Altered expression of amino acid transporter LATs of intestinal cells in 5-fluorouracil-induced intestinal mucosal inflammation

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Background: We hypothesized that amino acid transporters are associated with local inflammation in anticancer agent-induced intestinal mucosal injury. The L-type amino acid transporter (LAT) is an important gastrointestinal transporter for nutrient absorption because it non-selectively transports several types of amino acids, including essential amino acids.

Objective: We aimed to clarify the disease-dependent roles of LAT1 and LAT2.

Methods: We orally administered 5-fluorouracil to mice once daily (50 mg/kg/day) for 4 days. The severity of mucositis was evaluated based on intestinal length and, cell infiltration, and the immune response. LAT expressions were quantified in the small intestinal tissues using real-time polymerase chain reaction and Western blot.

Results: The 5-FU-treated mice demonstrated decreased body weight and stool volume as well as food and water consumption. Treatment with 5-FU increased the numbers of Peyer's patches and infiltrating cells and upregulated the mRNA expressions of interleukin (IL)-1β and IL-6 suggesting enhancement of the immune response associated with inflammatory cytokine production. After treatment with 5-FU, LAT1 expression considerably increased in the small intestine, whereas LAT2 expression decreased.

Conclusions: These findings suggest that because LAT1 expression increases in association with the inflammatory cytokine expression, it can be used as a marker of gastrointestinal inflammation.

Key words: L-type amino acid transporter, 5-fluorouracil, intestinal mucosal inflammation

Introduction

In recent years, the number of patients with cancer and the frequency of cancer-related deaths have been on the rise. The incidence of cancer has increased by 28% over the past 10 years.1 Anticancer treatments have been actively investigated and used in clinical practice. New anticancer agents have been developed, and new combinations of various anticancer agents have been investigated and are being used to treat patients with cancer. The median survival times of patients with various cancer types, including esophageal, gastric, and colorectal cancers, have been comparatively prolonged by using these new anticancer treatments than conventional options.2,3 However, the increased use of anticancer agents has been associated with increasing occurrences of adverse reactions in patients. There are several adverse reactions of anticancer agents, including gastrointestinal
mucosal disturbances (diarrhea, constipation, nausea, vomiting, and anorexia), cytopenia, and alopecia. In particular, anticancer agent-induced gastrointestinal mucosal disturbances often deprive patients of the pleasure of eating, leading to a very poor nutritional status, lower ability to perform activities of daily living, poor quality of life, and shorter survival time; therefore, measures against these adverse reactions are urgently required in clinical practice.

Nutrients, such as carbohydrates, lipids, and amino acids, from food are absorbed in the gastrointestinal tract. Transporters that transport nutrients and drugs are expressed in the epithelial cells of the gastrointestinal tract and play various roles in biological responses besides nutrient absorption and drug uptake. Amino acids also act as functional substances that mediate biological reactions and play an important role in the transport system. Amino acids are the essential elements of body proteins and have recently been shown to provide signals for biological reaction control. For example, obesity is associated with increased levels of amino acids in the liver and other organs and with the activation of the downstream mammalian target of rapamycin (mTOR) pathways. Thus, amino acids play important roles in intracellular signaling pathways leading to the synthesis of proteins required for the maintenance of life and regulation of autophagy.

Amino acid transporters have been studied since the 1960s. Various molecules have been described, thereby reflecting the diversity of amino acids. L-type amino acid transporter (LAT) 1 and 2 transport neutral amino acids, including essential amino acids, and exhibit broad substrate selectivity; thus, they are considered important gastrointestinal transporters for nutrient absorption. Therefore, we focused on these transporters in this study. LATs are sodium ion-dependent and are composed of transmembrane proteins, thereby forming heterodimers of 4F2hc as auxiliary subunits. LAT1 transports large transmembrane proteins, thereby forming heterodimers LATs are sodium ion-dependent and are composed of 12

In the present study, we used a model of anticancer agent-induced gastrointestinal mucosal injury to analyze the dynamics of LATs in the small intestine. The study aimed at elucidating changes in LAT1 and LAT2 expressions after treatment with anticancer agents that primarily affect the gastrointestinal tract, which may provide an understanding of amino acid uptake at the time of gastrointestinal mucosal injury. The gastrointestinal tract is very elastic, flexible, and very susceptible to the effects of edema and fibrosis. Because the intestine length tends to shorten during injuries, the intestine length was used as an index of mucosal injury severity. In future clinical studies, when LAT1 will be measured as a tumor marker, elucidation of LAT1 expression at mucosal injury sites may potentially lead to amino acid transporters becoming a treatment target for anticancer agent-induced gastrointestinal mucosal injuries. Therefore, the findings of this study may be helpful for designing future cancer therapies.

Materials and Methods

Overall experimental design and evaluation methods
Animal models with mucosal injury were prepared and evaluated using living body observation, histological observation of the intestinal tract, gene expression analysis, histological analysis, and Western blotting.

Mice and drug treatment
Seven-week-old male BALB/cAJcl mice (n = 6–10 each experimental group, n = 45 (total) were used for this study) were obtained from CLEA-Japan (Tokyo). All mice were bred under specific pathogen-free conditions at the School of Allied Health Sciences, Kitasato University, and maintained at 23 ± 3°C in a 12-hour light/12-hour dark cycle. The mice were provided with a commercial diet (CRF-1; Oriental Yeast, Tokyo) and studies have demonstrated the involvement of amino acid transporters in several diseases. Various tumor markers have been developed and extensively used in clinical practice to facilitate the early detection of cancer and an early understanding of disease progression. In particular, the relation of LAT1 with cancer has recently been studied. LAT1 is considered a major transporter in the supply of essential amino acids to tumor cells. Increased LAT1 expression in human cancer cells has been suggested to be related to tumor malignancy and survival outcomes in patients with liver cancer, myelodysplastic syndrome, esophageal cancer, lung cancer, and gastric cancer. These findings suggest that LAT1 can be used as a tumor marker.

In the present study, we focused on LATs with extensive substrate selectivity.

Recent studies have shown that a histidine transporter expressed on B cells inhibits inflammation and that dysfunction of the glutamate transporter may play a role in the development of neuropsychiatric diseases.
water ad libitum. All experiments were performed according to the Institutional Guidelines for the Care and Use of Laboratory Animals in Research and with the approval of the local ethics committee of the Kitasato University. 5-fluorouracil (5-FU) was suspended in 0.5% carboxymethylcellulose (CMC) solution and prepared immediately before use. Thereafter, 5-FU was administered orally by gavage (50 mg/kg) once daily for 4 days. The control animals received 0.5% CMC instead of 5-FU. Body weight, stool volume, food consumption, and water consumption were measured once a day before administration of 5-FU on days 0−4. Four researchers who were skilled in handling mice conducted all animal experiments, such as oral administration and specimen collection (Each researchers are the experts in anatomy, gastroenterology or animal experimentation). In addition, the specialized researchers with ≥10 years of experience performed the observation of specimens and gene expression analysis.

**Histological analyses**

Small intestinal tissues were isolated and divided into halves by approximation. The upper half region was considered as the jejunum and the lower half was considered as the ileum. The length of the small intestine and the number of Peyer's patches were measured at full length. The tissues were immediately fixed for 24 hours in freshly prepared 4% paraformaldehyde in PBS. After fixation, 4-μm paraffin sections were stained with hematoxylin-eosin (H&E). Villus height was measured from the basement membrane of the mucosa to the brush border at 10 sites of the tissue section of each mouse, and the mean value and standard deviation (SD) were calculated. Invasive cells in the proliferative zone were counted based on the number of hematoxylin-positive cells in the 10 crypts of the tissue sections of each mouse, and the mean value and SD were calculated.

The sections were stained with periodic acid Schiff (PAS) stain, which stains the neutral mucin a reddish violet color. The 10 sites of the mucosal epithelium and PAS-positive cells of each mouse were assessed using an image analysis software (Image J, National Institutes of Health, Bethesda, ML, USA). The area ratio was calculated as the area of PAS-positive cells to that of the villus cells in the mucosal epithelium.

The paraffin sections were also immunostained with nutrient transporter-related antibodies. For immunohistochemical staining, endogenous peroxidase activity was blocked with 0.3% H2O2 and the tissue was then sequentially incubated with Protein block (Agilent Technologies, Santa Clara, CA, USA), anti-LAT1 (TransGenic, Fukuoka), anti-LAT2 (Abcam, Cambridge, UK), and anti-sodium-glucose transporter 1 (SGLT1) (Abcam) with antibody diluent (Agilent Technologies) and VECTASTAIN Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA). The reaction was detected using ImmPACT DAB Substrate (Vector Laboratories). Counterstaining was performed using H&E. The immunohistochemical reactivity of the antibodies was assessed under an optical microscope (Olympus, Tokyo).

**Real-time reverse transcription polymerase chain reaction**

Transcripts encoding interleukin (IL)-1β, IL-4, IL-6, IL-10, IL-13, tumor necrosis factor-α (TNF-α), LAT1, LAT2, 4F2hc, SGLT1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were examined using real-time reverse transcription polymerase chain reaction (RT-PCR). Briefly, total RNA was purified from the tissues using TRIzol RNA isolation reagents (Thermo Fisher Scientific).

**Supplementary Table 1.** Primer sequences used for RT-PCR

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The primers were picked from DNA sequences in the NCBI database using the Primer designing tool (Primer3).
Elevated LAT1 in inflammatory mucosa

Scientific, Waltham, MA, USA). Single-stranded cDNA was generated from the total RNA by reverse transcription using the PrimeScript RT reagent kit (Takara Bio, Shiga), according to the manufacturer’s instructions. Quantitative PCR amplification was performed with SYBR Select Master Mix (Thermo Fisher Scientific). The gene-specific primers are listed in the Supplementary Table 1. Data were normalized to the level of GAPDH in each sample.21

Western blotting
Tissues were added to ice-cold lysis buffer (150 mM NaCl, 1.0% NP-40 or 0.1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 50 mM Tris-HCl [pH 8.0], and protease inhibitors) and homogenized with an electric homogenizer. The lysed tissues were then maintained with constant agitation for 2 hours at 4°C. The homogenates were centrifuged for 20 minutes at 12,000 rpm at 4°C in a centrifuge and the supernatant was aspirated. Protein concentrations were estimated using the BCA Protein assay kit (Thermo Fisher Scientific). Samples containing 10 μg of protein were added to an equal volume of 2× SDS-PAGE sample buffer and boiled for 5 minutes at 100°C. Samples were loaded onto 5%–20% gradient gels (ATTO, Tokyo). The gels were then transferred onto a polyvinylidene fluoride (PVDF) membrane using the Trans-blot turbo system (Bio-Rad Laboratories, Hercules, CA, USA) and blocked with a PVDF blocking reagent (Toyobo, Osaka). The antibodies used were anti-LAT1 (1:500 dilution) (Trans Genic, Fukuoka) and anti-LAT2 (1:1000 dilution) (Abcam). Primary antibodies were incubated overnight in Can Get Signal immunoreaction enhancer solution (Toyobo). Secondary antibodies were added using the VECTASTAIN Elite ABC Kit (Vector). The signals

Figure 1. Changes in the physical condition of the mice after treatment with 5-FU
Differences between the control group and the 5-FU treatment group in (A) body weight, (B) stool volume, (C) food consumption, and (D) water consumption (Control group: n = 6, 5-FU group: n = 6). Data are shown as mean ± SD. *P < 0.05, **P < 0.01
were detected with Luminata crescendo western HRP Substrate (Merck Millipore, MA, USA), and the membrane was imaged using an Odyssey Infrared scanner (LI-COR, Lincoln, NE, USA), and quantified using ImageJ software.22

**Statistical analyses**

Data were expressed as mean ± SD. Data were analyzed using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). The normality of the distribution of continuous variables was evaluated using the Kolmogorov-Smirnov test. Repeated-measures analysis of variance and Student's two-tailed t-test were used for

**Figure 2.** Morphological changes in the small intestinal mucosa at day 4 after treatment with 5-FU (A) The full length of the small intestine was measured. (B) Number of mucus cells stained with H&E and villus height was measured, and (C) the number of PAS-positive cells was counted (Control group: n = 6, 5-FU group: n = 6). The continuous mucosal injury after treatment with 5-FU produced a decrease in the defensive function of epithelial cells. Data are shown as mean ± SD. *P < 0.05, **P < 0.01

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statistical comparisons (P < 0.05). Post-hoc sample size calculations confirmed that the study had adequate statistical power to evaluate the outcomes studied (n = 6 vs. n = 6, n = 8 vs. n = 10, or n = 4 vs. n = 4).

Results

Biological changes after treatment with 5-FU
We measured the body weight, fecal excretion, food intake, and water intake of the mice over a 4-day period to examine the systemic effects after treatment with 5-FU. The body weight of the mice in the control (C) group was approximately 30 g throughout the study period and did not fluctuate. In contrast, the body weight of the mice in the 5-FU group was approximately 26 g 4 days after the treatment, indicating a decrease of 13%. Therefore, the body weights differed significantly between the C group and 5-FU group 4 days after treatment (Figure 1A). Fecal excretion in the C group did not considerably change in the C group but decreased by 53% in the 5-FU group 4 days after treatment. Therefore, the difference in the fecal excretion between the groups was significant (Figure 1B). Food intake and water intake did not considerably change in the C group but decreased by approximately 30% 4 days after treatment in the 5-FU group, indicating that the differences between the groups in terms of food and water intake were significant (Figure 1C,D). Treatment with 5-FU decreased the food and water intake and fecal excretion, indicating that 5-FU treatment exerted systemic effects associated with decreased body weight.

Histological changes in the small intestine after treatment with 5-FU
We measured the overall lengths of the small intestines and compared the results of the mice in the C and 5-FU groups to examine the macroscopic changes after 5-FU treatment. The overall length of the small intestine was significantly shorter in the mice in the 5-FU group (average, 37 cm) than that in those in the C group (average, 41 cm) (Figure 2A).

The histologic heterogeneity of the epithelial cells of the jejunal villi of the mice in the 5-FU group increased after treatment, and most of the crypts had collapsed compared with those in the control group (Figure 2B). Similar changes were also observed in the ileum. The villi in the jejunum and ileum of the mice in the 5-FU group were approximately 17% and 66% shorter than those in the jejunum and the ileum of the mice in the C group, respectively (Figure 2C). These findings suggest that the epithelial cells of the small intestinal mucosa were injured by the 5-FU treatment.

PAS staining was performed to examine the severity of mucosal injury. As compared with the C group, the 5-FU group demonstrated decreased numbers of PAS-positive goblet cells in the jejunum and the ileum (Figure 2B). In the jejunum, the percentage of PAS-positive cells per villus was 10% in the C group, which decreased to 4% in the 5-FU group. In the ileum, the percentage of PAS-positive cells significantly decreased from 18% to 4% after treatment with 5-FU (Figure 2C). These results suggest that continuous mucosal injury after treatment with 5-FU decreased the number of goblet cells, thereby resulting in decreased defensive function of the epithelial cells.

Effects of 5-FU on the immune response in the small intestine
We compared the number of Peyer’s patches in the small intestine between the C and 5-FU groups. The mean number of Peyer’s patches in the small intestine increased to approximately 7 in the 5-FU group as compared with 5 in the C group. This difference was significant (Figure 3A). Using H&E-stained specimens, we compared the numbers of infiltrating cells in the cell proliferation zones between the groups. The area covering three villi was considered as one region, the number of infiltrating cells was counted in 10 regions, and the average value was calculated. In the jejenum, the number of infiltrating cells increased to 74 in the 5-FU group as compared with 32 in the C group. In the ileum, the number of infiltrating cells significantly increased from 32 in the C group to 55 in the 5-FU group, similar to that observed in the jejunum (Figure 3B).

The resected small intestine was evenly split at 8 locations from the proximal to the distal intestine. Gene expression in each section of the tissue was analyzed using real-time PCR. An increased number of Peyer’s patches and increased cell infiltration were observed after treatment with 5-FU (Figure 3A,B), suggesting that some type of biological defense reaction had occurred. Therefore, we focused on the dynamics of cytokines as inflammatory mediators and investigated the changes in the mRNA expression of cytokines. Irrespective of the site in the small intestine, IL-1β mRNA expression was slightly detected in the C group; however, IL-1β expression was upregulated to approximately 10 times in the 5-FU group. In particular, IL-1β mRNA expression was significantly upregulated in the ileum. Similar to IL-1β, certain levels of IL-6 and TNF-α mRNA expression were detected in the C group, irrespective of the site in the small intestine. However, IL-6 and TNF-α mRNA
Figure 3. Effects of immunological reactions in small intestinal mucosa at day 4 after treatment with 5-FU

The number of (A) Peyer’s patches and (B) infiltrated cells was counted in the small intestine. (C) mRNA expression levels of the IL-1β, IL-6, TNF-α, IL-4, IL-7, and IL-13 were determined (Control group: n = 8, 5-FU group: n = 10 for each area of intestine). 5-FU causes mucosal injury associated with inflammation in the intestine. Data are shown as mean ± SD. *P < 0.05, **P < 0.01

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expression levels were upregulated by several times in the 5-FU group and tended to increase in the distal half of the small intestine. The mRNA expression levels of IL-4, IL-10, and IL-13 did not fluctuate even after treatment with 5-FU (Figure 3C).

Treatment with 5-FU increased the number of Peyer's patches and infiltrating cells in the small intestinal mucosa and upregulated the expression levels of IL-1β, IL-6, and TNF-α, which are inflammatory cytokines. These findings suggest that treatment with 5-FU results in mucosal injury associated with inflammation in the small intestine. In addition, cytokine expression tended to be upregulated in the distal half of the small intestine, suggesting that the inflammatory reaction is greater in the ileum than in the jejunum.

Changes in amino acid transporter expression after treatment with 5-FU

The mRNA expressions of LATs in the tissue specimens of the small intestine were analyzed using real-time PCR. In the steady state (C group), a certain level of LAT1 expression was found in the small intestinal mucosa, regardless of the site of the small intestine. LAT2 expression gradually increased at the center of the small

Supplementary Figure 1. Expression of the amino acid transporter in the small intestine of naïve mice

(A) LAT1 and LAT2 gene transcriptions were analyzed by RT-PCR, and (B) protein expressions were determined by immunohistochemistry (Control group: n = 8, 5-FU group: n = 10).
intestine and then tended to decrease in the distal half (Suppl. Figure 1). After treatment with 5-FU, LAT1 expression was upregulated by approximately 20 times. In particular, LAT1 expression significantly increased in the ileum. In contrast, LAT2 expression was significantly downregulated in the 5-FU group compared to that in the C group. LAT2 expression was significantly downregulated at all the sites examined. The expression of 4F2hc, an auxiliary subunit of LATs, showed no distinct site-related trend in the small intestine in the C group but was downregulated several times throughout the entire small intestine after treatment with 5-FU (Figure 4).

Because LATs showed fluctuations in protein expression, Western blot analysis was performed. The sites of the small intestine that showed remarkable differences in mRNA expression between the groups were studied. Section no. 7 of the small intestine was used for LAT1 analysis, whereas section no. 3 was used for LAT2 analysis. After treatment with 5-FU, LAT1 expression

Figure 4. mRNA expression of the amino acid transporters after treatment with 5-FU. mRNA expression levels of LAT1, LAT2, and 4F2hc are determined (Control group: n = 8, 5-FU group: n = 10). 5-FU causes upregulation of LAT1 expression and downregulation of LAT2 expression in the small intestine. Data are shown as mean ± SD. *P < 0.05, **P < 0.01

Figure 5. Protein level of the amino acid transporter after treatment with 5-FU. Protein expression levels of LAT1, LAT2, and 4F2hc were analyzed by (A) Western blotting and by (B) immunohistochemistry (Control group: n = 4, 5-FU group: n = 4). 5-FU treatment causes upregulation of LAT1 expression and downregulation of LAT2 expression in the small intestine. Data are shown as mean ± SD. *P < 0.05, **P < 0.01
in the small intestinal tissue was upregulated by approximately 1.5 times, whereas LAT2 expression was downregulated to approximately one-fifth (Figure 5A). To examine the site of LAT expression, immunohistochemical analysis was performed using LAT antibodies. In the small intestinal mucosa of the C group mice, LAT1 was found to be expressed in the epithelial cells and the outer margin of the nuclei. In particular, LAT1 expression was confirmed in the lower half of the villi (Suppl. Figure 1). Treatment with 5-FU upregulated LAT1 expression as expected (Figure 5B). In the small intestinal mucosa of the C group mice, LAT2 expression was significantly upregulated in the lateral cell membrane and in the basement membrane of the epithelial cells in the jejunum. However, LAT2 expression was weak in the lateral cell membrane of the ileum. Instead, LAT2 expression was significantly expressed in the brush border and the apical membrane of the ileum (Suppl. Figure 1). As expected, 5-FU treatment downregulated LAT2 expression in both the jejunum and ileum (Figure 5b).

To demonstrate that the effects of 5-FU on amino acid transporter expression in the present study were not merely caused by the loss of epithelial tissue, we examined the expression of other nutrient transporters. No changes were observed in the mRNA or protein expressions of SGLT1 in the immunohistochemical analyses even after treatment with 5-FU (Suppl. Figure 2).

Our findings suggest that the 5-FU-induced injury of epithelial cells likely affects the transport of many neutral amino acids by LATs and particularly decreases the uptake of amino acids. Thus, while LAT2 expression was downregulated in the presence of inflammation, LAT1 expression was significantly upregulated. These conflicting results suggest that the up- and downregulation of LAT expression could be used as an index of mucosal inflammation in the gastrointestinal tract.

Discussion

Chemotherapy for gastrointestinal cancer can often cause adverse reactions such as inflammation of the gastrointestinal mucosa, which is associated with not only stomatitis and ulcers but also functional symptoms, e.g., nutritional and metabolic disorders. In addition, the prolonged continuation of anorexia due to factors, such as taste changes and gastrointestinal pain, often deteriorates the patient’s nutritional status and interferes with treatment. 5-FU acts by blocking thymidylate synthase, thereby terminating DNA synthesis and inhibiting cell proliferation. 5-FU also acts on intestinal epithelial cells with a high turnover, thereby destroying epithelial cells. This is one of the adverse reactions to anticancer agents. In clinical practice, methods for reducing adverse reactions as much as possible are being explored while obtaining good treatment outcomes for cancer. To date, many studies have reported on the enhancement of the efficacy of anticancer agents23,24 and on improvements in mucosal disturbances25,26; however, relatively few studies have examined the developmental mechanism of mucosal injury caused by anticancer agents. We previously reported that enteral nutrient preparations can reduce anticancer agent-induced gastrointestinal injury27,28 and speculated that disturbed uptake of nutrients in the intestine is a cause of mucosal disturbances. In the present study, we focused on molecules involved in the mechanism of nutrient uptake.
rather than that of the action of anticancer agents.

The mouse model of 5-FU-induced gastrointestinal mucosal injury demonstrated decreased body weight, suggesting malnutrition. Moreover, the villi were found to be shortened with lowered epithelial cell proliferation, and the histological architecture of the villi in the small intestine was found to be collapsed after treatment with 5-FU. Moreover, the goblet cells disappeared, suggesting decreased mucin production that led to decreased mucosal protection. Similar results were obtained in our previous study conducted using a rat model of 5-FU-induced mucosal injury. The findings in the present study suggest that some 5-FU-induced adverse effects occurred in the mechanism of nutritional intake, resulting in gastrointestinal malabsorption and decreased food intake owing to anorexia.

Apart from physical stimulation, gastrointestinal injury after treatment with anticancer agents may be caused by factors, such as abnormal immune response, decreased mucosal defense, and reduced intestinal flora. In the present study, 5-FU was administered orally; therefore, the oral cavity was first exposed to 5-FU; and, noteworthy, the pharmacological sensitivity to 5-FU was higher in the jejunum than in the ileum. Therefore, the jejunum would likely be more seriously injured than would the ileum. However, in our model of tissue injury, inflammation associated with the upregulation of IL-1β, IL-6, and TNF-α tended to be more severe in the distal half of the small intestine. The reason for this phenomenon was that immunity-related organelles, such as Peyer's patches (a component of the gastrointestinal lymphoid tissue), involved in the control of immune response to substances, such as enteric bacteria, in the intestine, are more developed in the ileum than in the jejunum. Therefore, there is a relatively higher immune response to tissue injury and a marked production of acute inflammatory proteins such as IL-1β in the ileum than that in the jejunum.

Gastrointestinal epithelial cells frequently transport various substances, such as nutrients and drugs, from the external environment and therefore express transporters that facilitate the transport of those substances. In the present study, we focused on LATs involved in the uptake of essential amino acids. First, we analyzed the mRNA expression of LATs in the small intestinal tissue of normal mice and found that LAT2 expression was upregulated in the midsection of the small intestine. In particular, the uptake of neutral amino acids, including essential amino acids, via LAT2 is thought to primarily occur in the midsection of the small intestine. Because tissue injury caused by 5-FU downregulated LAT2 expression, uptake of amino acids via LAT2 was most likely reduced.

However, LAT1 expression was upregulated by treatment with 5-FU. The upregulation of LAT1 expression in injured tissues is noteworthy and suggests that amino acid fluctuations occur particularly under inflammatory conditions. LAT1 is an inducible isoform, and high LAT1 expression is induced by lymphocyte activation and stimulation by hormones. Therefore, LAT1 is thought to be a transporter whose expression is adjusted to supply amino acids that are needed to meet the demands of cells. LAT1 expression is believed to be upregulated to compensate for the decreased amounts of amino acids transported by LAT2. However, the immunohistochemical analyses results revealed that LAT1 and LAT2 are not expressed at the same sites of the mucosa. Induction of LAT1 does not completely compensate for LAT2 and most likely adjusts the amounts of amino acids in the entire intestinal mucosa. Moreover, our previous study in a rat model with 5-FU-induced gastrointestinal mucosal injury showed that adverse reactions were alleviated by providing the rats an amino acid preparation that included essential amino acids. This finding suggests that amino acid dynamics play a role in intestinal wound healing. However, the substrate-binding site of LAT1 can bind to amino acid analogs with large side chains, such as the thyroid hormone and melphalan, besides amino acids. Therefore, we cannot conclude that the LAT1 induction observed in the present study is solely related to the promotion of the supply of amino acids.

As previously mentioned, it is clear that amino acids are intimately involved in physiological phenomena such as various metabolic signals. For example, cysteine, an amino acid derivative, suppresses body temperature elevation and inhibits inflammation. Histidine inhibits enteritis. Branched-chain amino acids are involved in inflammation and endoplasmic reticulum stress. Thus, amino acids control many biological phenomena. Therefore, amino acids that are transported via LAT, the target of the present study, may play functional roles specific to the in vivo environment.

Similar to LAT, TAT (T-type amino acid transporter) and BAT (basic amino acid transporter) exhibit extensive substrate selectivity. However, because LATs transport essential amino acids and are expressed in the blood-brain and placental barriers, thereby serving as drug transporters, we focused on LATs as target molecules that uptake various substances in the intestine in our experimental model of anticancer agent-induced mucosal injury. Because LATs are expressed in tumor cells, they can be considered as target molecules for cancer therapy.
The upregulation of LAT1 expression can be interpreted in two different ways. First, LAT1 expression may have been upregulated to supply various amino acids required to repair the injured mucosa. In that case, if the lacking amino acids are supplied, it is no longer necessary to promote the uptake of amino acids by upregulating LAT1 expression. Consequently, LAT1 expression would not be induced.

However, in the present study, we used a model in which mucosal injury was caused by continuous treatment with 5-FU. It is difficult to state that LAT1 expression occurred as an event in the repair process. In addition, because the mice had free access to a normal diet without any limitation, an extreme deficiency of essential amino acids is unlikely to have occurred even if food intake decreased.

The second factor is the mechanism that inflammatory reactions upregulate LAT1 expression. We support the latter interpretation because the expression levels of inflammatory cytokines, such as IL-1β, were markedly upregulated in the distal portion of the small intestine. The upregulation of LAT1 expression also showed a similar trend. Therefore, LAT1 expression is considered to be correlated with the severity of mucosal injury, i.e., the severity of inflammation. However, cytokines must be appropriately produced and should act at the injury site to achieve tissue repair. Given that the continuous mild invasion observed in the present model may have activated infiltrating lymphocytes and accelerated tissue repair, LAT1 induction can be considered a phenomenon associated with these findings. In other words, the required amino acids may be selected as the immune response of the living body bearing mucosal injury, and the amounts absorbed might have been adjusted. In any case, the upregulation of LAT1 expression and downregulation of LAT2 expression are phenomena observed at the time of anticancer agent-induced injury of the gastrointestinal mucosa and can be considered as indices of injury. In addition, we were interested in investigating whether inflammatory proteins, such as cytokines in the intestine, control LAT expression, and we considered the upregulation of LAT1 expression in the inflamed mucosa an interesting finding.

In the present study, 5-FU-induced injury of the small intestinal mucosa was associated with a change in the balance of amino acid absorption from the small intestine. For example, in the state of starvation with deficiency of all amino acids, LAT1 and LAT2 may act to increase blood amino acid concentrations, and both LAT1 and LAT2 may be upregulated. However, as the first step in the state of energy deficiency, rather than the aggressive intake of amino acids from food, amino acid metabolism is most likely suppressed. Further studies are warranted to examine LAT expression in other disease models and/or in the presence of inflammation caused by other anticancer agents. The dynamics of LAT observed in the present study may represent important fundamental knowledge on tissue injury caused by 5-FU, which is widely used in clinical practice. However, because the sample size used in this study is relatively low, it is interpreted that further research studies are needed to generalize these results for humans.

The causes of cancer include genetic factors, lifestyle habits (e.g., eating habits), environmental factors (e.g., exposure to hormones and other substances), and the presence of continuous local inflammation. LAT1 is intimately involved in the pathogenic mechanism of cancer, and its expression is upregulated in various cancer types. Therefore, LAT1 has been suggested as a useful tumor marker. The results of the present study suggest that LAT1 expression is upregulated even at inflammatory sites considered pre-cancerous. Thus, LAT1 can be considered a marker of gastrointestinal inflammation during chemotherapy and used as a treatment target along with anticancer drugs. LAT1 may also be useful for understanding the mechanism of carcinogenesis from the initial inflammation and may facilitate the early detection of pre-cancerous lesions. Finally, these results suggest that the amino acid balance of the body is altered at the time of mucosal injury. This deviation implies that the preferential uptake of amino acids is required for mucosal healing. Therefore, these results may contribute to the development of nutritional therapies in which amino acids required for mucosal healing are preferentially administered in clinical practice.

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References


