Green Tea Polyphenol Administration Partly Ameliorates Chemotherapy-Induced Side Effects in the Small Intestine of Mice

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Abstract
The chemotherapeutic agent irinotecan (IT) is highly effective against several types of cancer, although its use is limited due to severe intestinal toxicity. The aim of this study was to evaluate inflammatory and oxidative stress-related processes contributing to small intestinal mucosa damage and to determine the extent to which green tea polyphenols could ameliorate the detrimental effects induced by IT. In Expt. 1, mice were challenged intraperitoneally with IT or saline on 2 consecutive days. For time kinetic measurements, the IT-treated mice were killed at 3, 24, 48, 72, and 96 h after the 2nd dose of IT. Three hours after IT administration, the ileum glutathione concentration dropped significantly. Lipid peroxidation and inflammation, as measured by macrophage inflammatory protein-2 content, myeloperoxidase activity, and nuclear factor-κB translocation, were highest between 24 and 48 h after IT treatment. In Expt. 2, green tea polyphenols (1 g/L) were supplied via drinking water for 7 d before and 3 d after treatment with IT. Green tea polyphenols significantly affected the glutathione:glutathione disulfide ratio but not lipid peroxidation, macrophage inflammatory protein-2 levels, myeloperoxidase activity, or nuclear factor-κB activation. Our study reveals that IT administration is associated with oxidative stress and inflammation, both occurring simultaneously to IT-induced mucosal damage. The antioxidative defense is affected soon after IT administration. Green tea polyphenols supplied orally protected against oxidation in our experimental model and could be one approach to reducing the risk of IT-induced side effects in the clinical setting. J. Nutr. 137: 634–640, 2007.

Introduction
The treatment of cancer using chemotherapeutic agents is often accompanied by side effects caused by the occurrence of oxidative stress. Enhanced lipid peroxidation, reduction of antioxidant vitamins, free radical trapping capacity in plasma, and a marked reduction of tissue glutathione (GSH)1 levels are frequently detected during chemotherapy (1–3). The overwhelming production of reactive oxygen species (ROS) damages healthy tissues and is therefore considered to be one of the causes for the toxic side effects of chemotherapeutic agents. In particular, tissue and cells with a high proliferation rate, such as the epithelium of the gastrointestinal tract, the bone marrow, and hair follicles are especially affected by the oxidative insult (4). Furthermore, ROS generated during cancer chemotherapy may also diminish the efficacy of the treatment by interfering with cellular processes, such as cell cycle progression and drug-induced apoptosis, which are important for chemotherapeutic agents to exert their optimal effect on cancer cells (5,6).

Camptothecin is a well-described alkaloid isolated from the bark and wood of the Chinese tree Camptotheca acuminata. The semisynthetic and water-soluble derivative irinotecan (IT) and its active metabolite SN-38 display cytostatic activity by preventing the enzyme DNA topoisomerase I to release the torsional strain occurring during DNA replication, which finally leads to double-strand DNA breaks and cell death (7). Its main field of application is the treatment of metastatic colorectal cancer, where IT is used after the failure of 5-fluorouracil chemotherapy or as first-line treatment together with 5-fluorouracil and leucovorin (8,9). Unfortunately, the application of IT is limited by the induction of gastrointestinal toxicities, such as severe late-onset diarrhea and the destruction of the intestinal epithelium, which is accompanied by the production of proinflammatory cytokines like tumor necrosis factor-α (TNF-α) and the infiltration of leukocytes (10). Several immuno-modulating substances are currently being investigated to overcome treatment-associated intestinal toxicity. The oral administration of RDP58, a D-isomer decapeptide with potent anti-inflammatory activity, allows for doubling of the maximum tolerated dose in tumor-bearing mice leading to enhanced efficacy of IT (11). A novel but controversial strategy to prevent

1 Abbreviations used: EGCG, epigallocatechin-3-gallate; GSH, glutathione; GSSG, glutathione disulfide; GTP, green tea polyphenols; IT, irinotecan; MIP-2, macrophage inflammatory protein-2; MPO, myeloperoxidase; NF-κB, nuclear factor-κB; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances; TNF-α, tumor necrosis factor-α.

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chemotherapy-induced toxicity relies on the administration of antioxidants to reduce side effects caused by the occurrence of oxidative stress (12,13). Plant polyphenolic compounds like green tea flavanoids display significant antioxidant as well as anti-inflammatory activities by directly scavenging ROS or by influencing signaling pathways, such as the nuclear factor κB (NF-κB) pathway (14). Furthermore, green tea polyphenols (GTP) have attracted interest because of their potency as a cancer chemopreventive and cancer therapeutic agent (15,16). Interestingly, it has been shown that 25% of patients with prostate carcinoma resort to nonprescription (so-called alternative) therapies, including green tea and green tea extracts (17). Gastrointestinal toxicity can be regarded as the main and dose-limiting side effect during cancer treatment with IT and remains an unresolved problem. Therefore, an understanding of the mechanisms leading to the toxicity of chemotherapeutic agents on healthy tissue is of interest in attempting to ameliorate side effects without affecting the anti-tumor capacity of these agents. Our study aimed to develop strategies for preventing the intensity of side effects by evaluating the extent to which inflammatory as well as oxidative stress-related processes were induced in IT-treated mice. As GTP are known to exert anti-inflammatory effects, we investigated whether GTP have the potential to serve as useful nutritional supplements during IT chemotherapy to counteract detrimental effects to the small intestine.

Materials and Methods

Diets and treatment. Female BALB/c mice (6–8 wk of age, weighing 15–20 g) were obtained from Charles River Laboratories. In Expt. 1, mice were randomly assigned to 3 groups. After an adaptation period of 7 d, group 1 was challenged intraperitoneally on 2 consecutive days with 0.17 g IT-hydrochlorid-trihydrat (Campto; Aventis)/kg body weight. At the same time points, group 2 and 3 (controls) received equal amounts of physiological saline (Fresenius) with the difference that group 3 (pair-fed control) was restricted to the mean food intake of group 1 (IT) (Fig. 1A). All mice were fed a commercial pelleted maintenance diet (Altromin 1324 FORTI, Altromin GmbH) with the following proximate composition (g/100 g): crude protein, 19; fat, 4; fiber, 6; mineral, 7; carbohydrates, 50; water, 14 (18). For time kinetic measurements, IT-treated mice (5 mice/time point) were killed by cervical dislocation at 3, 24, 48, 72 and 96 h after the 2nd dose of IT. Control mice were killed immediately after the 2nd dose of saline.

In Expt. 2, mice were randomized into 4 treatment groups. For 10 d overall, group 1 (water/saline) and group 2 (water/IT) had free access to normal drinking water, whereas group 3 (GTP/saline) and group 4 (GTP/IT) had free access to drinking water containing 1 g/L GTP. GTP was a decaffeinated, water-soluble extract from Camellia sinensis (Sunphenon DCF-2; Taiyo Kagaku) containing 85% polyphenols by weight. The polyphenols comprise epigallocatechin gallate (47.2% of the total polyphenols), epigallocatechin (11.0%), gallocatechin gallate (11.0%), epicatechin gallate (10.8%), gallocatechin (8.6%), epicatechin (8.4%), and catechin (3.0%). Mice were fed with Altromin 1324 standard maintenance diet, whereby group 1 (water/saline) was restricted in food intake to the mean of group 2 (water/IT) and group 3 (GTP/saline) received the mean food intake of group 4 (GTP/IT). After the prefeeding period of 7 d, mice of groups 2 and 4 were challenged intraperitoneally with 0.17 g IT/kg body weight on 2 consecutive days, whereas mice of groups 1 and 3 received saline. All mice were killed 48 h after the 2nd dose of IT or saline (Fig. 1B).

In both experiments, measurements of body weight and food intake were performed daily during the study period. At the times mice were killed, the ileal part of the small intestine was removed, rinsed with cold physiological saline, frozen, and stored in liquid nitrogen until further analysis (unless otherwise indicated). This study was carried out in accordance with NIH guidelines on the use of laboratory animals, with the approval of the Austrian Federal Ministry for Education, Science, and Culture, and upon the recommendation of the committee overseeing experimentation on animals located at the Institute of Biomedical Research at the Medical University of Vienna, Austria.

Histological examinations. A tissue sample was cut from the ileum, the middle of the 2nd-most distal small intestinal section of each animal. After rinsing the samples with cold physiological saline, they were placed in Tissue-Tek OCT Compound (Sakura Finetek, Zoeterwoude), embedded in 1-butyl ethanol, frozen in liquid nitrogen, and stored at −80°C. The tissue samples were cut using a cryostat microtome, and the slices were placed on SuperFrost microscope slides (Menzel-Gläser). Air-dried at room temperature, and stained with hematoxylin and eosin on the following day. The stained samples were studied under a light microscope (Nikon Mikrophot-FXa) at 20× magnification.

NF-κB translocation. Preparation of nuclear extracts and measurement of NF-κB by electrophoretic mobility shift assay was performed as described previously for neural tissue (19). Quantification of radioactive bands in dried gels was performed on a Molecular Imager FX Pro Plus Phosphor Imager using the Quantity One software, version 4.2.2 (BioRad). Density of bands was expressed as adjusted volume × count × mm² (AV × CNT × mm²).

Macrophage inflammatory protein-2. Small intestinal tissue was homogenized in Tris buffer (10 mmol/L; pH 7.4) containing 1 mmol/L EDTA, 0.5 g/L sodium azide, 10 mL/L Tween-80 and a protease inhibitor cocktail (P8340, Sigma Chemicals). Macrophage inflammatory protein-2 (MIP-2) concentrations were quantified using a commercially available enzyme immunoassay (Quantikine, Mouse MIP-2 Immunoassay, MM200, R&D Systems).

Myeloperoxidase activity. Tissue samples were homogenized in potassium phosphate buffer (20 mmol/L; pH 7.4). After centrifugation of the homogenates (21,000 × g; 20 min at 4°C), the remaining pellet was suspended in potassium phosphate buffer (50 mmol/L; pH 6.0) containing 5 g/L hexadecyltrimethyl-ammonium bromide (Sigma Chemicals). Myeloperoxidase (MPO) activity was assessed using the o-dianisidine method as described previously (20). The reaction was performed on a microtiter plate, and the change in absorbance at 450 nm was tracked for 5 min on a Wallac 1420 Victor 2 multilabel plate reader (PerkinElmer Life and Analytical Sciences). MPO activity was expressed as the change in absorbance/(min · g wet tissue).

Figure 1 Experimental overview to study the effects of IT in Expt. 1 (A) or green tea polyphenols in Expt. 2 (B) administered to mice.

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Determination of intestinal GSH by HPLC. For GSH determinations, the samples were homogenized in 65 g/L sulfosalicylic acid solution (Merck). The supernatant was stored at -80°C until further analysis. The separation of low molecular mass thiol-bimane adducts was achieved by HPLC as described previously (21). The resultant profiles were quantified on the basis of peak areas and compared with those of authentic external standards of GSH.

Enzymatic determination of intestinal GSH and GSSG. Levels of GSH and its oxidized form glutathione disulfide (GSSG) were determined using the Calbiochem GSH/GSSG Ratio Assay Kit (Calbiochem) with some modifications in sample preparation. Small intestinal samples were homogenized in cold 100 g/L m-phosphoric acid (Fluka). After allowing the mixture to stand for 5 min at room temperature, homogenates were centrifuged (10,000 × g; 10 min at 4°C). Supernatants were frozen at -80°C until further analysis (stable for 6 mo). Neutralization was achieved by adding 5 μL of triethanolamin (4 mmol/L, Sigma Chemicals) to a 100 μL sample. For GSSG analysis, 5 μL of the thiol-scavenging reagent, 1-methyl-2-vinylpyridinium trifluoromethanesulfonate, was added to 25 μL of sample and incubated for 10 min at room temperature. Then both GSSG and GSH (GSH + GSSG) samples were diluted using the provided phosphate buffers and measured following the manufacturer’s instructions.

Lipid peroxidation. Samples were homogenized in 11.5 g/L potassium chloride (Merck). To measure the concentration of thiobarbituric acid reactive substances (TBARS), we followed the method of Mihara et al. (22) with slight modifications. Therefore, tissue homogenate, n-phosphoric acid (Merck), thiobarbituric acid (Sigma Chemicals), butyrylated hydroxytoluene (Sigma Chemicals), and EDTA (Gibco BRL) were heated on a boiling water bath for 45 min, cooled down on ice, and extracted with n-butanol (Merck). The butanol phase was separated by centrifugation (15,000 × g; 15 min at 4°C), and absorbance at 535 nm was measured using a U-3000 spectrophotometer (Hitachi High Technologies). Absorbances were converted to nmol malondialdehyde/g wet tissue using increasing concentrations of 1,1,3,3-tetramethoxypropane (Sigma Chemicals) to yield a standard curve.

Statistical analysis. Values are means ± SD. Data were analyzed using the SPSS software package (SPSS for Windows, version 10.0.0). When necessary, data were log-transformed to achieve stabilized variance. In Expt. 1, differences in body weight between the 3 groups were tested separately at time points −1, 0, 1, 2, 3, and 4 d after the 2nd dose of IT using 1-way ANOVA followed by Tukey post-hoc analysis. Time effects in each group were measured using repeated measures ANOVA (Dunnett’s t test) with comparisons made to time point −1. Thus, a total of nine 1-way ANOVA were conducted. To control for the experiment-wise error, the level for each 1-way ANOVA was set at 0.005 so that the overall experiment-wise error was 0.005 × 9 = 0.045, which is < 0.05. For all other variables, examining only time effects, P < 0.05 was considered statistically significant. For examining time (4 time points) as well as treatment effects (4 groups) on body weight in Expt. 2, the level for each 1-way ANOVA was set at 0.005 so that the overall measure of error was < 0.05. For all other variables of Expt. 2, a 2-way ANOVA was performed regarding GTP and IT as factors. Tukey’s post-hoc analysis was performed when the interaction (GTP × IT) was significant.

Results

Time course of IT effects and related toxicities (Expt. 1)

Daily food intake and body weight. The normal food intake of healthy control mice was 2.8 ± 0.1 g throughout the study period. Administration of IT reduced daily food intake from 2.8 ± 0.1 g to 1.0 ± 0.1 g (−64% of d −1, P < 0.001) and remained at this level until d 3 after the 2nd application of IT. On d 4, food intake returned to normal levels. Food of pair-fed control mice was artificially restricted to these amounts. A fall in body weight accompanied the diminished ingestion of food in IT-treated and pair-fed control mice, which was most pronounced on d 3 (IT: −16% of d −1, P < 0.001; pair-fed: −11% of d −1, P < 0.001)

Mice regained weight but did not return to their normal weight within the observation period (Fig. 2A).

Histological examination. As we observed a steady decrease in body weight up to the 3rd day after the 2nd dose of IT, 24, 48, and 72 h were chosen to perform histological examinations. The treatment of mice with IT led to a massive destruction of small intestinal (ileal) mucosa that was most pronounced 48 h after the 2nd dose of IT. Pathological changes include villus shortening, massive destruction of epithelial layer, and cellular debris. With respect to visible signs of inflammation, such an enhanced infiltration of neutrophils, only minimal differences between controls and IT-treated mice could be detected (Fig. 3).

Temporal sequence of inflammatory processes. Similar to the human IL-8, mouse macrophage inflammatory protein-2 (MIP-2) exhibits potent neutrophil chemotactic activity and may be a key mediator of neutrophil recruitment in response to tissue injury and infection. MIP-2 levels in small intestinal tissue were enhanced from undetectable levels to 11.3 ± 7.1 μg/mg protein (P < 0.001) 24 h after IT administration. Thereafter, MIP-2 concentration showed a steady decline but did not reach control values on d 4 (P < 0.05) (Fig. 4A).

Enhancement of MIP-2 activity was used to quantify the infiltration of neutrophils into the small intestine. The time response of MIP-2 activity was parallel to that of MIP-2 concentrations and showed a maximal increase 24 h after the 2nd dose of IT (+106%, P < 0.001). MIP-2 activity stayed above baseline values at 48 and 72 h (+58% and +55%, P < 0.01), but returned to control levels on d 4 (Fig. 4B).

The influence of IT on activation of the redox-sensitive transcription factor NF-κB was studied because NF-κB plays a...
critical role in regulating gene expression of cellular mediators involved in inflammatory and oxidative stress-induced processes. The administration of IT caused a marked NF-κB activation in the ileum 24 h after the 2nd dose (194 6 9% of time 0 h, P < 0.05), reaching its maximal activation at 48 h (298 6 21% of time 0 h, P < 0.01) and not returning to control levels within 96 h (235 6 19% of time 0 h, P < 0.05) (Fig. 4C). Interestingly, maximal NF-κB activation was delayed when compared with the highest increase of MIP-2 concentrations and MPO activity.

Modulation of oxidative stress variables. The measurement of GSH representing the main intracellular antioxidant and the determination of lipid peroxidation as a marker for oxidative tissue damage were chosen as variables to evaluate oxidative stress induced by IT administration. GSH concentration in ileal samples decreased as early as 3 h after treatment with IT (−35%, P < 0.01), but returned to control levels within 24 h (Table 1).

IT administration increased lipid peroxidation as shown by measurement of thiobarbituric reactive substances (TBARS). Forty-eight hours after the 2nd dose of IT, TBARS of ileal samples were enhanced (+26%, P < 0.05), whereas at 96 h, the TBARS concentration was less than that of control mice at 0 h (−28%, P < 0.05) (Table 1).

Anti-inflammatory and antioxidative effects of green tea polyphenols (Expt. 2)

Daily food intake and body weight. Similar to the findings from Expt. 1, the administration of IT led to a reduction of food intake in water- and in GTP-administered mice (water: −60% of d 1, P < 0.001; GTP −64% of d 1, P < 0.001), which was associated with a loss in body weight (water: −8% of d 1, P < 0.001; GTP: 13% of d 0, P < 0.001). Pair-feeding of the respective saline-treated group mimicked the loss in food intake and body weight of IT-treated mice. The 4 treatment groups did not differ in daily food intake and in body weights of mice (Fig. 2B).

Inflammatory variables. As mucosal damage, lipid peroxidation and NF-κB activation were maximal, and MPO activity as well as MIP-2 concentration was still enhanced at 48 h in Expt. 1, we chose this time-point to evaluate the influence of GTP. After treatment with IT-enhanced MIP-2 levels from undetectable levels to 10.7 6 3.6 mg/mg protein (P < 0.001), which was not prevented by an oral supply of 1 g/L GTP, MPO activity did not differ between groups. Similarly, the supplementation of drinking water with GTP did not prevent NF-κB activation induced by IT treatment (Table 2).

Oxidative stress variables. From our data from the time course experiments, we concluded that IT exerts its pro-oxidative effects via affecting the GSH:GSSG ratio. Therefore, GSH as well as GSSG concentrations of ileum were measured in healthy and IT-treated mice 48 h after the challenge to investigate the influence of GTP. The consumption of water containing 1 g/L GTP during the study period did not affect ileum GSH and GSSG concentrations or GSH/GSSG ratio in saline-treated mice. However, GTP supplementation reduced GSSG concentrations in IT-treated mice (−48%, P < 0.05) but did not affect GSH levels, leading to a significant increase in the GSH/GSSG ratio (+140%, P < 0.01). However, the addition of GTP to drinking water did not reduce IT-induced lipid peroxidation, which still was greater than in saline-treated and GTP-fed controls (+23%, P < 0.05) (Table 2).

Discussion
The present study showed that administration of the chemotherapeutic agent IT to healthy mice resulted in severe small intestinal mucosa damage. Mucosa atrophy in the ileum was...
associated with enhanced oxidative stress as shown by an initial decrease of GSH concentration followed by an enhancement of lipid peroxidation. Furthermore, inflammatory processes were elicited such as activation of the transcription factor NF-κB, increase of MIP-2, and enhancement of MPO activity, indicating the recruitment of neutrophils to sites of inflammation.

IT has been applied in the clinics for the treatment of solid tumors for several years. However, the administration of IT at higher doses was associated with an unexpected and significant incidence of late-onset diarrhea, which has been recognized as the rate-limiting toxicity of this compound (23–25). In this respect, several possible mechanisms have been proposed, such as structural changes in ileum and cecum, altered large intestinal absorption rates, and influences on intestinal bacteria by increasing intestinal β-glucuronidase activity, which leads to an enhanced conversion of IT to its 1000-fold more potent metabolite SN-38 (26–28). In our study, histological examination of ileum revealed that IT-induced damage was most pronounced 48 h after administration of a nonlethal dose of IT and was manifested by villus-shortening, massive destruction of epithelial layer, and enhanced cellular debris at the luminal site.

Although evidence indicates that administration of various cytostatic agents leads to an excessive generation of free radicals, we were interested in whether IT-induced mucosal damage was associated with enhanced oxidative stress. Our data showed that ileal GSH, which represents the main intracellular antioxidant, declined as early as 3 h after administration of IT to mice. This is in line with a previous study showing that the inhibition of GSH synthesis with buthionine sulfoximide is associated with an increased sensitivity of V79 hamster lung fibroblasts to IT (29). Interestingly, GSH levels were recovered within 24 h after the treatment. These findings lead to the hypothesis that oxidative stress induced by IT, as shown by an initial decrease of GSH, might be counter-regulated by enhancing GSH synthesis. Additionally, IT administration could exert pro-oxidative effects through enhancement of GSSG levels rather than through a drop in GSH levels. Because we were limited with respect to available amounts of small intestinal tissue, we considered this point only in the 2nd set of experiments, where we assessed both GSH and GSSG levels.

We chose the measurement of lipid peroxidation as a parameter for oxidative tissue damage. In contrast to GSH, TBARS of ileum were not enhanced until villous atrophy reached its maximum (48 h). Consistent with our data, Sadzuka et al. (30) showed that IT administration enhances lipid peroxidation in various organs of mice and rats. However, in our model, lipid peroxidation was decreased 96 h after treatment, which fits to the observed increase in GSH concentration and strengthens the hypothesis of an upregulation of antioxidative pathways after a pronounced oxidative insult.

The administration of IT resulted in a strong activation of NF-κB reaching its maximum after 48 h without returning to control levels within 96 h. The transcription factor NF-κB was shown to be involved in the regulation of inflammatory as well as apoptotic conditions (31,32). Most cancer therapeutics function by killing cells through the induction of apoptosis, whereby the relation between cytostatic agents, apoptosis, and NF-κB is well documented. Our results are in line with investigations showing NF-κB activation after exposure of cells to chemotherapeutics (33). However, NF-κB activation is also thought to mediate the resistance of tumor cells to treatment with IT, insofar as its inhibition resulted in a markedly increased level of apoptosis (34). In this respect, the activation of NF-κB strongly suppressed the apoptotic potential of IT to human fibrosarcoma cells (35). Regarding the IT-induced damage to healthy tissues, proinflammatory processes might also play an important role.

The destruction of intestinal epithelium after administration of lethal doses of IT to mice has been shown to be accompanied by massive neutrophil infiltration (36). Using a lower and nonlethal dose of IT in the present study, histological investigations did not reveal visible signs of enhanced accumulation of neutrophils in the ileum. Nevertheless, MPO activity, another commonly accepted marker for neutrophil infiltration, was maximally increased 24 h after treatment with IT, and remained enhanced at 48 and 72 h. The lower sensitivity of the histological examination compared with the measurement of MPO activity

<table>
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<tr>
<th>Table 1</th>
<th>IT-modulated indices of oxidative stress in the ileum of mice (Expt. 1)¹</th>
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<td>Time after IT administration, h</td>
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<td>Glutathione, μmol/g tissue</td>
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<td>TBARS, nmol/g tissue</td>
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¹ Values are means ± SD, n = 5. Asterisks indicate different from 0 h: * P < 0.05 and ** P < 0.01.

<table>
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<th>Table 2</th>
<th>Influence of GTP and IT on inflammation and oxidative stress in mice (Expt. 2)¹</th>
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<td>Water</td>
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<td>MIP-2, μg/mg protein</td>
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<td>MPO activity, ΔOD(min - g tissue)</td>
<td>0.28 ± 0.10</td>
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<td>NF-kB², AV × CNT × mm²</td>
<td>11.260 ± 4680</td>
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<td>GSH, μmol/mg tissue</td>
<td>3.24 ± 0.37ab</td>
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<td>GSSG, nmol/mg tissue</td>
<td>0.049 ± 0.019ab</td>
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<td>GSH/GSSG</td>
<td>69.6 ± 25.2ab</td>
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<td>TBARS, nmol/g tissue</td>
<td>29.8 ± 3.9</td>
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¹ Values are means ± SD, n = 8–12. Means in a row with superscripts without a common letter differ, P < 0.05.
² AV, adjusted volume; CNT, count.
might explain this discrepancy. The involvement of inflammatory processes was also confirmed by an endoscopic examination of a patient with severe diarrhea after IT chemotherapy, which revealed severe inflammation of the ileo-cecal region (37). Furthermore, the oral administration of the anti-inflammatory D-amino acid decapetide RDP58 significantly decreased the incidence of diarrhea and improved the survival rates of mice treated with toxic doses of IT or 5-fluorouracil by inhibiting local overproduction of TNF-α, IFN-γ, and IL-12 in the intestine (11).

In the clinical setting, loperamide is used as symptomatic drug for the treatment of IT-induced diarrhea, but there is currently no prophylactic therapy to ameliorate or prevent the gastrointestinal toxicity (7). However, some attempts have been made to prevent structural changes in the intestine by inhibiting inflammatory processes (11,38). We wanted to assess the impact of GTP administered orally to IT-treated mice because GTP have a proven anti-inflammatory and antioxidative potential, as shown in numerous experimental studies (39). Additionally, the green tea constituent, epigallocatechin-3-gallate (EGCG), inhibits topoisomerase I activity in human colon carcinoma cells, which suggests that a combination of green tea with other topoisomerase inhibitors could be an improved strategy for the treatment of colon cancer (40).

Although MPO activity and MIP-2 levels were maximally increased after 24 h, the 48-h time point was chosen for evaluating the effects of GTP administration, because mucosal damage, lipid peroxidation, and NF-κB activation were maximal at this time point and MPO activity and MIP-2 levels were still enhanced. In IT-treated mice, the supplementation of GTP did not reduce MPO activity, MIP-2 levels, or NF-κB activation. Comparable in vitro studies showed that the relevant plasma concentration of EGCG inhibits neutrophil migration through endothelial cell monolayers and GTP almost completely block liver MPO activity caused by ischemia-reperfusion (41,42). The topical application of EGCG to mice before a single dose of UV-radiation inhibits the infiltration of leukocytes and MPO activity (43). At a dose of 0.5 g of green tea polyphenols/kg body weight, lipopolysaccharide-induced lethality of mice was completely blocked, possibly by diminishing NF-κB-induced TNFα production (44). The blunted effect of GTP administration on inflammatory parameters could be due to a lower dose of GTP used in our study. However, 1 g/L GTP was chosen in our experimental setting because this concentration reflects clinically achievable, nontoxic doses in humans (45).

We could not detect any differences in ileal GSH concentration 48 h after IT treatment. In Expt. 2 we assessed both GSH and GSSG concentrations using an enzymatic test system instead of HPLC analysis. The consumption of GTP displayed a marked influence on GSH metabolism insofar as it did not alter GSH levels, whereas reducing GSSG levels leading to the doubling of GSH:GSSG ratio represented the most abundant intracellular redox buffer (46). Direct antioxidative properties of tea polyphenols have been demonstrated in numerous in vitro and experimental studies (47,48), whereas the influence of GTP on GSH metabolism is not very well investigated. Despite affecting the GSH:GSSG ratio, which plays an important role in protecting against oxidative injuries, GTP were not able to prevent the oxidative damage of lipids as shown by enhanced levels of TBARS. Obviously, GTP exert differential effects on GSH and lipid peroxidation, which was also shown in an in vitro model using PC12 cells (49).

In summary, our study revealed that oxidative as well as inflammatory processes are involved in the IT-induced destruction of small intestinal epithelium in healthy mice. We showed that, after an initial drop of GSH, inflammatory pathways are activated and lead to NF-κB activation and enhancement of MPO activity, which finally leads to oxidative tissue damage. Oral administration of GTP during the study period prevented the IT-induced increase of GSSG and MPO activity in the ileum, whereas lipid peroxidation was unaffected. Therefore, the supply of GTP displays partial protection against IT-induced gastrointestinal toxicity. Further studies using a tumor model are needed to investigate a possible synergistic chemotherapeutic effect between GTP and IT.

**Literature Cited**


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