

Oral Administration of Cystine and Theanine Attenuates 5-Fluorouracil-Induced Intestinal Mucositis and Diarrhea by Suppressing Both Glutathione Level Decrease and ROS Production in the Small Intestine of Mucositis Mouse Model

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Abstract

Background

Chemotherapy is frequently used in cancer treatment; however, it may cause adverse events, which must be managed. Reactive oxygen species (ROS) have been reported to be involved in the induction of intestinal mucositis and diarrhea, which are common side effects of treatment with fluoropyrimidine 5-fluorouracil (5-FU). Our previous studies have shown that oral administration of cystine and theanine (CT) increases glutathione (GSH) production *in vivo*. In the present study, we hypothesized that CT might inhibit oxidative stress, including the overproduction of ROS, and attenuate 5-FU-induced mucositis and diarrhea.

Methods

We investigated the inhibitory effect of CT administration on mucositis and diarrhea, as well as its mechanism, using a mouse model of 5-FU-induced intestinal mucositis.

Results

CT administration suppressed 5-FU-induced diarrhea and weight loss in the studied mice. After 5-FU administration, the GSH level and the GSH/GSSG ratio in the small intestine mucosal tissue decreased compared to normal control group; but CT administration improved the GSH/GSSG ratio to normal control levels. 5-FU induced ROS production in the basal region of the crypt of the small intestine mucosal tissue, which was inhibited by CT. CT did not affect the antitumor effect of 5-FU.

Conclusions

CT administration suppressed intestinal mucositis and diarrhea in a mouse model. This finding might be associated with the antioxidant characteristics of CT, including the improved rate of GSH redox and the reduced rate of ROS production in the small intestine mucosal tissue. CT might be a suitable candidate for the treatment of gastrointestinal mucositis associated with chemotherapy.

Background

Although chemotherapy is an effective treatment for several types of cancer, it is also associated with a relatively high risk of adverse events [1]. Many types of anticancer drugs induce cancer cell death by inhibiting DNA and/or RNA synthesis, which suppresses cancer cell proliferation; as the rate of cancer cell division is higher than that of normal cells, chemotherapy can be selectively attack the former while sparing the latter. However, some normal tissue cells, for example, the oral and intestinal mucosa, also

divide frequently and are thus more likely to be affected by the activity of anticancer drugs, resulting in the development of adverse events such as mucositis [2].

Patients with gastrointestinal mucositis tend to develop symptoms such as abdominal pain, malabsorption, vomiting, diarrhea, ulceration, and hemorrhage [3]. Among them, diarrhea interferes with nutrient and water absorption, induces electrolyte imbalance, malnutrition, and dehydration, which reduce patients' quality of life and increase the risk of infection and sepsis. These symptoms can lead to chemotherapy dose reduction or discontinuation, potentially reducing treatment efficacy and increasing the socioeconomic cost of treatment due to its prolongation and associated hospitalization [4].

The fluoropyrimidine 5-fluorouracil (5-FU) is commonly used in the treatment of several cancer types, including head and neck, breast, prostate, pancreas, and liver cancers as well as cancers of the genitourinary and gastrointestinal systems [5].

The mechanism of 5-FU cytotoxicity has been implicated in the induction of apoptosis by inhibition of thymidylate synthase (TS) and incorporation of its metabolites into RNA and DNA [6]. Concurrently, 5-FU-induced intestinal mucositis is among the most common side effects of chemotherapy; in fact, 5-FU-induced oxidative stress, including reactive oxygen species (ROS) production [7], and the associated inflammation are risk factors for mucositis [8].

Cystine-theanine (CT) is a combination of the two amino acids at a weight ratio of 5:2. Cystine is a sulfur-containing amino acid, composed of two cysteine molecules linked via disulfide bonds. Cystine is reduced intracellularly and converted to cysteine. Meanwhile, theanine (g-glutamylethylamide), an amino acid abundant in green tea, is metabolized to glutamic acid and ethylamine in the intestine or liver [9]. Cysteine and glutamic acid are constituents of glutathione (GSH), the most abundant type of non-protein thiol that protects healthy tissues against the effects of oxidative stress [10]. Studies have shown that the availability of cysteine is the key determinant of the rate of GSH synthesis [11, 12]; in addition, CT intake has been shown to promote GSH biosynthesis [13].

Furthermore, GSH is an important determinant of redox signaling and has been shown to be essential for the detoxification of foreign bodies. In addition, it has been shown to regulate the rate of cell proliferation and apoptosis, and to contribute to the immune function and fibrosis [14].

Oxidative stress is defined as the imbalance between the rate of oxidant production and elimination [15]. GSH is a major cellular non-enzymatic antioxidant. The oxidation of GSH to disulfide (GSSG), followed by a subsequent decrease in the level of coupled GSH-GSSG is a useful indicator of cellular oxidative stress [16]. The GSH/GSSG redox state governs cell transition from the quiescent state to the proliferative state as well as growth arrest, differentiation, and apoptosis [17]. In addition, if the redox environment is highly oxidized, it promotes apoptosis or necrosis [18].

In our previous human study, we have shown that CT administration may suppress the increase and decrease in the number of neutrophils and lymphocytes, respectively, that result from heavy exercise in

athletes [19]. CT also had a tendency to reduce the incidence rate of side effects, including diarrhea and hand foot syndrome (HFS) induced by capecitabine treatment in colorectal cancer patients [20]. Using a surgical mouse model, we have also shown that perioperative CT administration may suppress inflammatory responses by inhibiting the surgically induced decrease in GSH biosynthesis in the small intestine, thus promoting recovery [21, 22].

Based on these findings, we hypothesized that CT administration could suppress the 5-FU-induced intestinal mucositis and diarrhea by limiting the decrease in GSH levels in the intestinal tissue and reducing oxidative stress, including ROS production. In this study, we investigated the inhibitory effect of CT on 5-FU-induced mucositis and the resulting diarrhea, using the mouse model of 5-FU-induced enteromucositis.

Methods

Animals

Animals: Six-week-old male BALB/c mice (Charles River Japan, Inc.) were housed under specific pathogen-free conditions with food and water ad libitum, and were kept on a 12/12-hr light/dark cycle. The mice were given a standard laboratory diet, CRF-1 (Oriental Yeast Co., Ltd., Tokyo, Japan), which contained L-methionine (0.45 g/100 g diets) and L-cystine (0.35 g/100 g diets) as sulfur amino acids (data provided by Oriental Yeast Co., Ltd.). All mouse studies were performed according to the animal experimental guidelines for laboratory issued by Animal Care Committee of Ajinomoto Co., Inc., and all of the experimental procedures were reviewed and approved by the Animal Care Committee of Ajinomoto Co., Inc. (approval number: 2017107 and 2018104).

Administration of L-cystine and L-theanine

L-Cystine (Ajinomoto Co., Tokyo, Japan) was suspended in 0.5% methylcellulose. L-Theanine (Taiyo Kagaku Co., Ltd., Yokkaichi, Japan) was dissolved in 0.5% methylcellulose. 280 mg/kg of L-Cystine and L-theanine suspension (CT: cystine 200 mg/kg, theanine 80 mg/kg) was administered orally to the mice. The control mice were administered 0.5% methylcellulose orally during the same period.

Induction of intestinal mucositis

Intestinal mucositis was induced in male BALB/c mice by a single intraperitoneal injection of 5-FU (120-150 mg/kg). 280 mg/kg of CT (cystine 200 mg/kg, theanine 80 mg/kg) or the equivalent volume of 0.5% methylcellulose was orally administered once daily, starting 3 days before 5-FU administration until the end of the experiment. Disease severity was evaluated daily by measuring body weight and diarrhea scores. Disease severity was scored using the following scale, 0: solid stool (Normal), 1: soft stool (Normal), 2: slightly wet and soft stool (Mild), 3: wet and unformed stool (Moderate), 4: watery stool (Severe).

Small intestine histology

On days 1, 3, 4, 6, and 8 after 5-FU injection, mice were euthanized, and intestinal samples were collected, fixed with 4% paraformaldehyde overnight, and embedded in paraffin. The samples were cut into 5- μ m thick sections, and stained with hematoxylin and eosin for histological analysis. The villus length and crypt depth were measured using Sensiv Measure Imager software (micro square Inc.) on images captured using an OLYMPUS BX61 microscope and an OLYMPUS DP71 camera (OLYMPUS). Ten intact and well-oriented villi and crypts were measured and averaged.

Apoptosis analysis and proliferating cell nuclear antigen immunohistochemistry

Mice were sacrificed 1, 3, 4, 6 or 8 days after 5-FU injection, and intestinal tissue samples were collected, fixed with 4% paraformaldehyde overnight, and embedded in paraffin. The samples were cut into 5- μ m thick sections. Apoptosis was detected by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) assay using the TaKaRa In Situ Apoptosis Detection Kit (TaKaRa: MK500). For each sample, the number of TUNEL-positive cells was counted from 10 crypts/mouse under a microscope, and the average value of 4-6 mice in each group was taken as the apoptosis index. Cell proliferation was determined immunohistochemically using the anti-proliferating cell nuclear antigen (PCNA) antibody (Sigma-Aldrich Inc., U.K.). The immunocomplex was visualized by TaKaRa POD conjugate anti-mouse, for mouse tissue. Sections were counter-stained with hematoxylin. The numbers of PCNA-positive cells were counted under a light microscope (BX-50, Olympus) from 10 crypts /mouse and the average value of 4-6 mice in each group was taken as the mitotic index.

Measurement of GSH and GSSG levels in small intestinal tissue

The mice were randomly assigned to 3 groups: (1) a no treatment group (normal control group: Normal Control, n=6), (2) a group receiving 150 mg/kg of 5-FU once (day 0) intraperitoneally (5-FU group: 5-FU, n=6), or (3) a group receiving 150 mg/kg of 5-FU once (day 0) intraperitoneally and 280 mg/kg of CT once daily for 4 days (day -3–day 0) orally (5-FU+CT group: 5-FU+CT, n=6). Mice were sacrificed 18 h after 5-FU injection and the jejunum was collected. One hundred mg of the collected tissue was homogenized in 0.1 ml of 5% sulfosalicylic acid and centrifuged at 8000 \times g for 10 minutes. GSH and GSSG concentrations of the supernatants were measured using the GSSG/GSH Quantification Kit (Dojindo).

Identification of ROS in the small intestine

Male Balb/c mice aged 7-9 weeks were subjected to the ligated intestinal loop experiment 18 h after 5-FU administration. The mice were fasted from the day before surgery, but they had access to water until immediately before surgery. Under inhalation anesthesia with 3% isoflurane (Mylan Inc.), midline laparotomy was performed and an intestinal loop with a 1-cm length was carefully prepared in the jejunum as to not to injure or ligate blood vessels. The Oxidative Stress Detection Reagent (2 μ M, ROS-ID Total ROS/Superoxide Detection Kit Enzo) was dissolved in saline and 150 μ L of the solution was injected into the loop by inserting a winged needle (27G x1/2 0.40 x 13 mm TERUMO) into the intestinal lumen. After removing the injection needle, the intestine was ligated on the side close to the other ligation

avoiding leakage of the solution from the hole made by the needle. Then, the skin incisions were closed using a suture staple. After leaving to rest for one hour, the abdomen was re-opened, the small intestine was excised, and the loop region was taken out and washed with saline. The opened intestine was embedded in O.C.T. compound and 10- μ m thin frozen sections were prepared. The sections were immediately observed under a fluorescence microscope and photographed, and 3 visual fields were selected per animal and ROS-positive crypts were counted.

Anti-tumor efficacy of 5-FU in tumor-implanted mice.

CT26 tumor (a murine colon cancer cell line created in Balb/c mice)- bearing mice were prepared by the following methods. CT26 cells (1×10^6 each) were transplanted subcutaneously into the back of BALB/c mice under light isoflurane anesthesia and, 10 days later, these mice intraperitoneally received 5-FU at 150 mg/kg. From day 8 post transplantation, CT(280mg/kg) or vehicle (Methylcellulose) was orally administered once daily for the consecutive 10 days. On day 18, mice were sacrificed and measured tumor weight.

Statistical analyses.

All data were expressed as the mean \pm SEM. The Prism version 7.05 software package (Graph pad software, CA, USA) was used for a two-tailed Student's t-test (Fig.2 b and Fig.3a,b), one-way ANOVA followed by the Tukey test (Fig.4), Mann-Whitney test (Fig.1 b and Fig.5 b), Dunn's test (Fig.1 a), or Dunnett's test (Fig.6) as a multiple comparison test, with P-values < 0.05 regarded as significant.

Results

In the 5-FU group, body weight loss occurred already the day after treatment administration; the mean body weight decreased by approximately 82.3% (from 25.4 g to 20.9 g) by day 8 of the study. Diarrhea was observed from day 5 after 5-FU administration; the diarrhea score reached a plateau (mean score, 2.25 ± 0.86) between days 6 and 8. CT (280 mg/kg) was orally administered once daily, starting 3 days before the administration of 5-FU. In the 5-FU + CT group, the rate of body weight loss tended to improve after day 3 from the administration of 5-FU and after day 6, a statistically significant difference of body weight loss was found compared with the 5-FU group ($p < 0.05$; Fig. 1a). Concurrently, the diarrhea score did not increase in the CT treatment group after day 6; and, a statistically significant difference was observed between the treatment groups on days 6 and 7 (both $p < 0.05$; Fig. 1b).

CT impact on 5-FU-induced damage in the small intestine

The small intestine villus on day 6 after 5-FU (120 mg/kg) administration is shown in Fig. 2a. The villus length of the 5-FU group was substantially shorter than that of the normal control group; however, the degree of this shortening was relatively reduced in the 5-FU + CT group. Time-dependent changes in the length of the small intestine villi are shown in Fig. 2b. The length of the villi in the 5-FU group was approximately 30% shorter than that in the control group on day 3; although it subsequently increased, it

failed to return to its baseline length (mean of normal control). Similarly, in the 5-FU + CT group, the length of the intestinal villi decreased by 24% on day 3, which was similar to the decrease observed in the 5-FU group; however, the villi recovered to 86% and 100% of their baseline length (mean of normal control) on days 4 and 6, respectively. The small intestine villus length of the 5-FU + CT group was significantly longer than that of the 5-FU group on days 4 and 6 (both $p < 0.05$)

Impact of CT on the small intestine crypt cell apoptosis and proliferation

In our experiments, on days 1 and 4 following 5-FU administration, TUNEL-positive cells were observed in the 5-FU group. In addition, the number of TUNEL-positive cells observed in this group was greater on day 1 than on day 4. Meanwhile, the number of TUNEL-positive cells in the 5-FU + CT group was not significantly different from that in the 5-FU group on day 1; however, it significantly decreased on day 4 ($p < 0.05$; Fig. 3a). Concurrently, 5-FU administration reduced the number of PCNA-positive crypt cells by 21% compared to that observed in the normal control mice. Significant improvement was seen in the 5-FU + CT group compared to the 5-FU group on day 3 ($p < 0.05$), and the number of PCNA-positive cells peaked on day 4 (Fig. 3b).

Impact of CT on glutathione production in the small intestine

The levels of oxidized and reduced glutathione levels (GSSG and GSH, respectively) in the small intestine villi were measured 18 h after 5-FU administration. In the 5-FU group, the GSH level was significantly lower than that in the normal control group ($p < 0.001$). This reduction in GSH level tended to recover with CT administration (Fig. 4a). However, no significant differences were observed in GSSG levels among the groups (Fig. 4b). While as shown in Fig. 4c, 5-FU administration significantly decreased the GSH-GSSG ratio compared to the normal control group ($p < 0.01$), CT treatment returned it to its normal control level.

Impact of CT on ROS production in the small intestine

To investigate the influence of CT on ROS production in the small intestine, tissue ROS was visualized using an ROS-specific fluorescent probe. In the 5-FU group, ROS-positive crypts were observed in the basal region of crypts 18 h after administration (Fig. 5a). In the 5-FU + CT group, the number of ROS-positive crypts were decreased compared with that in the 5-FU group ($p < 0.01$), suggesting that CT induced the scavenging of ROS (Fig. 5b).

Impact of CT on 5-FU antitumor effects

The induction of oxidative stress, including the production of ROS, in tumor tissue contributes to the antitumor effects of several anticancer drugs; thus, the effect of CT administration on the antitumor action of 5-FU was investigated using a subcutaneous transplant model of CT26, which is a mouse colon cancer. The tumor weight was significantly reduced in the 5-FU and in the 5-FU + CT groups compared

with the CT26 transplanted control group (both $p < 0.01$; Fig. 6). However, no significant difference in tumor weight was observed between the 5-FU and 5-FU + CT groups.

Discussion

Our study showed that CT administration reduced diarrhea in a mouse 5-FU-induced mucositis model. The results of our study are consistent with our previous human studies, CT broadly alleviate the adverse effects of chemotherapy, including diarrhea in cancer patients, thereby reducing the incidence of withdrawal / discontinuation of chemotherapy and increasing the treatment completion rate [23]. CT administration reduced the shortening of small intestinal villi and the destruction of crypts, which are the morphological features of 5-FU-induced mucositis. In addition, CT administration improved the GSH / GSSG ratio to normal control levels and inhibited 5-FU-induced ROS production in intestinal tissue. This suggests that CT administration suppressed mucositis by improving the redox state, resulting in suppression of diarrhea.

In the mucositis model used in this study, 5-FU administration induced and inhibited villus stem cell apoptosis and proliferation, respectively, resulting in the shortening of the small intestine villus length. Body weight loss occurred already on the day following 5-FU administration, suggesting that its underlying mechanism is associated with 5-FU toxicity rather than diarrhea. The intestinal villus length and crypt cell proliferation rate decreased from the day after 5-FU administration, with and without CT administration. The recovery of the small intestine villus length on day 4 in the 5-FU + CT group was consistent with the improved rate of cell proliferation observed on day 3. In particular, in the 5-FU + CT group, the PCNA-positive cell number/crypt value exceeded that of the normal control tissue on days 3 and 4, suggesting the presence of hyperplasia associated with oxidative colitis [24]. CT administration promoted the proliferation of crypt cells and repaired the damage caused by 5-FU to the small intestine villus tissue. These results indicate that the inhibitory effects of CT on 5-FU-induced diarrhea may be due to the restoration and protection of the small intestinal villus tissue, which is achieved by the induction of crypt cell growth.

5-FU is an antimetabolite anticancer drug, while the inhibition of DNA/RNA synthesis is considered the key factor in the induction of apoptosis. Recently, it has been reported that 5-FU-induced ROS production leads to apoptosis [25]. In our study, 5-FU increased the number of apoptotic cells in the intestinal crypt on days 1 and 4, but CT administration did not suppress apoptosis on day 1. In fact, CT could not suppress apoptosis in the acute phase mediated by apoptosis-related factors such as inhibited DNA/RNA synthesis. In contrast, the second peak of the number of apoptotic cells that appeared on day 4 was suppressed by CT administration. Since the contribution of 5-FU remaining in the body is expected to decrease on day 4 after 5-FU administration, the apoptosis observed on day 4 might have occurred due to the effect of ROS rather than due to the direct effect of 5-FU. These results are supported by a previous report showing that the second peak of the number of 5-FU-induced apoptosis observed on day 3 was significantly lower in Nox1KO mice in which ROS production was suppressed by Nox1 deficiency [25].

In the present study, GSH and GSSG levels were measured at 12 to 24 hours after 5-FU administration. 5-FU administration reduced GSH levels in the small intestine tissue, where it tended to increase with CT administration. In addition, CT administration returned the GSH/GSSG ratio to normal levels. These results suggest that CT has the ability to improve oxidative stress in the small intestine tissue. The growth of crypt cells (Fig. 2b) and the rapid recovery of villus length (Fig. 3b) might have resulted from the promotion of GSH production due to the continuous CT administration and the accompanying improvement in the redox environment of the small intestine tissue. We initially hypothesized that a CT dose of 280 mg/kg, which was shown to reduce the rate of diarrhea in animal studies, would significantly increase GSH levels. However, no significant increase in GSH levels was observed upon CT administration. We speculate that the levels of GSH did not increase after CT administration because they were oxidized immediately after production at the inflamed site.

ROS are produced in small amounts in the body and are involved in cell regulation, homeostasis maintenance, and functions such as signal transduction, gene expression, and receptor activation [26]. However, excess production of ROS causes cell dysfunction such as loss of energy metabolism, disruption to cell signaling, and gene mutations, promoting reduced biological activity, immune activation, and inflammation [27]. In the present study, we used a ROS-specific fluorescent probe to investigate ROS production as early as 18 hours after 5-FU administration. After 5-FU administration, a strong ROS signal-positive crypt was detected in the mucosal tissue of the small intestine. These signals were reduced by CT administration, suggesting that CT may inhibit ROS production. The suppression of cell dysfunction associated with reduced ROS production and concurrent tissue protection might be the mechanisms underlying CT mucositis and diarrhea symptom reduction. Sonis's a five-stage process of pathobiology of mucositis supports our data and ideas. They proposed the biological sequence of mucositis can be divided into five phases including initiation, signal transduction, amplification, ulceration, and healing, and these biological events occur sequentially. [28, 29]. In addition, chemotherapy-induced DNA damage and ROS production are two characteristic events at the initiation phase [30, 31] and suppressing the initiation step is most effective in reducing mucositis [32]. CT might be thought to have suppressed mucositis by suppressing the production of ROS and stopping the biological sequence of mucositis at the initiation phase. Furthermore, We hypothesis that the attenuation of ROS production by CT administration may have suppressed the progression to the second and third steps of the five-stage process. However, further studies should examine the inhibitory effects of CT on the activation of transcription factor and inflammatory cytokine production in this process to verify this hypothesis.

CT may suppress oxidative stress by promoting intracellular GSH production, thus suppressing the antitumor effect of 5-FU. Therefore, the influence of CT administration on the antitumor activity of 5-FU was examined using a tumor-transplanted mouse model. Treatment with 5-FU strongly prevented the growth of subcutaneously implanted tumors in the study mice. However, the administration of CT did not affect the antitumor activity of 5-FU (Fig. 6). For normal cells, the amount of GSH produced by CT administration was sufficient to compensate for the decrease in GSH production caused by 5-FU administration. This finding suggests that CT administration might have shifted the redox environment of

the tissue from the oxidized form to the reduced form, preventing extensive tissue damage. However, the amount of GSH present in cancer cells is known to be larger than that present in normal cells [33]. It is plausible that CT administration did not affect the antitumor effect of 5-FU because the amount of GSH promoted by CT administration was insufficient to affect cancer cells.

Conclusion

In conclusion, our results indicate that CT administration suppresses 5-FU-induced mucositis by improving the glutathione redox environment and inhibiting ROS production in the small intestine, resulting in the alleviation of diarrhea. Concurrently, CT does not reduce the antitumor effects of 5-FU. Overall, our results suggest that the intake of CT, the source of antioxidant GSH, may be a promising supportive care for patients undergoing chemotherapy.

List Of Abbreviations

ROS, Reactive oxygen species

5-FU, 5-fluorouracil

CT, cystine and theanine

GSH, glutathione

GSSG, Glutathione-S-S-Glutathione

TUNEL, TdT-mediated dUTP nick end labeling

TS, Thymidylate synthase

PCNA, Proliferating cell nuclear antigen

HFS, Hand foot syndrome

Declarations

Ethics approval and consent to participate

a. All animal experimental procedures were reviewed and approved by the Anima Care Committee of Ajinomoto Co., Inc. (2017107 and 2018104)

b. All studies were carried out in compliance with the ARRIVE guidelines (<https://arriveguidelines.org>) for the reporting of animal experiments.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

Amino Acids Cystine and Theanine is a supplement manufactured and sold by Ajinomoto Co., Inc. All authors are employee thereof at the time the study was performed. The authors declare no other competing interests.

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Authors' contributions

All authors contributed to the study conception and design. J.Y. performed material preparation, data collection and analysis and S.N. contributed statistical analysis. S.K. contributed to laboratory assessment. The first draft of the manuscript was written by J.Y., S.N. & S.K. commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

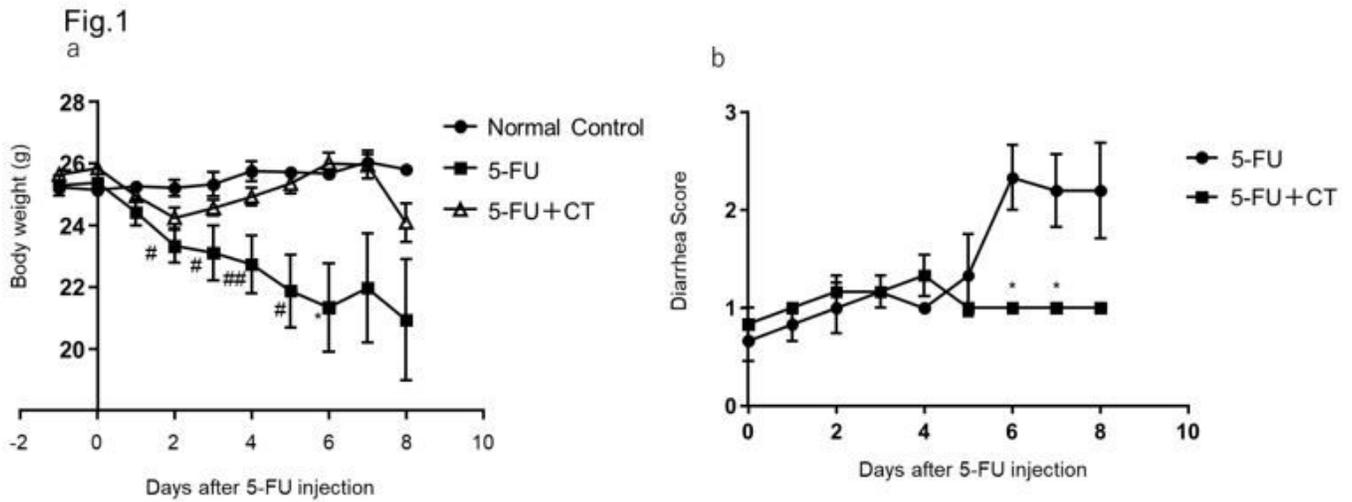
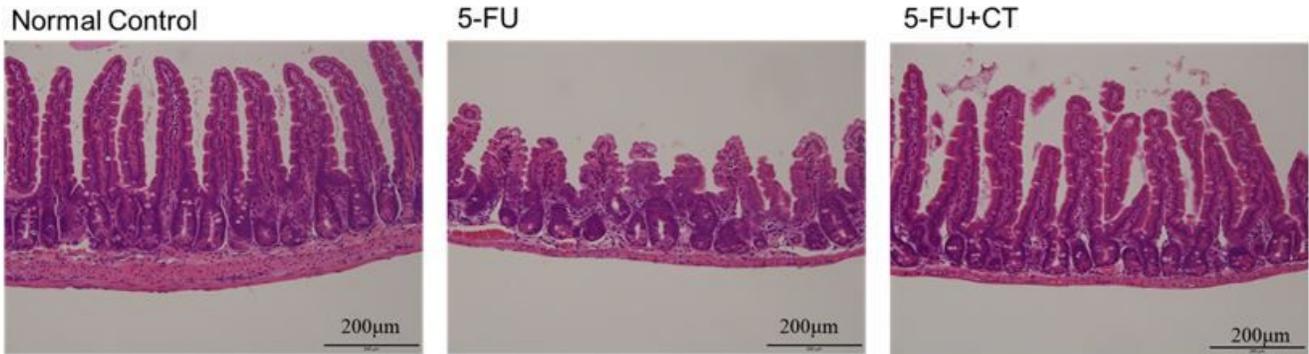


Figure 1

Effects of CT on body weight change and diarrhea development in 5-FU-induced small intestinal mucositis model mice. a) Time-course changes in body weight. Values are expressed as the mean \pm SEM. N=5–6. #p < 0.05 vs normal control, ## p < 0.01 vs normal control, *p < 0.05 vs. 5-FU+CT by Dunn’s Multiple comparison tests b) Diarrhea score: Diarrhea symptoms were observed every day and the severity of diarrhea was scored using the following scale, 0: solid stool (Normal), 1: soft stool (Normal), 2: slightly wet and soft stool (Mild), 3: wet and unformed stool (Moderate), 4: watery stool (Severe). Values are expressed as the mean \pm SEM. N=5–6. * p<0.05, compared with 5-FU by the Mann-Whitney U test

Fig.2

a



b

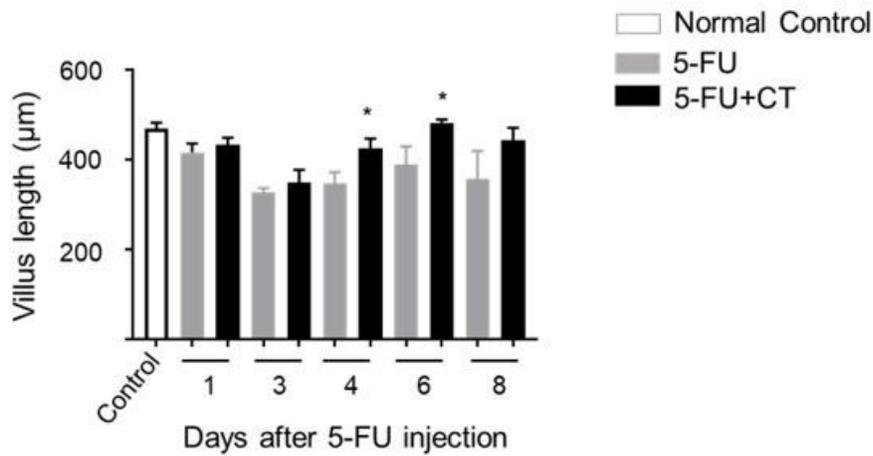


Figure 2

Small intestinal villus-protecting effects of CT in 5-FU-induced small intestinal mucositis model. a) Typical small intestinal villus tissue in each group. The tissue was excised on day 6 after 5-FU administration and subjected to hematoxylin-eosin staining. b) Time-course changes in small intestinal villus length (from the top of villus to the villus-crypt junction). H/E-stained small intestinal villus tissue was observed under a light microscope. Ten villi with relatively normal morphology were selected per animal, the villus length was measured, and the mean was regarded as individual mice data. Values are expressed as the mean \pm SEM. N=5-6. * $p < 0.05$ vs 5-FU by the two-tailed Student's t-test

Fig.3

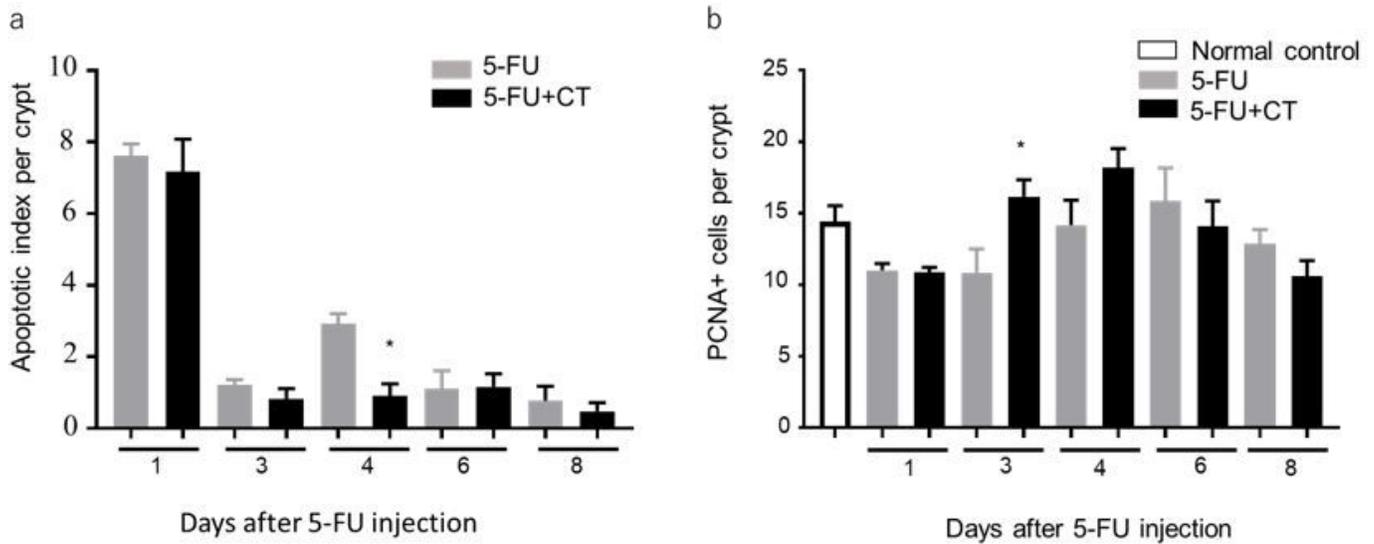


Figure 3

Effects of CT on the induction of apoptosis and proliferation of small intestinal crypt cells in 5-FU-induced small intestine mucositis model mice. a) Apoptosis was evaluated by the TUNEL assay. TUNEL-positive cells were counted in 10 small intestinal crypts per specimen under a light microscope. Values are expressed as the mean \pm SEM. N=5-6.*p < 0.05 vs 5-FU by the two-tailed Student's t-test. b) Cell proliferation activity was immunohistochemically measured using the anti-PCNA antibody. PCNA-positive cells were counted in intestinal crypts under a light microscope. Values are expressed as the mean \pm SEM. N=5-6.*p < 0.05 vs 5-FU by the two-tailed Student's t-test

Fig.4

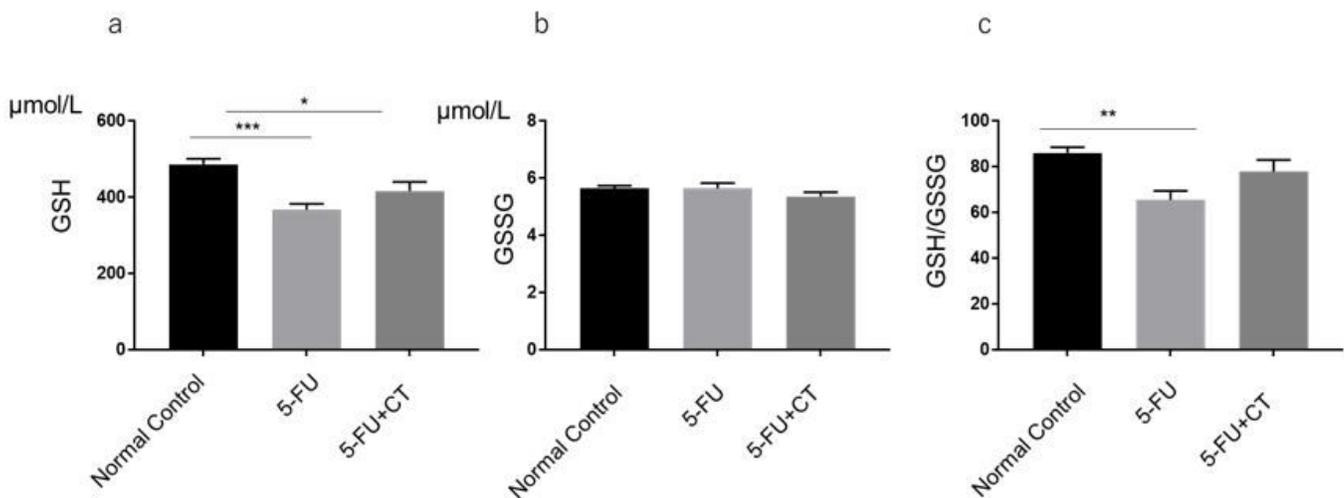


Figure 4

Fig.6

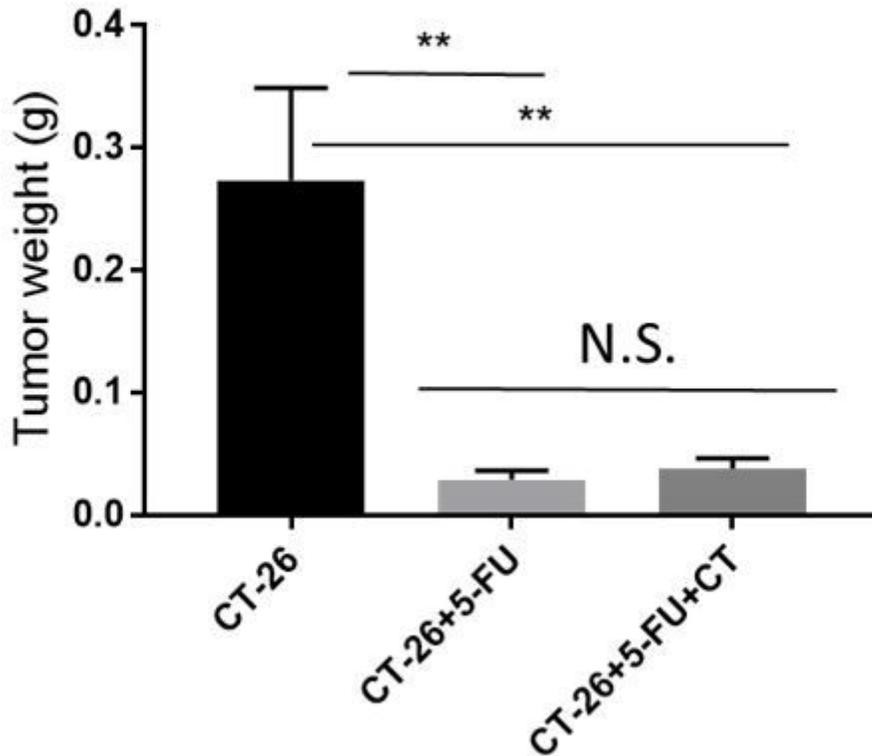


Figure 6

Influence of CT on the antitumor efficacy of antitumor drug in a tumor-bearing mouse model. CT26 murine colon cancer cells (1×10^6) were subcutaneously transplanted into male BALB/c mice. From Day 8 post transplantation, mice were orally administered CT (280mg/kg) or methyl cellulose (the vehicle for CT) once daily for 10 days. The mice received 5-FU (150 mg / kg , i.p.) on day 10 after transplantation. The data is presented as the mean \pm SEM of 6 mice. CT26 group vs CT26+5-FU and CT26 group vs CT26+5FU+CT group: ** < 0.01. CT26+5-FU group vs CT26+5-FU +CT group: No significant difference. (Dunnet's multiple composition test)