Leukotriene A_4 Hydrolase in Rat and Human Esophageal Adenocarcinomas and Inhibitory Effects of Bestatin

Xiaoxin Chen, Ning Li, Su Wang, Nan Wu, Jungil Hong, Xiaolong Jiao, Mark J. Krasna, David G. Beer, Chung S. Yang

Background: **Esophageal adenocarcinoma (EAC) is increasing at the most rapid rate of any cancer in the United States. An esophagogastroduodenal anastomosis (EGDA) surgical model in rats mimics human gastroesophageal reflux and results in EAC. Leukotriene A_4 hydrolase (LTA_4H), a protein overexpressed in EAC in this model, is a rate-limiting enzyme in the biosynthesis of leukotriene B_4 (LTB_4), a potent inflammatory mediator. We used this model and human EAC and non-tumor tissues to elucidate the expression pattern of LTA_4H and to evaluate it as a target for chemoprevention.** ***Methods:*** **LTA_4H expression was examined by western blotting and immunohistochemistry. The functional role of LTA_4H in carcinogenesis was investigated by use of an LTA_4H inhibitor, bestatin, in the rat EGDA model. All statistical tests were two-sided.** ***Results:*** **LTA_4H was overexpressed in all 10 rat EACs examined, compared with its level in normal rat tissue; it was also overexpressed in four of six human EAC tumor samples, compared with its level in adjacent non-tumor tissue. In tissue sections from 20 EGDA rats and 92 patients (86 with EAC, one with dysplasia, and five with columnar-lined esophagus), LTA_4H was expressed in infiltrating inflammatory cells and overexpressed in the columnar cells of preinvasive lesions and cancers, especially in well-differentiated EACs, as compared with the basal cells of the normal esophageal squamous epithelium. Bestatin statistically significantly inhibited LTB_4 biosynthesis in the esophageal tissues of EGDA rats (without bestatin = 8.28 ng/mg of protein; with bestatin = 4.68 ng/mg of protein; difference = 3.60, 95% CI = 1.59 to 5.61; *P* = .002) and reduced the incidence of EAC in the EGDA rats from 57.7% (15 of 26 rats) to 26.1% (6 of 23 rats) (difference = 31.6%, 95% CI = 0.3% to 56.2%; *P* = .042).** ***Conclusion:*** **LTA_4H overexpression appears to be an early event in esophageal adenocarcinogenesis and is a potential target for the chemoprevention of EAC. [J Natl Cancer Inst 2003;95:1053–61]**

Esophageal adenocarcinoma (EAC) has a yearly increase in incidence of 4%–10%, which is the most rapidly increasing incidence rate in the United States for any cancer *(1)*. Despite the progress made in clinical therapy, the 5-year survival rate of EAC has not improved appreciably during the past 20 years and remains around 10% *(2)*. Consequently, it is critical to study the mechanisms of esophageal adenocarcinogenesis so that effective approaches for its prevention and treatment can be designed. Our previous studies with animal models suggested that oxidative stress plays an important role in the formation of EAC, a situation similar to that in humans with gastroesophageal reflux and iron overload *(3,4)*. In a recently established rat surgical model known as esophagogastroduodenal anastomosis (EGDA), EAC is preceded by gastroesophageal reflux disease, columnar-lined esophagus (CLE, or Barrett's esophagus), and CLE with dysplasia. This staged process of chronic inflammation–associated carcinogenesis provides a good system for mechanistic and chemopreventive studies of EAC *(5,6)*.

Aberrant arachidonic acid metabolism is an important event in chronic inflammation. A close relationship has been demonstrated between aberrant arachidonic acid metabolism and many types of human cancers *(7)*. Many inhibitors of the key arachidonic acid metabolizing enzymes suppress cancer formation or cancer cell growth *(8)*. These nonsteroidal anti-inflammatory drugs are believed to exert their chemopreventive effects mainly by inhibiting arachidonic acid metabolism, although other mechanisms have also been suggested *(9)*. Most studies have

Affiliation of Authors: X. Chen, N. Li, S. Wang, N. Wu, J. Hong, C. S. Yang, Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, the State University of New Jersey, Piscataway; X. Jiao, M. J. Krasna, Division of Thoracic Surgery, University of Maryland Medical System, Baltimore; D. G. Beer, Section of General Thoracic Surgery, Department of Surgery, University of Michigan, Ann Arbor.

Correspondence to: Chung S. Yang, Ph.D., Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, the State University of New Jersey, 164 Frelinghuysen Rd., Piscataway, NJ 08854 (e-mail: csyang@rci.rutgers.edu).

See "Notes" following "References."

Journal of the National Cancer Institute, Vol. 95, No. 14, © Oxford University Press 2003, all rights reserved.

Downloaded from https://academic.oup.com/jnci/article/95/14/1053/2520331 by guest on 24 April 2024

focused on cyclooxygenase and 5-lipoxygenase pathways because their metabolites, prostaglandin E_2 and leukotriene B_4 (LTB_4), respectively, are among the most potent inflammatory mediators. Cyclooxygenase 2 is overexpressed in human EAC *(10)* and in EGDA-induced rat EAC *(11)*. Cyclooxygenase 2 expression increased substantially when biopsy samples of human preinvasive lesions were exposed to pulses of gastric acid or bile acids in an organ culture system, and this effect was attenuated by NS-398, a selective cyclooxygenase 2 inhibitor *(12)*. NS-398 also induces apoptosis in human EAC cell lines in a cyclooxygenase 2- and prostaglandin E_2–dependent manner *(13)*. Sulindac, a nonselective cyclooxygenase inhibitor, substantially inhibited esophageal adenocarcinogenesis in rat surgical models *(11,14)*.

The 5-lipoxygenase pathway of arachidonic acid metabolism bifurcates into three pathways leading to the production of LTB_4 (leukotriene A_4 hydrolase–dependent), $LTC_4/D_4/E_4$ (leukotriene C_4 synthase–dependent), and 5-hydroxyeicosatetraenoic acid. Inhibition or knockout of 5-lipoxygenase suppresses inflammation *(15–17)*, cell growth *(18)*, or tumor formation in various animal models of cancers *(19,20)*. Such a chemopreventive effect has been associated with inhibition of LTB_4 biosynthesis *(21)* and can be stronger than that of a cyclooxygenase inhibitor *(22)*. However, the 5-lipoxygenase pathway has not yet been studied in EAC. In human gastroesophageal reflux disease and CLE, the levels of LTB_4 and prostaglandin E_2 were higher in biopsy samples of esophageal mucosa than in normal human esophageal tissue. Antacid treatment reduced the production of LTB_4 and prostaglandin E_2 *(23,24)*.

Leukotriene A_4 hydrolase (LTA_4H) was identified as an overexpressed protein in the EAC of EGDA rats in a previous study using two-dimensional gel electrophoresis and mass spectrometry *(25)*. Because of the important roles of LTB_4 in inflammation and potentially also in inflammation-associated carcinogenesis, we elucidated the expression pattern of LTA_4H, a rate-limiting enzyme in the biosynthesis of LTB_4, in rat and human EACs, and evaluated it as a target for chemoprevention of EAC. We compared LTA_4H protein expression in rat and human EAC tumor tissues with that in non-tumor esophageal tissues. The EGDA rat model was used to investigate bestatin, an LTA_4H inhibitor, as an agent for chemoprevention.

Patients and Methods

Proteomics

Frozen samples of duodenal epithelium, esophageal epithelium, and EAC tumors from two EGDA rats were used for the proteomic experiment. These samples were obtained from a previous experiment in which the animals developed visible EAC 40 weeks after EGDA without iron supplementation *(5)*. Chronic inflammation caused by EGDA-induced reflux of duodenal and gastric contents was present in the esophageal epithelia under microscopic examination. Inflammation at the squamocolumnar junction was very severe.

Two-dimensional gel electrophoresis with 50 μg of protein was performed as described previously *(25)*. The images were analyzed with Phoretix 2D Full software version 4.0 (Nonlinear Dynamics, Durham, NC), so that differing spots and all major unchanged spots were outlined, quantified, and matched on all gels. Protein spots of interest (more than threefold higher in EAC tumor than in duodenal epithelium) were excised, digested in the gel by trypsin, and analyzed with matrix-assisted laser desorption/ionization mass spectrometry in a PerSeptive Voyager DE-RP mass spectrometer (PerSeptive Biosystems, Framingham, MA) in the linear mode. The protein corresponding to the peptide masses detected was determined with an online database (http://www.ExPaSy.ch/).

Western Blot Analysis of LTA_4H

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and western blotting were performed as described previously *(26)*. A 20-μg protein sample was loaded per lane. A polyclonal rabbit anti-LTA_4H antiserum (1 : 1000 dilution; Cayman Chemical, Ann Arbor, MI) was used to detect rat and human LTA_4H by western blotting and immunohistochemistry. This antiserum detects one band of approximately 69 kd on a western blot and recognizes both rat and human LTA_4H because of the high degree of amino acid sequence homology (93%) between the two enzymes *(27)*. Ten sets of frozen rat samples (duodenum, esophagus, and tumor), including the two sets used for proteomics, were obtained from a previous experiment *(5)*. Six pairs of surgically resected human samples (EAC tumor and adjacent non-tumor tissue) were obtained from the Tissue Retrieval Service, Cancer Institute of New Jersey (New Brunswick, NJ). Written informed consent was obtained from all patients, and patient identifiers were coded to protect confidentiality. Hematoxylin and eosin staining confirmed the diagnosis: two well-differentiated EAC (samples 01 and 04), two moderately differentiated EAC (samples 03 and 06), and two poorly differentiated EAC (samples 02 and 05). All samples were glandular adenocarcinoma in nature. All adjacent non-tumor tissues contained large amounts of infiltrating inflammatory cells. Proteins from rat and human tissues were prepared for western blotting from five to 10 frozen sections between two histologically confirmed sections that were carefully scraped into microcentrifuge tubes.

LTA_4H Immunohistochemistry

The avidin–biotin–peroxidase complex method (Elite ABC kit; Vector Laboratories, Burlingame, CA) and polyclonal rabbit anti-LTA_4H antiserum (1 : 1000 dilution) were used for immunohistochemical staining of LTA_4H in archival formalin-fixed paraffin-embedded tissue sections from 20 EGDA rats and 92 patients. Written informed consent was obtained from all patients, and patient identifiers were coded to protect confidentiality. Each rat tissue section contained a variety of histologic types, including normal esophagus, esophagitis, CLE, dysplasia, and EAC. Among the 92 human esophageal samples, 86 were EAC, one was dysplasia, and five were CLE; 21 of the 92 patients had archival paraffin sections, and 71 had tissue spotted on one tissue microarray (Table 1). In this collection of tissue samples, some patients contributed samples of more than one type of histology (squamous epithelium, CLE, dysplasia, or EAC), six contributed samples of all four types of histology, but no one contributed more than one tissue sample with the same histology.

Histologic diagnosis was made by using established criteria *(28)*. Paraffin sections were treated with antigen-unmasking fluid (BD Pharmingen, San Diego, CA) and then incubated with the primary antibody. The cytoplasm and/or nuclei of cells expressing LTA_4H were stained dark brown. Staining was evaluated and graded by a single pathologist (S. Wang). Staining intensity in epithelial cells in human paraffin sections was rated

Downloaded from https://academic.oup.com/jnci/article/95/14/1053/2520331 by guest on 24 April 2024

Table 1. Histopathology and leukotriene A_4 hydrolase (LTA_4H) immunostaining of human esophageal samples*

Histology	No. of tissue samples	LTA_4H staining intensity (95% CI)
Squamous epithelium†	11	0.4 (0.0 to 0.5)‡
Columnar-lined esophagus (CLE)	21	1.7 (1.4 to 1.7)‡
Dysplasia	20	2.2 (1.8 to 2.2)
Total esophageal adenocarcinoma (EAC)	86	2.0 (1.7 to 2.0)‡
Well-differentiated EAC	29	2.4 (2.0 to 2.4)§
Moderately differentiated EAC	43	1.7 (1.4 to 1.7)§
Poorly differentiated EAC	12	1.7 (1.0 to 1.9)§
Mucinous EAC	2	3

*Staining intensity in epithelial cells was scored as follows: 0 = no positive staining or slight staining close to background, 1 = weakly positive staining, 2 = moderately positive staining, 3 = strongly positive staining. Only the mean intensity of LTA_4H staining was shown for mucinous EAC. All the other cases of EACs were glandular adenocarcinoma.

†Staining intensity was expressed as that in the basal cells of esophageal squamous epithelium.

‡With two-sided Student's t test, CLE, dysplasia, and EAC had statistically significantly higher levels of LTA_4H than the squamous epithelium ($P<.001$). Dysplasia expressed higher levels of LTA_4H than CLE ($P = .04$). There was no statistically significant difference between CLE and EAC ($P = .22$) or between dysplasia and EAC ($P = .42$).

§With two-sided Student's t test, well-differentiated EAC had a statistically significantly higher level of LTA_4H than moderately differentiated EAC ($P = .003$) and poorly differentiated EAC ($P = .041$). There was no statistically significant difference between moderately differentiated EAC and poorly differentiated EAC $P = .92$).

on the following scale: 0 = no positive staining or slight staining close to background, 1 = weakly positive staining, 2 = moderately positive staining, and 3 = strongly positive staining. The area of maximal staining intensity was used for grading as long as it was composed of more than 10% of the region of interest (esophageal epithelial tissues). We did not quantify the percentage of positively stained cells because the staining was homogeneous in general. Polymorphonuclear neutrophils, especially those in blood vessels, were used as internal positive controls. These cells express high levels of LTA_4H, with a staining intensity of 3.

Short-Term Effect of Bestatin, an LTA_4H Inhibitor

Six-week-old male Sprague-Dawley rats from Taconic Farms (Germantown, NY) were housed two per cage, given the AIN93M diet (Research Diets, New Brunswick, NJ) and water *ad libitum,* and maintained on a 12-hour light/12-hour dark cycle. Rats were allowed to acclimate for 2 weeks before surgery. Solid food was withheld for 2 days, from 1 day before surgery to 1 day after surgery. EGDA was performed under general anesthesia (80 mg of ketamin and 12 mg of xylazine per kg of body weight, intraperitoneally), as described previously *(5).* A 1.5-cm incision was made on both the esophagus and the duodenum, and then the two were anastomosed with accurate mucosal-to-mucosal opposition. This procedure was approved by the Animal Care and Facilities Committee at Rutgers University (protocol 94–017). Four weeks after surgery, eight EGDA rats were given iron dextran (12.5 mg of iron per kg) intraperitoneally to enhance reflux-induced inflammation and esophageal adenocarcinogenesis *(5,29).*

Bestatin, also termed Ubenimex, [*S*-(*R**,*S**)]-*N*-(3-amino-2-hydroxy-1-oxo-4-phenylbuty1)-L-leucine ($C_{16}H_{24}N_2O_4$; Zhejiang Kangyu Pharmaceutical Co., Dongyang, China), is a rapid and irreversible LTA_4H inhibitor *(30).* Bestatin was dissolved in phosphate-buffered saline (PBS, pH 7.2) and given intraperitoneally to EGDA rats at a dose of 10 mg/kg once a day for 7 days beginning at week 5 after surgery. Four rats were treated with bestatin, and the remaining four were treated with PBS. Six hours after the last injection, the rats were killed by CO_2 asphyxiation, and the esophageal tissues (upper esophagus and esophagoduodenal junction) were harvested for analysis of LTB_4 biosynthesis by enzyme immunoassay. The esophagoduodenal junction was defined as the 5-mm region above the anastomosis line.

Chemoprevention of EGDA-Induced Rat EAC by Bestatin

EGDA was performed on 60 rats, as described above, 54 of which survived the surgery. Rats were given iron dextran (12.5 mg of iron per kg) every 2 weeks intraperitoneally, starting 4 weeks after surgery and continuing for the duration of the experiment. The animals were weighed once every other week. One week after surgery, rats were randomly assigned to two groups. Twenty-seven EGDA rats were given bestatin at a dose of 10 mg/kg, intraperitoneally, three times a week (group C). The remaining 27 EGDA rats were used as untreated positive controls (group B). Ten non-operated rats were included as negative controls (group A). Forty weeks after surgery, these rats were killed; the esophagus was removed, opened longitudinally, and examined for gross abnormalities. Tumor volume ($4/3\ \pi r^3$, where r is the radius) was determined by measuring the height, length, and width of all visible tumors and using the average of the three measurements as the diameter. The esophagus was cut longitudinally, fixed in 10% buffered formalin, Swiss-rolled as previously described *(29),* processed, and embedded in paraffin. Five-micrometer sections were mounted on glass slides and used for pathologic analyses. A small piece of esophagoduodenal junction was frozen and stored at –80 °C for future analysis of LTB_4.

Histopathologic analysis was carried out on slides stained with hematoxylin and eosin (slides 1, 10, 20, and 30) by a single individual (S. Wang) who was blinded to the treatment assignments. CLE was characterized by the presence of intestinal columnar epithelium containing a villiform surface, mucous glands, and intestinal-type goblet cells, above the blue prolene suture. Dysplastic lesions were diagnosed by the partial loss of cell polarity and maturation, nuclear atypia, and an increased numbers of mitotic figures. EAC was diagnosed when dysplastic columnar epithelial cells invaded through the basement membrane *(28).*

LTB_4 Enzyme Immunoassay

Eight pairs of frozen rat samples (upper esophagus and esophagoduodenal junction) from the short-term experiment (bestatin-treated EGDA rats and non–bestatin-treated EGDA rats) and eight frozen rat samples (esophagoduodenal junction) from each group of the bestatin chemoprevention experiment were analyzed for LTB_4. Frozen tissue samples were analyzed immediately after removal from a –80 °C freezer. After being homogenized in a buffer containing 0.1 *M* Tris–HCl (pH 7.1) and 20 m*M* EDTA, the protein concentration and the LTB_4 concentration were determined. LTB_4 was measured as described by the manufacturer of an enzyme immunoassay kit

Downloaded from https://academic.oup.com/jnci/article/95/14/1053/2520331 by guest on 24 April 2024

(Cayman Chemical Co., Ann Arbor, MI). The amount of LTB_4 was expressed as nanograms per milligram of protein.

Statistical Analysis

Student's *t* test was used to analyze differences in the intensity of LTA_4H immunostaining and the tissue level of LTB_4, which were normally distributed. The results for tumor incidence were analyzed by the χ^2 test. The Wilcoxon signed rank test was used for the other analyses. Statview, version 4.2 (SAS Institute, Cary, NC) software was used for all statistical tests and calculations, and all statistical tests were two-sided.

RESULTS

Overexpression of LTA_4H in Rat and Human EAC

Fifty micrograms of protein from the tissue samples (EAC tumor and nearby esophageal and duodenal epithelia) of two EGDA rats were separated by two-dimensional gel electrophoresis. After silver staining and laser scanning, the intensities of protein spots on two-dimensional gels were visually estimated and then quantified with imaging software. As we described previously *(25),* the density of 12 spots was more than threefold higher in both pairs of tumor samples than in the duodenal epithelial samples. Eleven of the 12 spots were identified by mass spectrometry as known proteins in the database, including glucose-regulated protein 94 (Grp94), LTA_4H, Grp78, calnexin, heat shock protein 90β, ER61, transferrin, Rho GDP-dissociation inhibitor, creatine kinase M chain, vitamin D-binding protein, and annexin V. One of the 12 spots was not in the database. We first characterized overexpression of Grp94 in rat and human EAC *(25).* Because Grp94 inhibits apoptosis by maintaining intracellular calcium homeostasis, our data suggested an association of oxidative stress, Grp94 overexpression, and apoptosis regulation in esophageal adenocarcinogenesis.

In this study, we characterized the enzyme LTA_4H (EC 3.3.2.6; Swiss-Prot accession number P30349; theoretical pI = 5.70; molecular mass = 69 044 d), which has tumor/duodenum density ratios of 6 : 1 and 3.5 : 1 for the two sets of rat samples on the two-dimensional gels. Seven major LTA_4H peptide peaks (1150, 1373, 1476, 1698, 1808, 1943, and 2048 *m/z*) were identified by mass spectrometry (Fig. 1, A).

Western blotting with a polyclonal antiserum against LTA_4H confirmed the overexpression of this enzyme in EAC tumors compared with the expression in normal duodenal tissue (Fig. 1, B). A similar expression pattern was observed in all 10 sets of rat samples (data not shown). The level of LTA_4H in esophageal tissues was similar to (Fig. 1, B, lane E1 versus lane T1) or lower than (Fig. 1, B, lane E2 versus lane T2) that in EAC tumors.

The expression of LTA_4H was examined in six sets of frozen human samples of EAC tumors and adjacent non-tumor esophageal tissues. Overexpression of LTA_4H was observed in four human EACs (samples 01, 02, 04, and 05) as compared with the

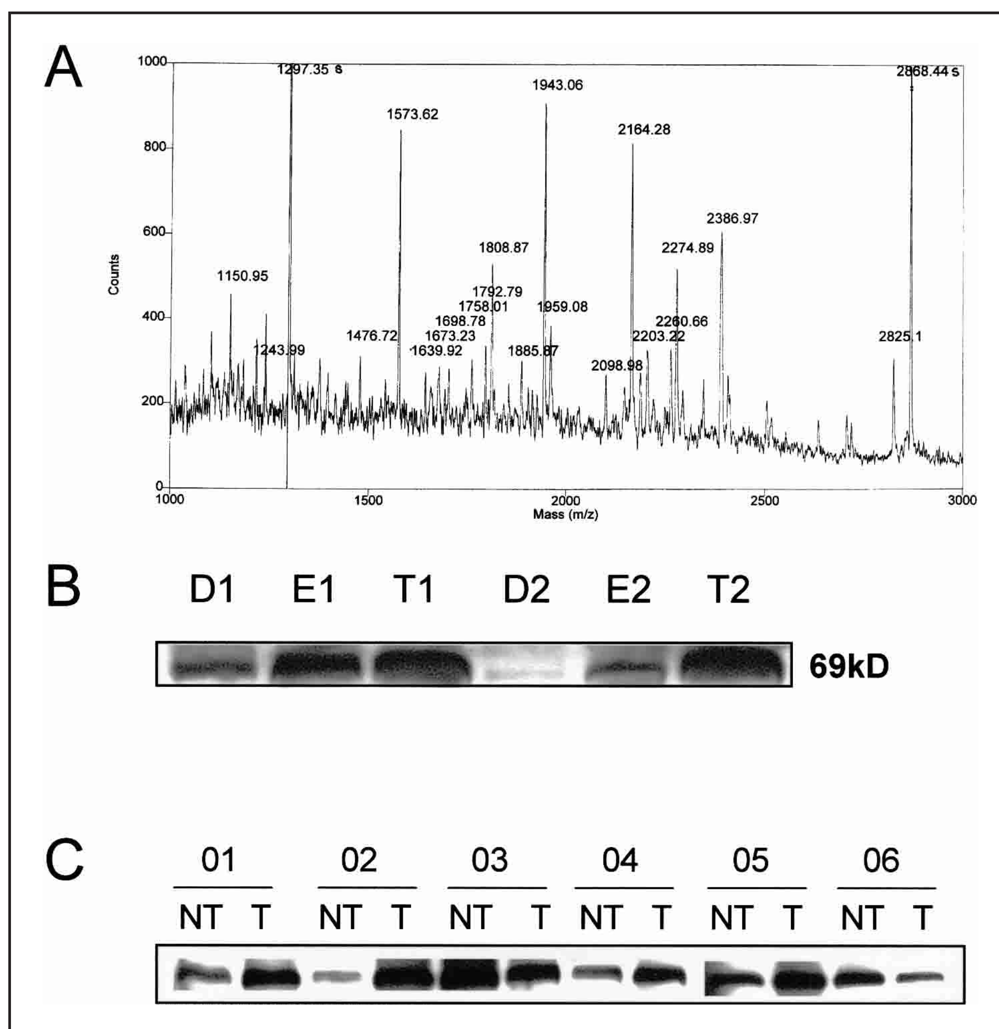

Fig. 1. Leukotriene A_4 hydrolase (LTA_4H) overexpression in rat and human esophageal adenocarcinoma (EAC). **A**) Matrix-assisted laser desorption/ionization mass spectrometry. The spectrum of one protein spot showed the presence of seven major mass peaks (1150, 1373, 1476, 1698, 1808, 1943, and 2048 *m/z*), which corresponded to LTA_4H in the SwissProt database. S = internal standard. **B**) LTA_4H western blotting in the EAC, duodenum, and esophagus of esophagogastroduodenal anastomosis (EGDA) rats. LTA_4H was overexpressed in two representative rat EAC tumors as compared with duodenum. A 20-μg protein sample was loaded onto each lane. D = duodenum; E = esophagus; T = EAC tumor. **C**) LTA_4H western blotting of human EAC and adjacent non-tumor tissue. LTA_4H was also overexpressed in four of six human EACs (samples 01, 02, 04, and 05) as compared with adjacent non-tumor tissue. T = EAC tumor; NT = adjacent non-tumor esophageal tissue.

Downloaded from https://academic.oup.com/jnci/article/95/14/1053/2520331 by guest on 24 April 2024

expression in corresponding adjacent non-tumor esophageal tissues. Another two EACs (samples 03 and 06) had a lower level of LTA_4H than corresponding adjacent non-tumor tissues (Fig. 1, C).

Expression of Rat and Human LTA_4H as Determined Immunohistochemically

LTA_4H expression was examined immunohistochemically in paraffin sections of rat and human tissues. Tissue sections from 20 EGDA rats, containing histologically normal tissue and CLE and EAC tissues, were used for this analysis. Consistent staining patterns of LTA_4H were found for all tissue types. In the normal rat esophagus, LTA_4H was not detected in epithelial cells (Fig. 2, A). In the squamous epithelium with inflammation, LTA_4H was strongly detected in the infiltrating inflammatory cells in the stroma (Fig. 2, B) but was not detected in squamous epithelial cells in the basal and parabasal cell layers. LTA_4H was detected in columnar cells of CLE (Fig. 2, C) and was strongly detected in EAC tumor cells (Fig. 2, D). When expressed, LTA_4H was detected in the cytoplasm and in the nucleus.

In the paraffin sections of human esophageal tissues (92 patients), LTA_4H was rarely detected in basal cells of the squamous epithelium but was usually detected in the parabasal cells of the esophageal squamous epithelium adjacent to the cancer tissue (Fig. 3, A). A moderate level of LTA_4H was detected in most cells in CLE (Fig. 3, B and C). The level of LTA_4H detected in dysplastic lesions (Fig. 3, D) and EAC (Fig. 3, E) was higher than that in CLE. However, in three EACs, little LTA_4H was detected in tumor cells but a high level of LTA_4H was detected in infiltrating inflammatory cells (Fig. 3, F).

To minimize bias in evaluating the staining intensity of the epithelial cells, polymorphonuclear neutrophils in the blood vessel on the same slide were used as an internal positive control (staining intensity = 3). Tissues that did not show strong LTA_4H staining in these cells were regarded as poorly preserved

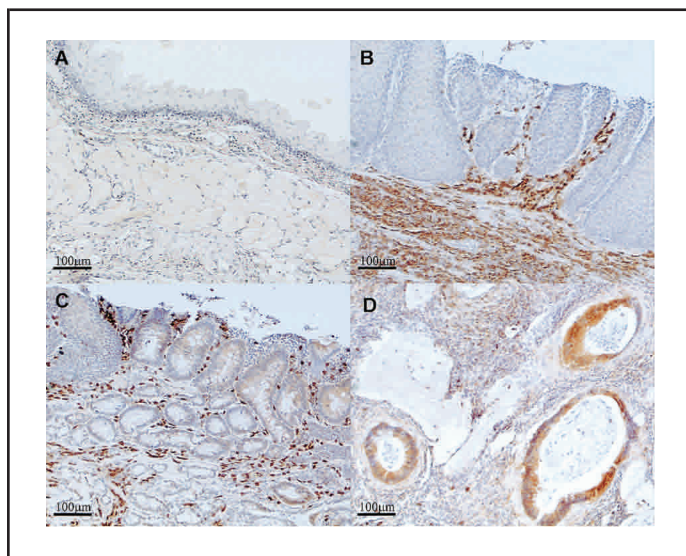

Fig. 2. Expression of leukotriene A_4 hydrolase (LTA_4H) in rat esophageal tissues detected by immunohistochemistry. Columnar cells of rat columnar-lined esophagus (**C**) and esophageal adenocarcinoma (**D**) overexpress LTA_4H, compared with the basal cells of the normal esophagus (**A**) and esophagitis (**B**). The cytoplasm and/or nuclei of cells expressing LTA_4H were stained dark brown. Infiltrating inflammatory cells were also strongly stained. All scale bars = 100 μm.

and excluded from analysis. When the staining intensity in epithelial cells was compared among different histologic stages, LTA_4H expression in CLE, dysplastic, and EAC tissues was statistically significantly higher than that in basal cells of squamous epithelium ($P<.001$). A higher level of LTA_4H was expressed in dysplastic lesions than in CLE ($P = .04$). No statistically significant difference was observed in the level of LTA_4H in dysplastic and EAC tissues ($P = .42$; Table 1). Among the six patients who contributed all four types of tissue, LTA_4H expression was statistically significantly higher in CLE, dysplastic, and EAC tissue than in basal cells of squamous epithelium ($P<.001$). No statistically significant difference was observed in the level of LTA_4H among CLE, dysplastic, and EAC tissues ($P = .109$). Because all patients had reflux esophagitis, no normal esophageal squamous epithelium was available. The parabasal cells of the esophageal squamous epithelium expressed a high level of LTA_4H (intensity score = 2.18, 95% CI = 1.78 to 2.30).

A higher level of LTA_4H was detected in well-differentiated EAC than in moderately differentiated EAC ($P = .003$) and poorly differentiated EAC ($P = .04$). No statistically significant difference in the level of LTA_4H was observed between moderately and poorly differentiated EAC ($P = .92$). LTA_4H was also highly expressed in two samples of mucinous EAC in this tissue collection (Table 1).

Chemoprevention of EGDA-Induced EAC in Rats by Bestatin

To assess the functional role of LTA_4H in esophageal adenocarcinogenesis, bestatin, an LTA_4H inhibitor, was tested for its effect on LTB_4 biosynthesis in EGDA rats. The level of LTB_4 in tissue from the esophagoduodenal junction of rats treated with bestatin (10 mg/kg, intraperitoneally, daily for 7 days) was statistically significantly lower (3.16 ng/mg of protein, 95% CI = 2.14 to 3.67 ng/mg) than that in untreated EGDA rats (7.64 ng/mg of protein, 95% CI = 6.22 to 8.11 ng/mg) ($P = .014$). The level of LTB_4 in the upper esophagus was not statistically significantly different in untreated rats (1.64 ng/mg of protein, 95% CI = 1.29 to 1.82 ng/mg) and rats treated with bestatin (1.58 ng/mg of protein, 95% CI = 0.96 to 1.89 ng/mg) ($P = .91$). Because EGDA rats always had severe inflammation at the esophagoduodenal junction and little or no inflammation in the upper esophagus, bestatin treatment appeared to inhibit LTA_4H activity in chronically inflamed esophageal tissues.

In a long-term chemoprevention study, the EGDA rats were active and healthy. Rats in group A were non-operated controls, rats in group B were assigned to receive EGDA, and rats in group C were assigned to receive EGDA and bestatin treatment. One rat from group B and four rats from group C died before the termination of the experiment. Body weight was slightly lower for EGDA animals (group B) than for the non-operated control animals (group A). Body weight for bestatin-treated EGDA animals (group C) was also lower than that of the EGDA group (group B) and the non-operated control group (group A) (Fig. 4). There was no statistically significant difference between group A and group B ($P = .10$) and between group B and group C ($P = .09$). However, group C had a statistically significantly lower body weight than group A ($P = .03$). At 40 weeks after EGDA, group B had a tumor incidence of 57.7% (15 of 26 rats), which was similar to that in our previous study *(5).* Treatment with bestatin at a dose of 10 mg/kg intraperitoneally, three times a week, statistically significantly reduced tumor incidence to

Downloaded from https://academic.oup.com/jnci/article/95/14/1053/2520331 by guest on 24 April 2024

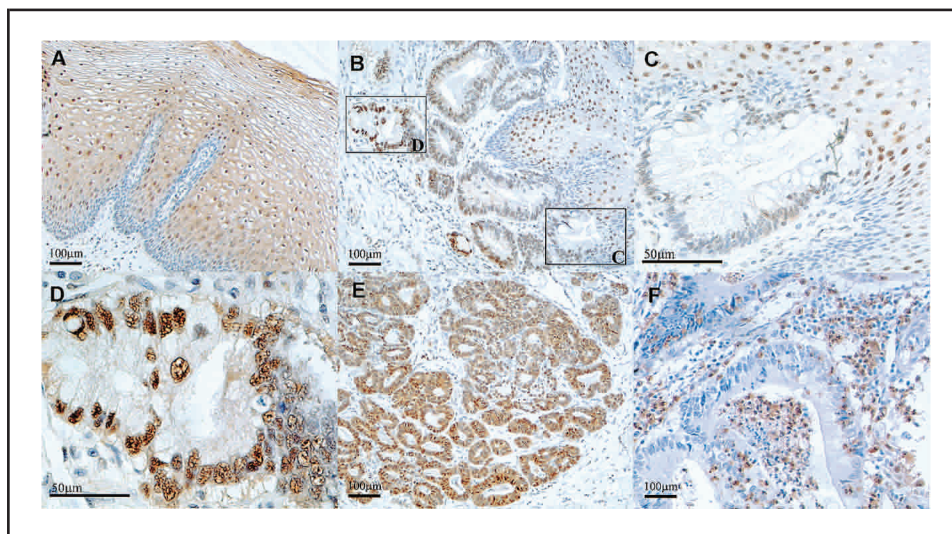

Fig. 3. Expression of leukotriene A_4 hydrolase (LTA_4H) in human esophageal tissues detected by immunohistochemistry. **A–E**) Sample 1, a well-differentiated esophageal adenocarcinoma (EAC). **F**) Sample 2, a poorly differentiated EAC. **A**) Parabasal cells (staining intensity = 3) and basal cells (staining intensity = 0) of human esophageal squamous epithelium. **B**) Columnar-lined esophagus (CLE; staining intensity = 1) and dysplasia (staining intensity = 3). **C** and **D**) Higher magnification of boxed parts of **panel B.** **E**) EAC tumor cells (staining intensity = 3). **F**) EAC tumor cells (staining intensity = 0) and infiltrating inflammatory cells (staining intensity = 3). Scale bars in **A, B, E,** and **F** = 100 µm; scale bars in **C** and **D** = 50 µm.

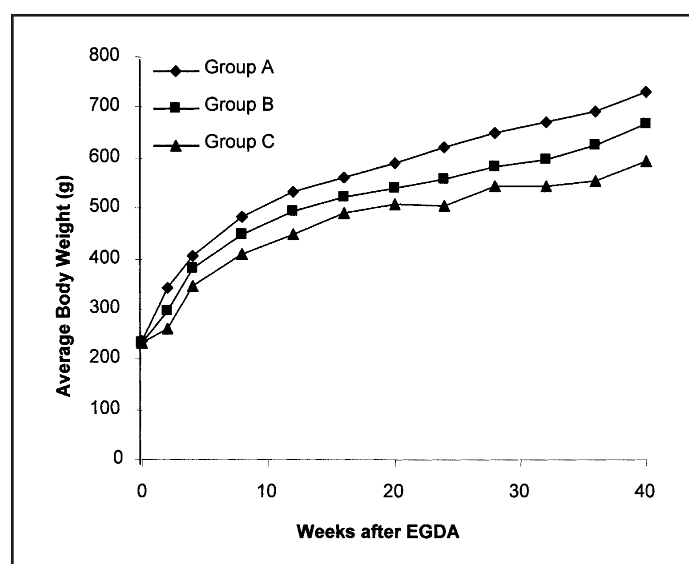

Fig. 4. Average body weight of rats after esophagogastroduodenal anastomosis (EGDA) in the long-term chemoprevention study. Group A = non-operated control group; group B = EGDA-treated group; group C = EGDA- and bestatin-treated group. Body weights at week 40 were as follows: group A = 730 g (95% CI = 702 to 739 g), group B = 665 g (95% CI = 636 to 671 g), and group C = 592 g (95% CI = 565 to 598 g). There was no statistically significant difference between group A and group B (P = .10) and between group B and group C (P = .09). However, group C had a statistically significantly lower body weight than group A (P = .03).

26.1% (6 of 23 rats) (difference = 31.6%, 95% CI = 0.3% to 56.2%; P = .042). Tumor volume of the visible tumors was also statistically significantly reduced (0.34 cm^3 in EGDA rats and 0.16 cm^3 in bestatin-treated EGDA rats; difference = 0.18, 95% CI = 0.084 to 0.276; P = .009; Table 2).

To associate the chemopreventive effect of bestatin with its enzyme-inhibitory effect, the level of LTB_4 in the rat esophageal tissue was determined with enzyme immunoassay. The level of LTB_4 in the esophageal epithelium of the non-operated control animals (group A) was as low as 0.68 ng/mg of protein. EGDA (group B) statistically significantly increased LTB_4 biosynthesis at the esophagoduodenal junction to 8.28 ng/mg of protein (difference between groups B and A = 7.60, 95% CI = 5.99 to 9.21; P<.001), and bestatin treatment after EGDA (group C) statistically significantly decreased the level of LTB_4 to 4.68 ng/mg of protein (difference between groups B and C = 3.60, 95% CI = 1.59 to 5.61; P = .002) (Table 2).

Discussion

In this study, we demonstrate the overexpression of LTA_4H in esophageal adenocarcinogenesis and the inhibition of carcinogenesis in a rat model by bestatin, an LTA_4H inhibitor. LTA_4H is a bifunctional zinc enzyme with epoxide hydrolase and aminopeptidase activities *(30).* The aminopeptidase activity of LTA_4H is generally assumed to process peptides related to inflammation and host defense *(31).* The epoxide hydrolase activity of LTA_4H hydrolyzes the epoxide LTA_4 to the diol LTB_4, which mainly functions as a chemoattractant and an activator of inflammatory cells in inflammation, immune responses, host defense against infection, platelet-activating factor-induced shock, and lipid homeostasis *(32–34).* LTB_4 acts as an autocrine and paracrine factor to the nearby cells that express the specific LTB_4 receptors (BLT1 and BLT2), with BLT1 being mainly involved in chemotaxis and BLT2 being possibly involved in leukocyte degranulation and superoxide production *(35).* Inhibition of LTA_4H by specific inhibitors or gene knockout decreases the effect of inflammatory diseases in animal models *(32,36,37).* Consequently, LTA_4H has long been regarded as an anti-inflammatory target *(38).*

Besides their involvement in inflammation, LTA_4H and LTB_4 are also associated with esophageal adenocarcinogenesis. The levels of LTB_4 and prostaglandin E_2 were substantially higher in human biopsy samples of preinvasive esophageal lesions than in biopsy samples of normal subjects *(24).* Using immunohistochemistry, we found overexpression of LTA_4H in the preinvasive and cancer cells of EGDA rats. We used a staining intensity scoring system to semi-quantify the expression levels of LTA_4H in the esophageal tissues of 92 human patients and found that LTA_4H was much higher in human CLE, dysplastic, and EAC tissues than in basal cells of the esophageal epithelium. LTA_4H overexpression appears to be an early event in esophageal adenocarcinogenesis. Rat esophageal squamous epithelial cells did not express LTA_4H (Fig. 2, A), even in the presence of reflux esophagitis (Fig. 2, B). However, LTA_4H was expressed in the parabasal cells of human esophageal squamous epithelium (Fig. 3, A). The reason for the difference is not known. One

Downloaded from https://academic.oup.com/jnci/article/95/14/1053/2520331 by guest on 24 April 2024

Table 2. Chemopreventive effects of bestatin on rat esophageal adenocarcinogenesis (EAC)

Group	Treatment*	No. of animals	EAC incidence, % (No. of animals with EAC/total No.)†	Tumor volume, cm^3 (95% CI)‡	LTB4, ng/mg of protein (95% CI)§
A	Negative control	10	—	—	0.68 (0.61 to 0.70)
B	EGDA	26	57.7 (15/26)	0.34 (0.28 to 0.35)	8.28 (6.51 to 8.91)
C	Bestatin-treated EGDA	23	26.1 (6/23)	0.16 (0.08 to 0.19)	4.68 (3.34 to 5.15)

*Esophagogastroduodenal anastomosis (EGDA) was performed as described previously *(5)*. Animals were treated with iron dextran intraperitoneally at 12.5 mg of iron per kilogram every 2 weeks, starting 4 weeks after surgery and continuing for the duration of the experiment. Bestatin was freshly dissolved in phosphate-buffered saline (pH 7.2) and administered at 10 mg/kg intraperitoneally three times a week. One rat in group B and four rats in group C died after surgery and before the termination of the experiment. — = none.

†Statistically significant difference between group B and group C by two-sided χ^2 test ($P = .042$).

‡Statistically significant difference between group B and group C by two-sided Wilcoxon signed rank test ($P = .009$). CI = confidence interval.

§Statistically significant differences between groups A and B ($P<.001$) and between groups B and C ($P = .002$), by two-sided Student's *t* test.

possibility is that, in the non-keratinized human squamous epithelium, LTA_4H may be more susceptible to induction by gastroesophageal reflux, as discussed below, whereas it is less responsive in the keratinized rat esophageal squamous epithelium. Although the level of LTA_4H in rat EAC tumors was not higher than that in esophageal epithelial samples (Fig. 1, B), possibly because of high expression of LTA_4H in infiltrating inflammatory cells, LTA_4H was still overexpressed in the tumor cells compared with the expression in the esophageal squamous epithelial cells (Fig. 2, B and D). Probably also because of high expression of LTA_4H in infiltrating inflammatory cells, some human EAC samples (03 and 06) expressed less LTA_4H protein than their respective adjacent non-tumor esophageal tissues (Fig. 1, C).

We found by immunohistochemical analysis that LTA_4H was also overexpressed in other human cancers, including colon and lung cancers (data not shown). LTB_4 was overproduced in human colon cancer tissue (mainly in the epithelial layer) compared with normal colon tissue *(39)*. LTB_4 at nanomolar levels stimulated the proliferation of colon cancer cells *(40,41)*; inhibited apoptosis of intestinal epithelial cells by regulating the expression of cyclooxygenase 2, β-catenin, and Bcl-2 *(42)*; enhanced oxidative stress *(43,44)*; and promoted cell spreading *(45)*. Topical application of LTB_4 to the skin leads not only to inflammation but also to substantial hyperplasia in the epidermis *(46,47)*. A BLT1 antagonist inhibited proliferation and induced apoptosis in human pancreatic cancer cells *(48)*. Thus, LTA_4H and/or LTB_4 may play an important role in esophageal adenocarcinogenesis by 1) augmenting inflammation in inflammatory cells through positive feedback mediated by its receptors and downstream signaling kinases *(49,50)* and 2) stimulating autocrine and paracrine growth of preinvasive and cancer cells. Results of this study and our previous study on cyclooxygenase 2 *(11)* indicate that aberrant arachidonic acid metabolism appears to play an important role in the development of EAC associated with chronic inflammation *(5,6)*.

LTA_4H is expressed in many types of cells in normal human, rat, and mouse tissues, including epithelial cells (e.g., from the gastrointestinal tract, skin, breast, prostate, and kidney and cells from other organs), inflammatory cells, endothelial cells, fibroblasts, muscle cells, liver cells, adipocytes, and neurons (data not shown). This pattern of cellular distribution is consistent with the ubiquitous involvement of LTB_4 in inflammatory conditions of many tissues. Under certain stimuli or physiologic or pathologic conditions, LTA_4H may be stimulated or inhibited by treatment with cytokines *(51,52)*, treatment with cyclosporin A *(53)*, smoking *(54)*, chorionic gonadotrophin during early pregnancy *(55)*, glomerulonephritis *(56)*, angiotensin-induced hypertension *(57)*, or bacterial infection of the oral mucosa *(58)*. LTA_4H expression in the esophagus may be stimulated by exposure of esophageal epithelium to gastroesophageal reflux. Bile acids, an important constituent in the gastroesophageal refluxate, increase LTA_4H expression and increase LTB_4 biosynthesis in the human EAC cell line SKGT4 (data not shown), in CaCo-2 colon cancer cells *(59)*, and in rat colonic mucosa *(60)*.

To further demonstrate the role of LTA_4H in esophageal adenocarcinogenesis, we investigated whether bestatin, an LTA_4H inhibitor *(30,61)*, was a chemopreventive agent for EAC in EGDA rats. Bestatin was chosen because it inhibits proliferation and induces apoptosis in cancer cell lines *(62–64)*, inhibits carcinogen-induced stomach tumorigenesis *(65)*, activates E-cadherin-mediated adhesion of breast cancer cells *(66)*, and promotes the immune reaction against cancer *(67)*. The low toxicity of bestatin is important for its use in chemoprevention. In this study, the injection of bestatin did not produce toxicity, although it slightly decreased the body weight of EGDA rats. This result is consistent with the results of a previous study *(68)* that showed that bestatin at a dose as high as 250 mg/kg given intravenously did not induce any signs of toxicity. In our short-term study, bestatin given at 10 mg/kg intraperitoneally daily for 7 days statistically significantly inhibited LTB_4 biosynthesis at the esophagoduodenal junction. In the long-term chemoprevention study in EDGA rats, bestatin inhibited LTB_4 production by 44% in rat esophageal tissues and reduced the incidence of EAC by 55% at 40 weeks after EGDA. These results are consistent with the hypothesis that bestatin suppressed the development of EAC by inhibiting the epoxide hydrolase activity of LTA_4H; however, other actions might also contribute to its chemopreventive effect *(69)*. Bestatin can also inhibit LTA_4H aminopeptidase activity *(30)*, which may play a role in carcinogenesis *(70,71)*. Inhibition of aminopeptidase N/CD13 by bestatin was associated with its immunomodulating effect for cancer therapy *(63,64,72,73)*.

Studies that use our rat EGDA model are relevant to human EAC. We have previously investigated the similarity of rat CLE and EAC to human diseases by studying histopathologic progression of rat esophageal adenocarcinogenesis, mucin-staining features, expression of differentiation markers, and mRNA expression profiles. As with human CLE and EAC *(74)*, most EGDA-induced rat CLE and EACs originated from metaplasia of the multipotential stem cells in the basal cell layer of the esophageal squamous epithelium (Su Y, Chen X, Klein M, Yang CS, Goyal RK: unpublished results). Using proteomics, we have

Downloaded from https://academic.oup.com/jnci/article/95/14/1053/2520331 by guest on 24 April 2024

identified Grp94 and LTA_4H as overexpressed proteins in rat and human EACs. As one of the stress proteins covalently attached to potential tumor-specific antigens, Grp94 may be used to mediate immunotherapy and immunoprevention of EAC *(25)*. As an important metabolizing enzyme of arachidonic acid, LTA_4H appears to be a potential target for the chemoprevention of human EAC. Recent studies of the crystal structure and molecular biology of LTA_4H *(75,76)* suggested that LTA_4H epoxide hydrolase activity could be targeted efficiently and specifically by some potent LTA_4H epoxide hydrolase inhibitors *(38,77)*. We urge that future studies test the chemopreventive effects of these inhibitors on EAC.

References

(1) Kubo A, Corley DA. Marked regional variation in adenocarcinomas of the esophagus and the gastric cardia in the United States. Cancer 2002;95: 2096–102.

(2) Farrow DC, Vaughan TL. Determinants of survival following the diagnosis of esophageal adenocarcinoma (United States). Cancer Causes Control 1996;7:322–7.

(3) Goldstein SR, Yang GY, Chen X, Curtis SK, Yang CS. Studies of iron deposits, inducible nitric oxide synthase and nitrotyrosine in a rat model for esophageal adenocarcinoma. Carcinogenesis 1998;19:1445–9.

(4) Chen X, Ding W, Yang GY, Bondoc F, Yang CS. Oxidative damage in an esophagoduodenal anastomosis (EDA) rat model. Carcinogenesis 2000;21: 257–63.

(5) Chen X, Yang GY, Ding W, Bondoc F, Curtis SK, Yang CS. An esophagogastroduodenal anastomosis model for esophageal adenocarcinogenesis in rats and enhancement by iron overload. Carcinogenesis 1999;20: 1801–7.

(6) Chen X, Yang CS. Esophageal adenocarcinoma: a review and perspectives on the mechanism of carcinogenesis and chemoprevention. Carcinogenesis 2001;22:1119–29.

(7) Steele VE, Hawk ET, Viner JL, Lubet RA. Mechanisms and applications of non-steroidal anti-inflammatory drugs in the chemoprevention of cancer. Mutat Res 2003;523–524:137–44.

(8) Hong SH, Avis I, Vos MD, Martinez A, Treston AM, Mulshine JL. Relationship of arachidonic acid metabolizing enzyme expression in epithelial cancer cell lines to the growth effect of selective biochemical inhibitors. Cancer Res 1999;59:2223–8.

(9) Chiu CH, McEntee MF, Whelan J. Discordant effect of aspirin and indomethacin on intestinal tumor burden in Apc(Min/+) mice. Prostaglandins Leukot Essent Fatty Acids 2000;62:269–75.

(10) Wilson KT, Fu S, Ramanujam KS, Meltzer SJ. Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett's esophagus and associated adenocarcinomas. Cancer Res 1998;58:2929–34.

(11) Chen X, Li N, Wang S, Hong J, Fang M, Yousselfson J, et al. Aberrant arachidonic acid metabolism in esophageal adenocarcinogenesis, and the effects of sulindac, nordihydroguaiaretic acid, and a-difluoromethylornithine on tumorigenesis in a rat surgical model. Carcinogenesis 2002;23: 2095–102.

(12) Shirvani VN, Ouatu-Lascar R, Kaur BS, Omary MB, Triadafilopoulos G. Cyclooxygenase 2 expression in Barrett's esophagus and adenocarcinoma: ex vivo induction by bile salts and acid exposure. Gastroenterology 2000; 118:487–96.

(13) Souza RF, Shewmake K, Beer DG, Cryer B, Spechler SJ. Selective inhibition of cyclooxygenase-2 induces apoptosis in human esophageal adenocarcinoma cells. Cancer Res 2000;60:5767–72.

(14) Buttar NS, Wang KK, Leontovich O, Westcott JY, Pacifico RJ, Anderson MA, et al. Chemoprevention of esophageal adenocarcinoma by COX-2 inhibitors in an animal model of Barrett's esophagus. Gastroenterology 2002;122:1101–12.

(15) Marleau S, Fruteau de Laclos B, Sanchez AB, Poubelle PE, Borgeat P. Role of 5-lipoxygenase products in the local accumulation of neutrophils in dermal inflammation in the rabbit. J Immunol 1999;163:3449–58.

(16) Goulet JL, Snouwaert JN, Latour AM, Coffman TM, Koller BH. Altered inflammatory response in leukotriene-deficient mice. Proc Natl Acad Sci U S A 1994;91:12852–6.

(17) Byrum RS, Goulet JL, Griffiths RJ, Koller BH. Role of the 5-lipoxygenase-activating protein (FLAP) in murine acute inflammatory responses. J Exp Med 1997;185:1065–75.

(18) Ghosh J, Myers CE. Arachidonic acid stimulates prostate cancer cell growth: critical role of 5-lipoxygenase. Biochem Biophys Res Commun 1997;235:418–23.

(19) Rioux N, Castonguay A. Inhibitors of lipoxygenase: a new class of cancer chemopreventive agents. Carcinogenesis 1998;19:1393–400.

(20) Moody TW, Leyton J, Martinez A, Hong S, Malkinson A, Mulshine JL. Lipoxygenase inhibitors prevent lung carcinogenesis and inhibit non-small cell lung cancer growth. Exp Lung Res 1998;24:617–28.

(21) Jiang H, Yamamoto S, Kato R. Inhibition of two-stage skin carcinogenesis as well as complete skin carcinogenesis by oral administration of TMK688, a potent lipoxygenase inhibitor. Carcinogenesis 1994;15:807–12.

(22) Castonguay A, Rioux N, Duperron C, Jalbert G. Inhibition of lung tumorigenesis by NSAIDS: a working hypothesis. Exp Lung Res 1998;24: 605–15.

(23) Marcinkiewicz M, Sarosiek J, Edmunds M, Scheurich J, Weiss P, McCallum RW. Monophasic luminal release of PGE2 in patients with reflux esophagitis under the impact of acid and acid/pepsin solutions. J Clin Gastroenterol 1995;21:268–74.

(24) Triadafilopoulos G, Kaczynska M, Iwane M. Esophageal mucosal eicosanoids in gastroesophageal reflux disease and Barrett's esophagus. Am J Gastroenterol 1996;91:65–74.

(25) Chen X, Ding Y, Liu CG, Mikhail S, Yang CS. Overexpression of glucose-regulated protein 94 (Grp94) in esophageal adenocarcinomas of a rat surgical model and humans. Carcinogenesis 2002;23:123–30.

(26) Bronstein I, Voyta JC, Murphy OJ, Bresnick L, Kricka LJ. Improved chemiluminescent Western blotting procedure. Biotechniques 1992;12: 748–53.

(27) Makita N, Funk CD, Imai E, Hoover RL, Badr KF. Molecular cloning and functional expression of rat leukotriene A4 hydrolase using the polymerase chain reaction. FEBS Lett 1992;299:273–7.

(28) Lewin KJ, Appelman HD. Barrett's esophagus, columnar dysplasia, and adenocarcinoma of the esophagus. In: Lewin KJ, Appelman HD, editors. Tumors of the esophagus and stomach. Washington (DC): Armed Forces Institute of Pathology Press; 1995. p. 99–144.

(29) Goldstein SR, Yang GY, Curtis SK, Reuhl KR, Liu BC, Mirvish SS, et al. Development of esophageal metaplasia and adenocarcinoma in a rat surgical model without the use of a carcinogen. Carcinogenesis 1997;18: 2265–70.

(30) Orning L, Krivi G, Fitzpatrick FA. Leukotriene A4 hydrolase: inhibition by bestatin and intrinsic aminopeptidase activity establish its functional resemblance to metallohydrolase enzymes. J Biol Chem 1991;266:1375–8.

(31) Haeggstrom JZ, Kull F, Rudberg PC, Tholander F, Thunnissen MM. Leukotriene A4 hydrolase. Prostaglandins Other Lipid Mediat 2002;68–69: 495–510.

(32) Byrum RS, Goulet JL, Snouwaert JN, Griffiths RJ, Koller BH. Determination of the contribution of cysteinyl leukotrienes and leukotriene B4 in acute inflammatory responses using 5-lipoxygenase- and leukotriene A4 hydrolase-deficient mice. J Immunol 1999;163:6810–9.

(33) Crooks SW, Stockley RA. Leukotriene B4. Int J Biochem Cell Biol 1998; 30:173–8.

(34) Dahlen SE. Leukotriene receptors. Clin Rev Allergy Immunol 1999;17: 179–91.

(35) Yokomizo T, Izumi T, Shimizu T. Leukotriene B4: metabolism and signal transduction. Arch Biochem Biophys 2001;385:231–41.

(36) Muskardin DT, Voelkel NF, Fitzpatrick FA. Modulation of pulmonary leukotriene formation and perfusion pressure by bestatin, an inhibitor of leukotriene A4 hydrolase. Biochem Pharmacol 1994;48:131–7.

(37) Tsuji F, Oki K, Fujisawa K, Okahara A, Horiuchi M, Mita S. Involvement of leukotriene B4 in arthritis models. Life Sci 1999;PL64:51–6.

(38) Penning TD. Inhibitiors of leukotriene A4 (LTA4) hydrolase as potential anti-inflammatory agents. Curr Pharm Des 2001;7:163–79.

(39) Dreyling KW, Hoppe U, Peskar BA, Morgenroth K, Kozuschek W, Peskar BM. Leukotriene synthesis by human gastrointestinal tissues. Biochim Biophys Acta 1986;878:184–93.

(40) Bortuzzo C, Hanif R, Kashfi K, Staiano-Coico L, Shiff SJ, Rigas B. The effect of leukotrienes B and selected HETEs on the proliferation of colon cancer cells. Biochim Biophys Acta 1996;1300:240–6.

Downloaded from https://academic.oup.com/jnci/article/95/14/1053/2520331 by guest on 24 April 2024

(41) Qiao L, Kozoni V, Tsioulias GJ, Koutsos MI, Hanif R, Shiff SJ, et al. Selected eicosanoids increase the proliferation rate of human colon carcinoma cell lines and mouse colonocytes in vivo. Biochim Biophys Acta 1995;1258:215–23.
(42) Ohd JF, Wikstrom K, Sjolander A. Leukotrienes induce cell-survival signaling in intestinal epithelial cells. Gastroenterology 2000;119:1007–18.
(43) Woo CH, You HJ, Cho SH, Eom YW, Chun JS, Yoo YJ, et al. Leukotriene B4 stimulates Rac-ERK cascade to generate reactive oxygen species that mediates chemotaxis. J Biol Chem 2002;277:8572–8.
(44) Steiner DR, Gonzalez NC, Wood JG. Leukotriene B(4) promotes reactive oxidant generation and leukocyte adherence during acute hypoxia. J Appl Physiol 2001;91:1160–7.
(45) Stockton RA, Jacobson BS. Modulation of cell-substrate adhesion by arachidonic acid: lipoxygenase regulates cell spreading and ERK1/2-inducible cyclooxygenase regulates cell migration in NIH-3T3 fibroblasts. Mol Biol Cell 2001;12:1937–56.
(46) Bauer RW, van der Kerhof PC, de Grood RM. Epidermal hyperproliferation following the induction of microabscesses by leukotriene B4. Br J Dermatol 1986;114:409–12.
(47) Ruzicka T, Burg G. Effects of chronic intracutaneous administration of arachidonic acid and its metabolites. Induction of leukocytoclastic vasculitis by leukotriene B4 and 12-hydroxyeicosatetraenoic acid and its prevention by prostaglandin E2. J Invest Dermatol 1987;88:120–3.
(48) Tong WG, Ding XZ, Hennig R, Witt RC, Standop J, Pour PM, et al. Leukotriene B4 receptor antagonist LY293111 inhibits proliferation and induces apoptosis in human pancreatic cancer cells. Clin Cancer Res 2002; 8:3232–42.
(49) Anthonsen MW, Andersen S, Solhaug A, Johansen B. Atypical lambda/iota PKC conveys 5-lipoxygenase/leukotriene B4-mediated cross-talk between phospholipase A2s regulating NF-kappa B activation in response to tumor necrosis factor-alpha and interleukin-1beta. J Biol Chem 2001;276: 35344–51.
(50) Surette ME, Krump E, Picard S, Borgeat P. Activation of leukotriene synthesis in human neutrophils by exogenous arachidonic acid: inhibition by adenosine A(2a) receptor agonists and crucial role of autocrine activation by leukotriene B(4). Mol Pharmacol 1999;56:1055–62.
(51) Montero A, Nassar GM, Uda S, Munger KA, Badr KF. Reciprocal regulation of LTA(4) hydrolase expression in human monocytes by gamma-interferon and interleukins 4 and 13: potential relevance to leukotriene regulation in glomerular disease. Exp Nephrol 2000;8:258–65.
(52) Zaitsu M, Hamasaki Y, Matsuo M, Kukita A, Tsuji K, Miyazaki M, et al. New induction of leukotriene A(4) hydrolase by interleukin-4 and interleukin-13 in human polymorphonuclear leukocytes. Blood 2000;96:601–9.
(53) Iversen L, Svendsen M, Kragballe K. Cyclosporin A down-regulates the LTA4 hydrolase level in human keratinocyte cultures. Acta Derm Venereol 1996;76:424–8.
(54) Munafo DA, Shindo K, Baker JR, Bigby TD. Leukotriene A4 hydrolase in human bronchoalveolar lavage fluid. J Clin Invest 1994;93:1042–50.
(55) Hattori N, Fujiwara H, Maeda M, Yoshioka S, Higuchi T, Mori T, et al. Human large luteal cells in the menstrual cycle and early pregnancy express leukotriene A4 hydrolase. Mol Hum Reprod 1998;4:803–10.
(56) Menegatti E, Roccatello D, Fadden K, Piccoli G, De Rosa G, Sena LM, et al. Gene expression of 5-lipoxygenase and LTA4 hydrolase in renal tissue of nephrotic syndrome patients. Clin Exp Immunol 1999;116: 347–53.
(57) Ishizaka N, Nakao A, Ohishi N, Suzuki M, Aizawa T, Taguchi J, et al. Increased leukotriene A(4) hydrolase expression in the heart of angiotensin II-induced hypertensive rat. FEBS Lett 1999;463:155–9.
(58) Eberhard J, Jepsen S, Tiemann M, Krause R, Acil Y, Albers HK. Leukotriene A(4)-hydrolase expression and leukotriene B(4) levels in chronic inflammation of bacterial origin: immunohistochemistry and reverse-phase high-performance liquid chromatography analysis of oral mucosal epithelium. Virchows Arch 2002;440:627–34.
(59) Dias VC, Shaffer EA, Wallace JL, Parsons HG. Bile salts determine leukotriene B4 synthesis in a human intestinal cell line (CaCo-2). Dig Dis Sci 1994;39:802–8.
(60) Craven PA, Pfanstiel J, DeRubertis FR. Role of reactive oxygen in bile salt stimulation of colonic epithelial proliferation. J Clin Invest 1986;77:850–9.
(61) Haeggstrom JZ. Leukotriene A4 hydrolase and the committed steps in leukotriene B4 biosynthesis. Clin Rev Allergy Immunol 1999;17:111–31.
(62) Ezawa K, Minato K, Dobashi K. Induction of apoptosis by ubenimex (bestatin) in human non-small-cell lung cancer cell lines. Biomed Pharmacother 1996;50:283–9.
(63) Sekine K, Fujii H, Abe F, Nishikawa K. Augmentation of death ligand-induced apoptosis by aminopeptidase inhibitors in human solid tumor cell lines. Int J Cancer 2001;94:485–91.
(64) Grujic M, Renko M. Aminopeptidase inhibitors bestatin and actinonin inhibit cell proliferation of myeloma cells predominantly by intracellular interactions. Cancer Lett 2002;182:113–9.
(65) Ebihara K, Abe F, Yamashita T, Shibuya K, Hayashi E, Takahashi K, et al. The effect of ubenimex on N-methyl-N'-nitro-N-nitrosoguanidine-induced stomach tumor in rats. J Antibiot 1986;39:966–70.
(66) Fujioka S, Kohno N, Hiwada K. Ubenimex activates the E-cadherin-mediated adhesion of a breast cancer cell line YMB-S. Jpn J Cancer Res 1995;86:368–73.
(67) Abe F, Schneider M, Black PL, Talmadge JE. Chemoimmunotherapy with cyclophosphamide and bestatin in experimental metastasis in mice. Cancer Immunol Immunother 1989;29:231–6.
(68) Talmadge JE, Lenz BF, Pennington R, Long C, Phillips H, Schneider M, et al. Immunomodulatory and therapeutic properties of bestatin in mice. Cancer Res 1986;46:4505–10.
(69) Scornik OA, Botbol V. Bestatin as an experimental tool in mammals. Curr Drug Metab 2001;2:67–85.
(70) Ishii K, Usui S, Sugimura Y, Yoshida S, Hioki T, Tatematsu M, et al. Aminopeptidase N regulated by zinc in human prostate participates in tumor cell invasion. Int J Cancer 2001;92:49–54.
(71) Hashida H, Takabayashi A, Kanai M, Adachi M, Kondo K, Kohno N, et al. Aminopeptidase N is involved in cell motility and angiogenesis: its clinical significance in human colon cancer. Gastroenterology 2002;122:376–86.
(72) Mishima Y, Matsumoto-Mishima Y, Terui Y, Katsuyama M, Yamada M, Mori M, et al. Leukemic cell-surface CD13/aminopeptidase N and resistance to apoptosis mediated by endothelial cells. J Natl Cancer Inst 2002; 94:1020–8.
(73) Ino K, Bierman PJ, Varney ML, Heimann DG, Kuszynski CA, Walker SA, et al. Monocyte activation by an oral immunomodulator (bestatin) in lymphoma patients following autologous bone marrow transplantation. Cancer Immunol Immunother 1996;43:206–12.
(74) Jankowski JA. Barrett's metaplasia. Lancet 2000;356:2079–85.
(75) Thunnissen MM, Nordlund P, Haeggstrom JZ. Crystal structure of human leukotriene A4 hydrolase, a bifunctional enzyme in inflammation. Nat Struct Biol 2001;8:131–5.
(76) Rudberg PC, Tholander F, Thunnissen MM, Samuelsson B, Haeggstrom JZ. Leukotriene A4 hydrolase: selective abrogation of leukotriene B4 formation by mutation of aspartic acid 375. Proc Natl Acad Sci U S A 2002; 99:4215–20.
(77) Penning TD, Russell MA, Chen BB, Chen HY, Liang CD, Mahoney MW, et al. Synthesis of potent leukotriene A(4) hydrolase inhibitors. Identification of 3-[methyl[3-[4-(phenylmethyl)phenoxy]propyl]amino]propanoic acid. J Med Chem 2002;45:3482–90.

Notes

Supported by Public Health Service grant CA75683 (to C. S. Yang) and grant CA72720 (Cancer Center Support; to C. S. Yang) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by grant ES05022 from the National Institute of Environmental Health Science Center.

Manuscript received November 26, 2002; revised May 5, 2003; accepted May 21, 2003.

Downloaded from https://academic.oup.com/jnci/article/95/14/1053/2520331 by guest on 24 April 2024