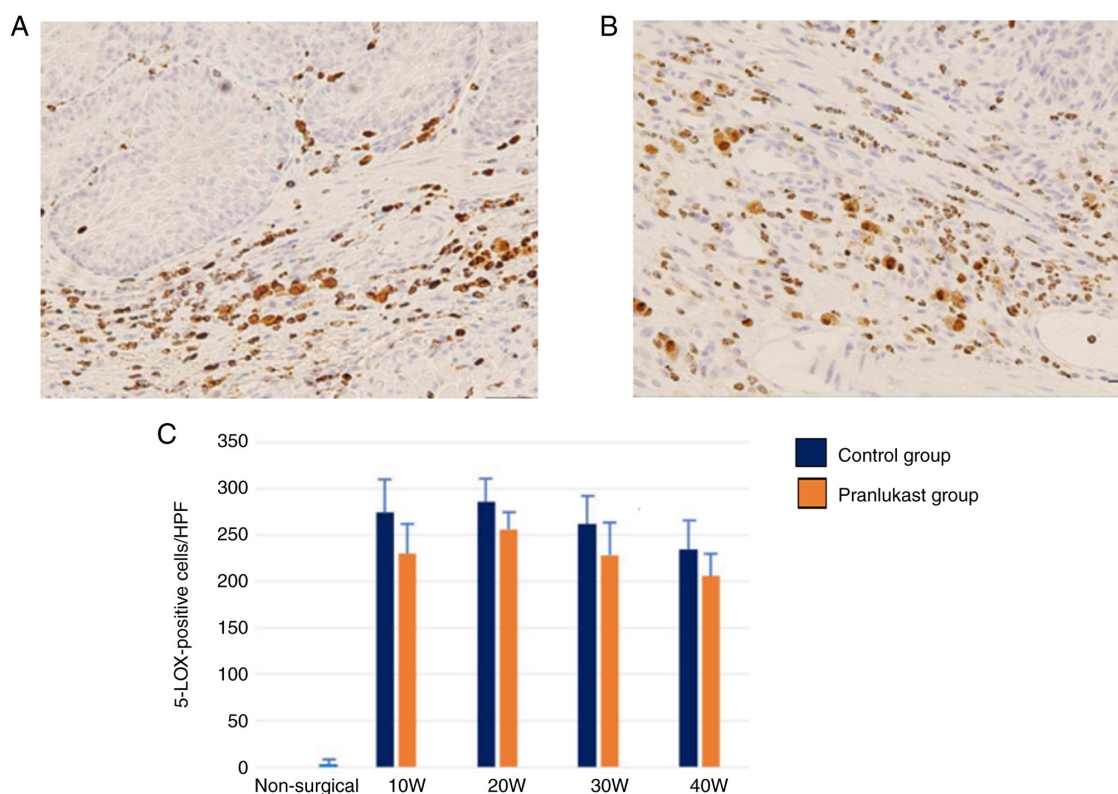


Figure 6. 5-LOX expression. 5-LOX is primarily expressed by the infiltrating macrophages. The 5-LOX expression has also been observed in other infiltrating leukocytes including neutrophils, mast cells and eosinophils. (A) The control group and (B) the pranlukast group: No significant difference in 5-LOX immunoreactivity was observed between the two groups. (magnification, x200). (C) 5-LOX expression levels were higher in rats with duodenogastroesophageal reflux compared with non-surgical rats. There was no significant difference in the 5-LOX expression between the control and pranlukast groups. LOX, lipoxigenase; HPF, high-power field.

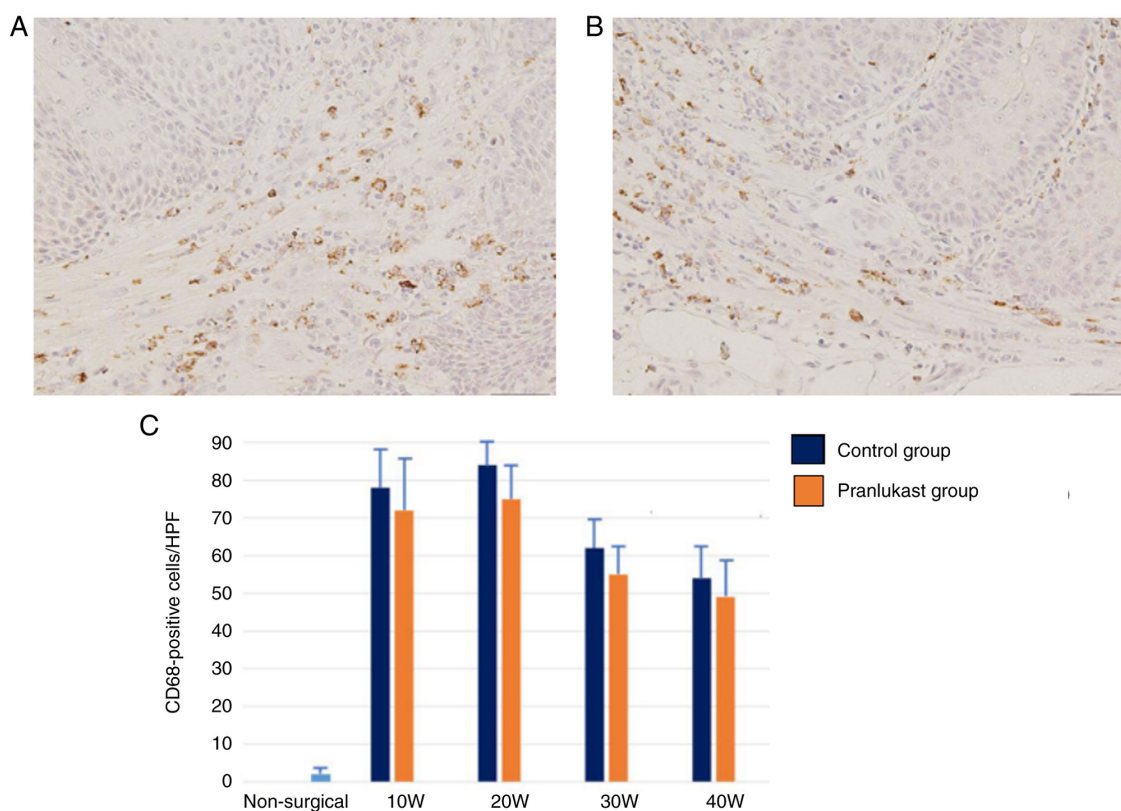


Figure 7. CD68 expression. (A) Control group and (B) pranlukast group: No significant difference in the immunoreactivity of CD68, indicating that macrophages were observed between the two groups (magnification, x200). (C) CD68 expression levels were higher in rats with duodenogastroesophageal reflux compared with non-surgical rats. No significant difference in CD68 expression was observed between the control and pranlukast groups. HPF, high-power field.



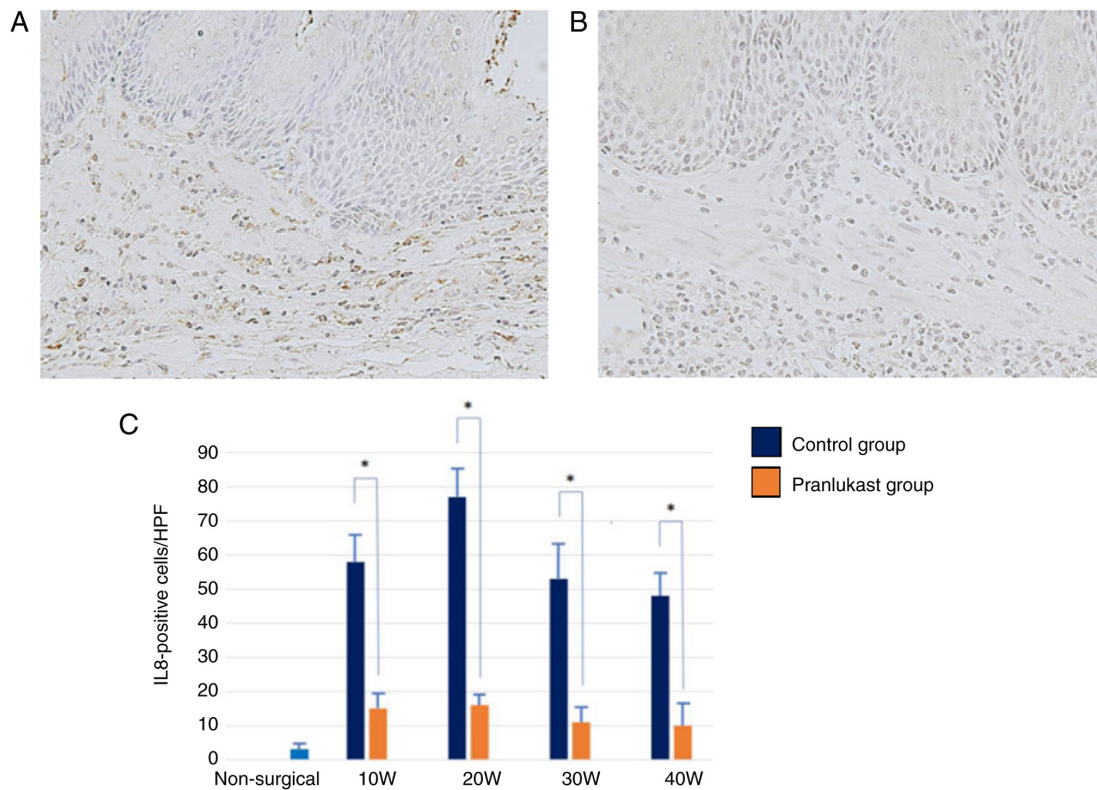


Figure 8. IL-8 expression. IL-8 is mainly detected in macrophages. (A) The control group and (B) pranlukast group: Immunoreactivity for IL-8 in the control group was stronger compared with that in the pranlukast group (magnification, x200). (C) IL-8 expression levels were higher in rats with duodenogastroesophageal reflux compared with non-surgical rats. IL-8 expression was significantly suppressed in the pranlukast group compared with the control group. \*P<0.05. HPF, high-power field.

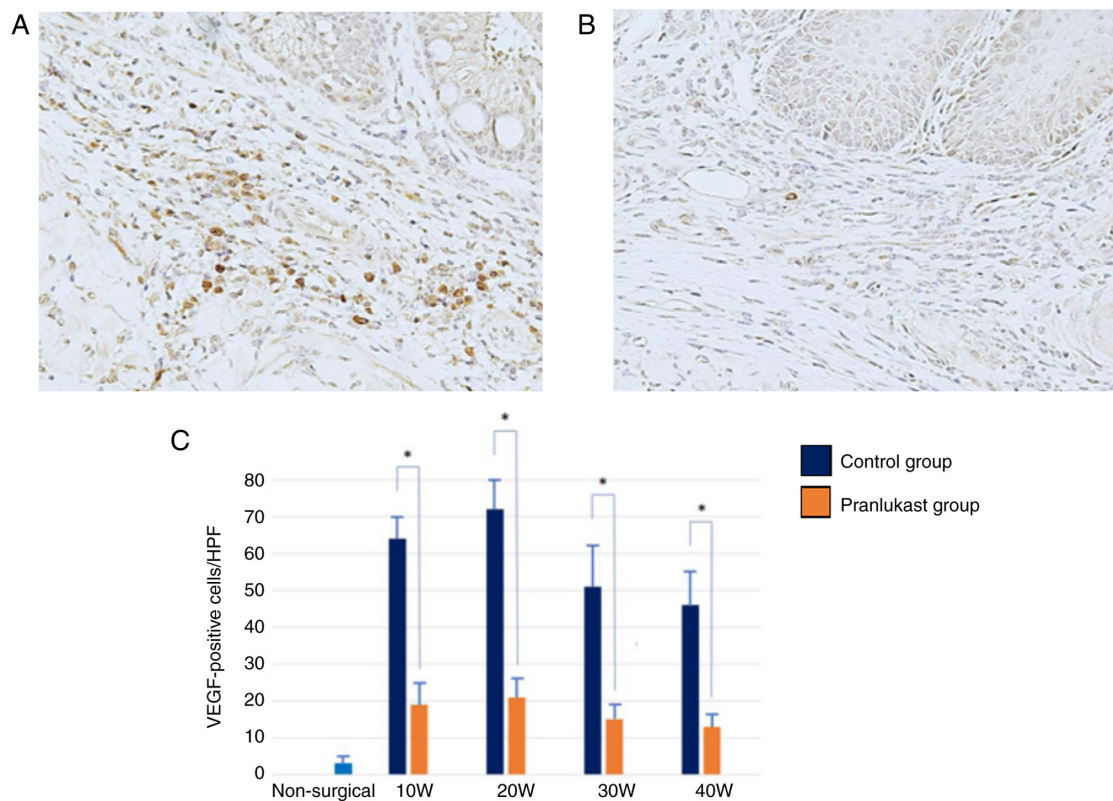


Figure 9. VEGF expression. VEGF is mainly detected in macrophages. (A) The control group and (B) pranlukast group: VEGF immunoreactivity in the control group was stronger compared with that in the pranlukast group (magnification, x200). (C) VEGF expression was higher in rats with duodenogastroesophageal reflux compared with in non-surgical rats. VEGF expression was significantly suppressed in the pranlukast group compared with the control group. \*P<0.05. HPF, high-power field.

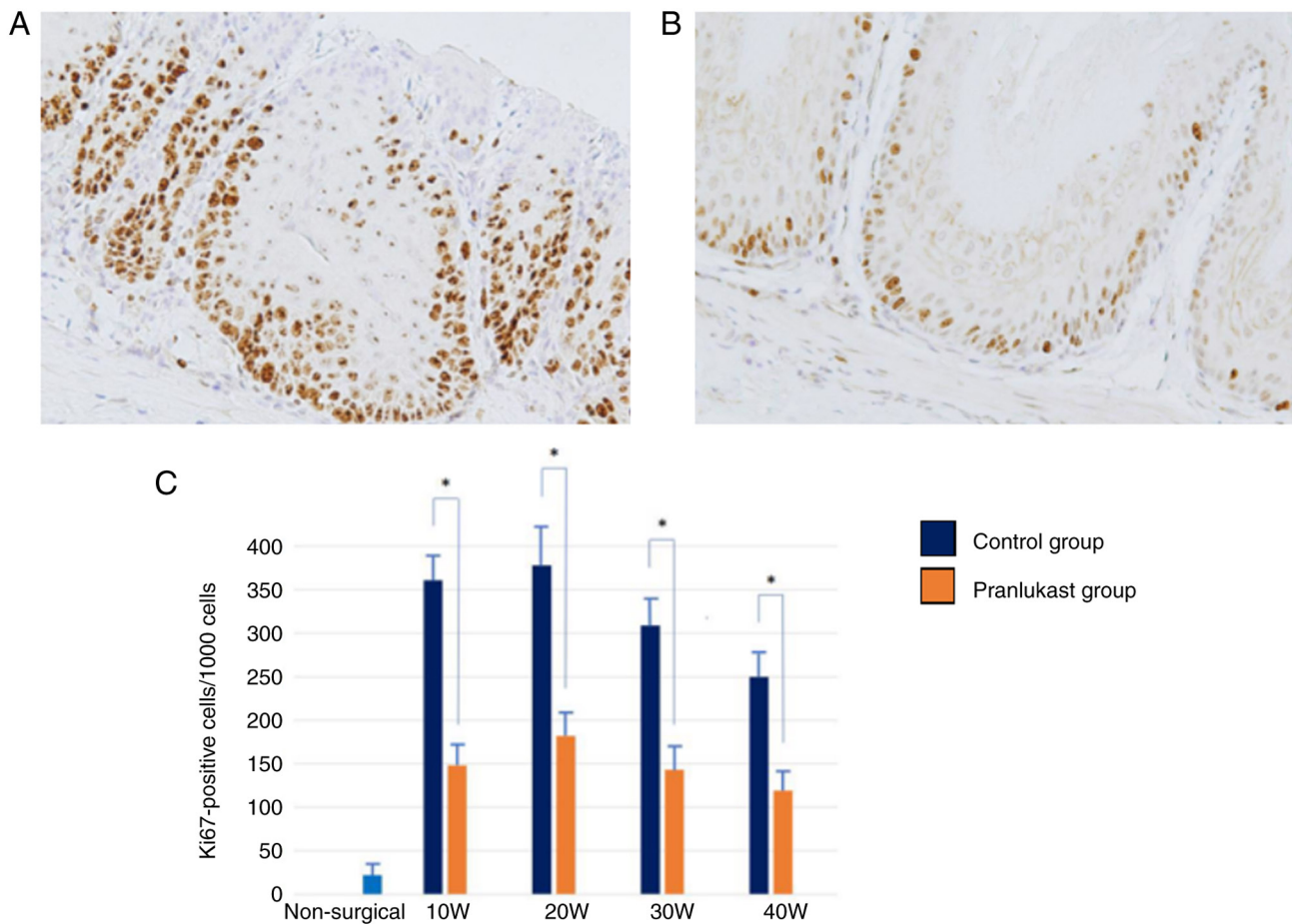


Figure 10. Proliferative activity. Ki-67 immunoreactivity was observed in the basal cells of the esophageal epithelium. (A) The control group and (B) pranlukast group: Ki-67 immunoreactivity in the control group was stronger compared with that in the pranlukast group (magnification, x200). (C) The proportion of Ki-67-positive cells was higher in the esophageal epithelium of rats with duodenogastroesophageal reflux compared with non-surgical rats. Pranlukast administration significantly decreased the Ki-67 labeling index. \* $P < 0.05$ .

inflammation-associated esophageal carcinogenesis. In tissues exposed to chronic inflammation, the continuous activation of the LOX pathway promotes epithelial cell proliferation.

The observed changes in this study are primarily triggered by inflammatory infiltrates, with 5-LOX and cytokine expression predominantly observed in the infiltrated macrophages. Similar findings have been reported in studies examining chronic kidney disease models, where macrophages were identified as the primary source of 5-LOX (34). Another study reported that macrophages mediate epithelial damage by producing 5-LOX (35). In the esophageal epithelium affected by inflammation, 5-LOX metabolites were strongly detected in the infiltrating inflammatory cells within the stroma, while they were not detected in the squamous epithelium (36).

Macrophages are the main sources of IL-8 and VEGF (37,38). In this study, macrophage infiltration was not influenced by the CysLT1R antagonist despite the significant suppression of IL-8 and VEGF expression. The current study reported that the inhibition of 5-LOX did not appear to change the infiltration of leukocytes, which is similar to the results of this our study (34). Although inflammatory cell infiltration was not influenced by pranlukast, it inhibited the production of inflammatory cytokines such as IL-8 and VEGF by suppressing the activity of infiltrating cells.

The interaction between CysLTs and CysLT1R in innate immune cells leads to the release of various inflammatory mediators, including IL-8 and VEGF (20). Several studies have investigated the role of IL-8 in esophageal carcinogenesis. Fitzgerald *et al* found increased IL-8 expression in patients with reflux esophagitis, particularly at the squamous-columnar junction where inflammation is most pronounced (39). Nguyen *et al* reported a correlation between elevated IL-8 expression and poor prognosis in esophageal cancer, indicating the pro-tumor role of IL-8 (40). In an *in vitro* study, IL-8 was significantly upregulated during esophageal carcinogenesis, and inhibiting the IL-8 receptor led to reduced invasiveness of esophageal adenocarcinoma (41). VEGF is also associated with esophageal carcinogenesis. Möbius *et al* observed increased VEGF expression in Barrett's esophagus and esophageal adenocarcinoma (42). LTD4, the CysLTs, induced VEGF production and enhanced VEGF release through CysLT1R activation in human monocytes/macrophages, and these effects were completely inhibited by pranlukast (43). In the present study, IL-8 and VEGF were found to be overexpressed under chronic inflammatory conditions and were effectively suppressed by pranlukast, consistent with previous findings. This strongly suggests that IL-8 and VEGF play a significant role in inflammation-induced carcinogenesis.



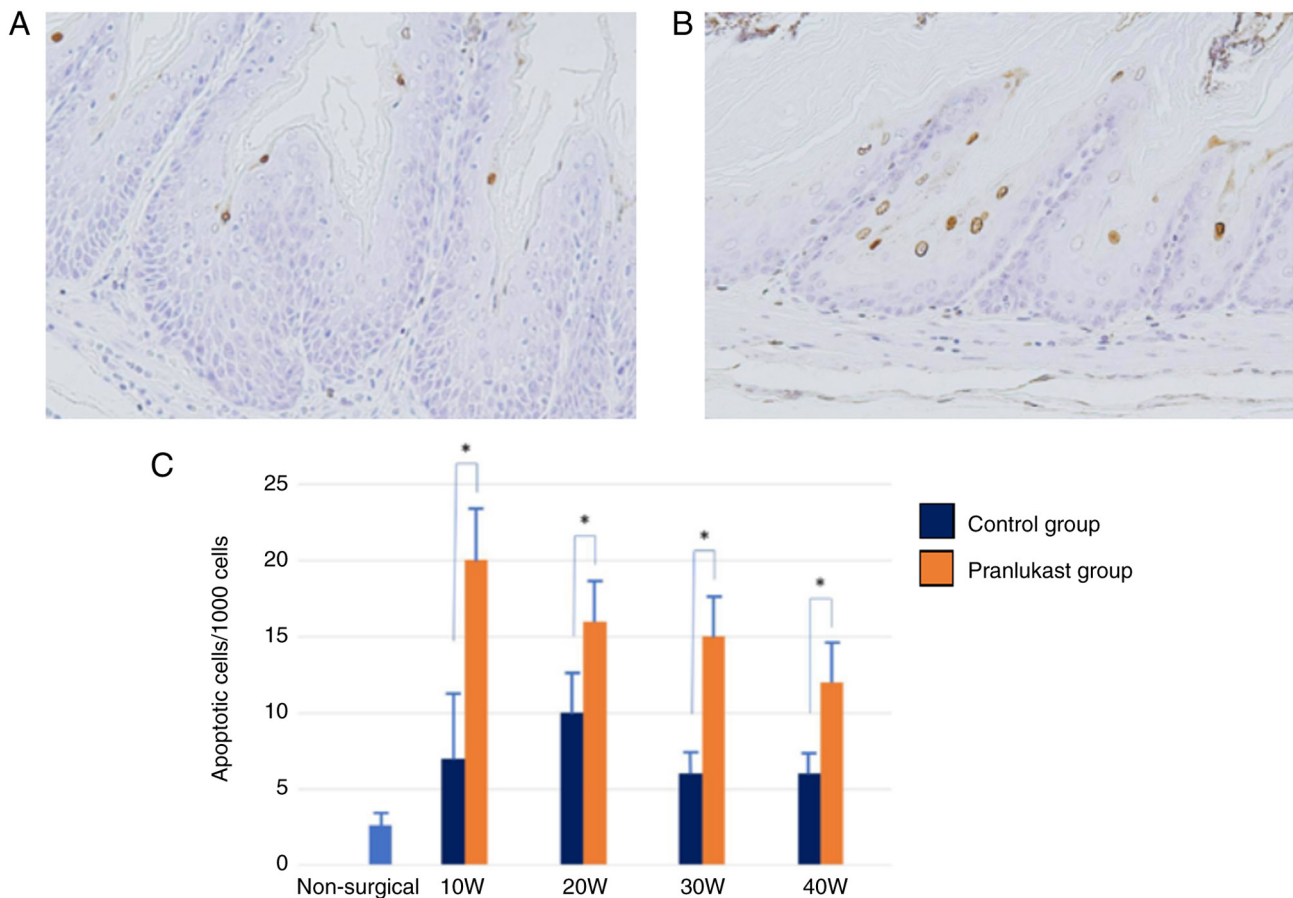


Figure 11. Apoptosis in the esophageal epithelium. Apoptosis in the esophageal epithelium was evaluated using the TUNEL assay. (A) The control group and (B) pranlukast group: The number of apoptotic cells was higher in the pranlukast group compared with the control group (magnification, x200). (C) The proportion of apoptotic cells increased in the esophageal epithelium of duodenogastroesophageal reflux rats compared with that in non-surgical rats. Pranlukast administration significantly increased the apoptotic index. \* $P < 0.05$ .

The chemopreventive effects of CysLT1R antagonists have been demonstrated in large retrospective cohort studies (44,45). Tsai *et al* showed that the use of a CysLT1R antagonist reduced cancer risk in a dose-dependent manner in patients with asthma compared to non-users of CysLT1R antagonists (44). In the present study, an increase in apoptosis cells was observed in the Barrett epithelium in the pranlukast group from 10 to 40 weeks postoperatively. These results suggest that pranlukast induces apoptosis not only in esophageal adenocarcinoma cells, but also in metaplastic cells of Barrett's esophagus or its progenitor atypical cells in a background of chronic inflammation. Previously, Hormi-Carver *et al* reported that all trans-retinoic acid induces via p38 and caspase pathway in a non-neoplastic, metaplastic Barrett's cell line, thus retinoid treatment inhibits carcinogenesis (46). Although further studies are needed to elucidate the mechanism by which pranlukast induces apoptosis of non-neoplastic metaplastic cells via inhibition of the 5-LOX pathway, the present findings indicate the potential use of CysLT1R antagonists for chemoprevention in Barrett's esophagus. Clinical studies on colorectal, pancreatic, urological, and breast cancers have reported upregulation of CysLT1R expression in cancer tissues compared to normal tissues, and this upregulation is associated with poor prognosis (25,26,47,48). CysLT1R expression

has also been detected in human colon cancer cell lines, and its overexpression enhances cell viability (25). Furthermore, Bellamkonda *et al* found that LTD4 promoted tumor growth induced by cancer-initiating cells in a xenograft model of nude mice injected with human colon cancer cells (49). The LOX pathway not only influences oncogenesis but also cancer progression through the interaction between cancer cells and stromal cells. While this study did not specifically assess CysLT1R-expressing cells, the CysLT1R antagonist is expected to have a dual antitumor effect by targeting both macrophages and cancer cells.

The dose of pranlukast administered to the DGER model in this study (3.3 mg/kg/day) was less than the human dose given in the treatment of asthma and allergic rhinitis (around 9 mg/kg/day). Also, given that the toxic dose in rats is considered to be 1000 mg/kg/day or higher, the dose used in our study is considered to be safe enough to be clinically applicable to humans.

In this study, we investigated the anti-carcinogenic effects of pranlukast in a rat model. These results indicate that the administration of a CysLT1R antagonist may suppress esophageal carcinogenesis associated with the IMA sequence. However, this topic has not yet been fully elucidated. Further studies are required to completely understand the relationship between esophageal carcinogenesis and the LOX pathway.

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## Availability of data and materials

All data generated or analyzed during the present study are included in the published article.

## Authors' contributions

TK, JK, KO, HS, MS, TT, DY, HM, NI and TO contributed to the study conception and design. Material preparation, data collection was performed by TK, KO, HS, and MS. Data analysis were performed by JK, TT, DY and HM. TK and KO confirm the authenticity of all raw data. The first draft of the manuscript was written by TK, NI and TO, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

All animal studies were reviewed and approved by The Ethics Committee of Kanazawa University (Kanazawa, Japan; approval no. 153650). All animal procedures were performed according to approved protocols from the Institutional Animal Care and Use Committee (IACUC) of Kanazawa University.

## Patient consent for publication

Not applicable.

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## Competing interests

The authors declare that they have no competing interests.

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