

Calcitonin Gene-Related Peptide and Substance P Regulate the Intestinal Radiation Response

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Abstract Purpose: Intestinal toxicity is important in the therapeutic use of radiation as well as in nontherapeutic radiation exposure scenarios. Enteric sensory nerves are critical for mucosal homeostasis and for an appropriate response to injury. This study assessed the role of the two major neuropeptides released by sensory nerves, calcitonin gene-related peptide (CGRP) and substance P, in the intestinal radiation response.

Experimental Design: Male rats received full-length CGRP, CGRP antagonist (CGRP₈₋₃₇), a modified substance P peptide (GR73632), a small-molecule substance P receptor antagonist (neurokinin-1 receptor antagonist, SR140333), or vehicle for 2 weeks after localized X irradiation of a 4-cm loop of small bowel. Structural, cellular, and molecular aspects of the intestinal radiation response were assessed.

Results: Intestinal CGRP and substance P transcript levels increased after irradiation. Multivariate analysis showed that CGRP and SR140333 ameliorated and CGRP₈₋₃₇ and GR73632 exacerbated intestinal radiation injury. Univariate analysis revealed increased radiation injury score, bowel wall thickening, and collagen III deposition after treatment with CGRP₈₋₃₇, whereas SR140333 ameliorated radiation injury score, loss of mucosal surface area, collagen III deposition, and mucosal inflammation.

Conclusions: The two major neuropeptides released by sensory neurons, CGRP and substance P, are overexpressed after irradiation and have opposing effects during development of intestinal radiation injury. Systematic studies to assess CGRP agonists and/or neurokinin-1 receptor blockers as protectors against intestinal toxicity during radiation therapy and after nontherapeutic radiation exposure are warranted.

Although advances in treatment planning and radiation delivery techniques have increased the ability to focus the radiation beam on the tumor, radiation therapy continues to be dose limited by the tolerance of surrounding normal tissues. Increased focus on the avoidance of treatment-related side effects in cancer patients (i.e., the concept of uncomplicated cancer cure), as well as the recently emerged need to develop medical countermeasures against radiological or nuclear accidents or terrorism, has resulted in a resurgence of interest in mechanisms of normal tissue radiation toxicity and in developing interventions that may help reduce normal tissue toxicity.

The intestine is an important dose-limiting organ during radiation therapy of tumors in the pelvis or abdomen. It was previously believed that the severity of intestinal radiation

toxicity solely depends on the extent of radiation-induced intestinal crypt cell death. This view has been supplanted by the recognition that radiation-induced changes in cellular function as well as a plethora of secondary alterations contribute substantially to the pathophysiologic manifestations of intestinal radiation toxicity (1).

Several lines of evidence suggest that enteric afferent (sensory) nerves and cross-talk between enteric nerves and cells of the immune system are important in the regulation of radiation-induced bowel toxicity (recently reviewed in ref. 2). Intestinal afferent nerves not only relay sensory information to the central nervous system but also exert many local effector functions that serve to maintain the integrity of the bowel mucosa and to ensure an appropriate response to injury. We recently reported that ablation of sensory neurons in rats before localized small bowel irradiation profoundly influenced the development of intestinal radiation toxicity and that this effect was partly dependent on interactions between sensory enteric neurons and resident mucosal mast cells (3).

Enteric neurons release a plethora of peptides that regulate neuroimmune processes either directly or indirectly. Calcitonin gene-related peptide (CGRP) and substance P are widely distributed in sensory nerve endings in the gastrointestinal tract and are presumed to be major neurotransmitters in capsaicin-sensitive neurons. Moreover, CGRP and substance P seem to be particularly important in terms of intestinal inflammation and injury-repair processes. In addition to interacting with inflammatory and immune cells, CGRP and

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substance P also act on microvascular endothelial cells and epithelial cells, both of which participate in the intestinal radiation response (4, 5). Substance P exerts its effects by binding to its receptor, the neurokinin-1 (NK-1) receptor. The NK-1 receptor is a widely expressed G protein-coupled receptor. The cellular receptor for CGRP is a heterodimer composed of the G protein-coupled calcitonin receptor-like receptor and receptor activity-modifying protein 1, a single-pass transmembrane protein.

In this study, a rat model of radiation enteropathy was used to evaluate the effect of exogenous administration of substance P and CGRP and of their receptor antagonists on the intestinal radiation response. The results show that, although both neuropeptides are overexpressed after irradiation, they have opposing effects on the development of radiation toxicity. These data suggest that the use of NK-1 antagonists or CGRP agonists should be explored as strategies to control intestinal side effects of radiation therapy or to ameliorate intestinal toxicity in the nontherapeutic radiation exposure situation.

Materials and Methods

Animals and experimental radiation enteropathy model

A total of 79 male Sprague-Dawley rats (Harlan, Indianapolis, IN) were housed in conventional cages in a pathogen-free environment with controlled humidity, temperature, and 12:12 light/dark cycle with free access to tap drinking water and chow (Harlan Teklad, Madison, WI). The experimental protocol was approved by the University of Arkansas for Medical Sciences Institutional Animal Care and Use Committee (Little Rock, AR).

The surgical model for localized small bowel irradiation was prepared as described previously (6). Briefly, rats underwent bilateral orchietomy; a loop of distal was sutured to the inside of the left part of the empty scrotum. The model creates a "scrotal hernia" that contains a 4-cm loop of small intestine that can be irradiated locally without additional surgery. The intestine remains technically within the abdominal cavity, and the surgical procedure does not cause appreciable long-term structural, functional, cellular, or molecular alterations. The model minimizes manipulation during irradiation and produces radiation-induced changes similar to those seen clinically. This model has been extensively used and validated in our laboratory.

After 3-week postoperative recovery, the rats were anesthetized with isoflurane inhalation and the transposed bowel segment within the scrotal hernia was sham-irradiated or exposed to 17 Gy localized, single-dose irradiation with a Seifert Isovolt 320 X-ray machine (Seifert X-Ray Corp., Fairview Village, PA), operated at 250 kVp and 15 mA, with 3-mm Al added filtration. The resulting half-value layer was 0.85-mm Cu, and the dose rate was 4.49 Gy/min. Dosimetric considerations have been described elsewhere (7). This radiation regimen was based on data from previous experiments and intended to elicit moderate to severe radiation enteropathy.

Administration of substance P, CGRP, and antagonists

Neuropeptides have been given both by s.c. injection and by use of s.c. miniosmotic pumps (8). Administration by miniosmotic pumps was initially considered for the present study. However, mass spectroscopy analysis of CGRP before and after 1-week storage at 37°C showed significant degradation (data not shown). Therefore, administration by twice-daily s.c. injection was chosen. Neuropeptides have significant effects on physiologic variables that may directly or indirectly influence radiosensitivity, such as mucosal microcirculation. Therefore, administration of the modifying compounds after irradiation, rather than before irradiation, was used. The daily doses of the neuropeptide agonists/antagonists were based on those used by others (8–10).

Rats were randomly assigned to CGRP agonist, rat CGRP1-37 ($n = 16$; American Peptide Co., Sunnyvale, CA), CGRP antagonist, rat CGRP₈₋₃₇ ($n = 14$; American Peptide), or physiologic saline control ($n = 15$). CGRP (75 µg/kg/d), CGRP₈₋₃₇ (75 µg/kg/d), or saline were given as follows: one fourth of the daily dose was given by i.p. injection and one fourth by s.c. injection within 15 minutes of irradiation, and the remaining half of the dose of the first day was given by s.c. injection in the late afternoon. Thereafter, both compounds were given by twice-daily s.c. injection until termination of the experiment on day 14.

Analogously, rats were randomly assigned to treatment with substance P agonist ($n = 12$), NK-1 receptor antagonist ($n = 12$), or saline control ($n = 10$). The substance P agonist GR73632 (Sigma, St. Louis, MO) is a modified substance P peptide, which is at least 10-fold more potent than native substance P. The NK-1 receptor antagonist SR140333 (Sanofi Recherche, Montpellier, France) is a small-molecule (nonpeptide) compound with high affinity for human and rat NK-1 receptor (11). GR73632 (15 µg/kg/d) was given using the same schedule as described for the CGRP agonist and antagonist (i.e., the first dose was given as divided i.p./s.c. injections followed by twice-daily s.c. injections). SR140333 (1 mg/kg) was given every other day by s.c. injection.

Assessment of the intestinal radiation response

The rats were euthanized 2 weeks after irradiation. This time point corresponds to early intestinal radiation injury (i.e., epithelial injury, inflammatory changes, and reactive fibrosis). Specimens of irradiated and shielded (unirradiated) intestine were procured. One part was snap frozen in liquid nitrogen and subsequently transferred to -80°C for RNA extraction and real-time PCR analysis. The remainder was fixed in methanol-Carnoy's solution for histologic, stereologic, and immunohistochemical studies.

Quantitative histopathology and morphometry

Radiation injury score. The overall severity of structural radiation injury was assessed using the radiation injury score (RIS) scoring system. The RIS is a composite histopathologic scoring system that provides a global measure of the severity of structural radiation injury. It has been extensively used and validated in our laboratory (12, 13). Briefly, seven histopathologic variables of radiation injury (mucosal ulcerations, epithelial atypia, thickening of subserosa, vascular sclerosis, intestinal wall fibrosis, ileitis cystica profunda, and lymph congestion) were assessed and graded from 0 to 3. The sum of the scores for the individual alterations constitutes the RIS. All specimens were evaluated in a blinded fashion by two separate researchers, and discrepancies in scores were resolved by consensus.

Mucosal surface area. Radiation-induced decrease in the surface area of the intestinal mucosa is a sensitive variable of small bowel radiation injury. Mucosal surface area was measured in vertical sections using a stereologic projection/cycloid method as described by Baddeley et al. (14) and adapted by us to our model system (15). This technique does not require assumptions about the shape or orientation distribution of the specimens and thus circumvents problems associated with most other procedures for surface area measurement.

Thickness of the intestinal wall and subserosa. Intestinal wall thickening is a measure of both reactive intestinal wall fibrosis and intestinal smooth muscle cell hyperplasia. In contrast, subserosal thickening mainly reflects reactive fibrosis. Intestinal wall thickness (encompassing submucosa, muscularis externa, and subserosa) and the subserosal thickness were measured with an eyepiece linear microruler. Five measurements, 500 µm apart, were obtained, and the average for each specimen was used as a single value for statistical calculations.

Quantitative immunohistochemistry/histochemistry and image analysis

Immunohistochemistry and computer-assisted image analysis (Image-Pro Plus, Media Cybernetics, Silver Spring, MD) were used to assess (a) neutrophil infiltration, (b) proliferation rate of intestinal smooth muscle cells, (c) deposition of collagen types I and III in the

intestinal wall as described in detail and validated previously (5, 16), (d) crypt cell labeling index, and (e) mucosal mast cell numbers.

Immunohistochemical staining was done with standard avidin-biotin complex technique, 3,3'-diaminobenzidine chromogen, and hematoxylin counterstaining. Appropriate positive and negative controls were included. The primary antibodies, incubation times, dilutions, and sources were as follows: polyclonal anti-myeloperoxidase antibody (2 hours, 1:100; DAKO, Carpinteria, CA), monoclonal antibody against proliferating cell nuclear antigen (PCNA; 2 hours, 1:100; Calbiochem, Cambridge, MA), and polyclonal antibodies against collagens I and III (2 hours, 1:100 and 2 hours, 1:100; Southern Biotechnology Associates, Birmingham, AL).

Myeloperoxidase. Myeloperoxidase is a well-documented inflammation marker. Myeloperoxidase enzymatic activity in leukocytes correlates directly with neutrophil number ($r = 0.99$), and myeloperoxidase activity in tissue extract correlates directly with cellular infiltration assessed histologically ($r = 0.94$; ref. 17). The number of myeloperoxidase-positive cells was determined by color thresholding and counting in 20 fields ($\times 40$ magnification), selected according to a predetermined grid pattern.

Smooth muscle cell proliferation. In the intestine, most collagen is produced by intestinal smooth muscle cells rather than by fibroblasts. Intestinal smooth muscle cell proliferation rate is very low at baseline but increases steeply after irradiation (18). Intestinal smooth muscle cell proliferation was assessed in the external muscle layer of irradiated intestine. The number of total smooth muscle cells and PCNA-positive smooth muscle cells was determined in 20 fields ($\times 40$ magnification) using color thresholding and normalized to PCNA-positive smooth muscle cells per thousand smooth muscle cells.

Collagen. After irradiation of many normal tissues, collagen accumulation is primarily a late end point. In the irradiated intestine, however, significant accumulation of collagen I and, particularly, collagen III occurs as a reactive change already after 2 weeks. The relative areas positive for collagen types I and III were determined in 20 fields ($\times 40$ magnification) according to Raviv et al. (19) as adapted to our model system (20).

Crypt cell labeling index. The intestinal radiation response correlates with crypt cell proliferation rate at the time of irradiation. On the other hand, increased enterocyte proliferation after irradiation is associated with enhanced epithelial recovery. The potential influence of the neuropeptide agonists/antagonists on intestinal crypt cell proliferation was analyzed in unirradiated (shielded) intestine using PCNA as proliferation marker. The total number of intestinal crypt cells (excluding Paneth cells) and the number of PCNA-positive cells were manually counted in 15 longitudinally sectioned crypts per specimen. Crypt cell labeling index (PCNA-positive cells/total crypt cells per crypt) was calculated for each crypt. The arithmetic mean in each specimen was considered a single value for statistical calculations.

Mucosal mast cells. Sensory enteric neurons and mucosal mast cells are closely associated both functionally and anatomically. Studies from our laboratory show that neuroimmune interactions between sensory nerves and mast cells regulate the intestinal radiation response (3, 18). The effects of the CGRP agonist/antagonist, the substance P agonist, and the NK-1 antagonist on mucosal mast cells were analyzed in unirradiated intestinal specimens. Color thresholding was used to identify and count the number of mast cells in 20 fields ($\times 40$ magnification) of intestinal mucosa in toluidine blue-stained (Sigma) specimens (3).

Fluorogenic probe reverse transcription-PCR

Radiation-induced changes in steady-state CGRP and substance P transcript levels were assessed with real-time quantitative PCR.

Total RNA was isolated from frozen unirradiated and irradiated intestinal samples and reverse transcribed. Real-time PCR was done using an ABI Prism 7000 sequence detection system (PE Applied Biosystems, Foster City, CA), Taqman Universal PCR Master Mix, and Assays-on-Demand Gene Expression kits for CGRP (Rn00569199_m1),

substance P (Rn00562002_m1), and 18S rRNA (Hs99999901_s1; PE Applied Biosystems). PCR amplification with 18S rRNA as internal control was done as described elsewhere (3). Relative transcript quantitation was done using the comparative threshold cycle method (21).

Statistical methods

Differences in end points as a function of radiation [irradiated versus shielded (unirradiated) intestine] across the various treatment groups (controls, CGRP, and CGRP antagonist in the first experiment and controls, substance P agonist, and NK-1 receptor antagonist in the second experiment) were assessed using fixed-factor ANOVA (NCSS 2004 for Windows, NCSS, Kaysville, UT). The effects of exogenous addition of agonist, vehicle, or receptor antagonist for substance P and CGRP were assessed by multivariate analysis with the Jonckheere-Terpstra test using the StatXact 5 software package for exact non-parametric inference (Cytel Software, Cambridge, MA). The Jonckheere-Terpstra test is similar to the Kruskal-Wallis test (nonparametric one-way ANOVA) but makes the additional assumption that the populations are not random but rather exhibit a trend (in this case that neuropeptide agonist and antagonist will change a particular variable in opposite directions relative to the vehicle-treated control group). Univariate comparisons of differences that were significant with the Jonckheere-Terpstra test were further assessed with the Mann-Whitney U test. P s < 0.05 were considered statistically significant.

Results

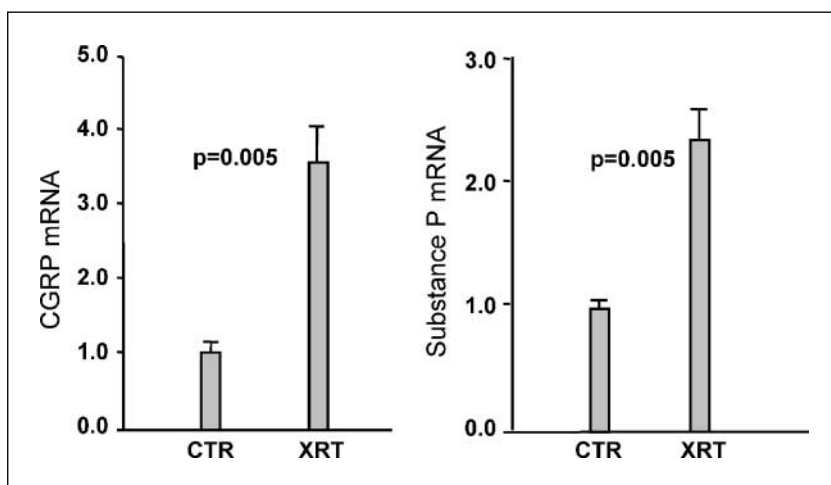
Effect of exogenous modulation of CGRP and substance P in unirradiated intestine. Routine histologic examination of intestinal sections from unirradiated (shielded) intestine after 2-week administration of neuropeptide agonists/antagonists did not reveal obvious structural changes at the light microscopic level. However, although the difference was small and perhaps not biologically significant, there was a statistically significant decrease in intestinal crypt cell labeling index in intestine from SR140333-treated rats compared with intestine from vehicle-treated control rats ($P = 0.0003$). Crypt cell proliferation was not influenced by GR73632, CGRP, or CGRP₈₋₃₇. Compared with vehicle-treated control rats, the neuropeptide agonists/antagonists did not affect the number of mucosal mast cells.

Effect of CGRP and substance P modulation on the intestinal radiation response. Radiation-induced changes in this study were similar to those observed previously by us and others (see for example ref. 15). At the histologic level, the predominant features were mucosal injury and ulcerations, reactive bowel wall thickening, and inflammatory cell infiltration. These changes were associated with a significant loss of mucosal surface area, increase in the number of myeloperoxidase-positive cells, increased smooth muscle cell proliferation, and increased deposition of collagen in the bowel wall ($P < 0.001$ for all variables). Real-time PCR revealed a highly significant radiation-induced increase in substance P and CGRP transcript levels in intestinal tissue ($P = 0.005$ for both transcripts; Fig. 1).

Overall, examination of irradiated intestine from the six experimental groups showed that the two neuropeptides, CGRP and substance P, had opposing influence on the intestinal radiation response (i.e., CGRP ameliorated injury whereas substance P exacerbated injury).

Multivariate analysis showed that, compared with vehicle-treated controls, exogenous administration of CGRP attenuated, whereas CGRP₈₋₃₇ exacerbated, structural radiation-induced

Fig. 1. CGRP and substance P transcripts in unirradiated and irradiated small bowel. Real-time PCR was used to measure relative steady-state mRNA levels for substance P and CGRP in RNA extracted from unirradiated (shielded) and irradiated (17 Gy single dose) intestinal tissue 2 weeks after irradiation. 18S rRNA was used as normalizer.



intestinal injury (RIS, $P = 0.003$; Fig. 2). Consistent with this finding, CGRP attenuated, whereas CGRP₈₋₃₇ exacerbated, inflammatory cell infiltration ($P = 0.02$; Fig. 2), serosal thickening ($P = 0.03$), intestinal wall thickening ($P = 0.03$), and collagen III accumulation ($P = 0.003$; Fig. 3). The differences in loss of mucosal surface area and PCNA-positive smooth muscle cells did not reach statistical significance (data not shown). Subsequent univariate analysis revealed that receptor blockade with CGRP₈₋₃₇ had a more pronounced effect on the level of injury than exogenous administration of CGRP, with a significant effect of CGRP₈₋₃₇ on RIS ($P = 0.05$), subserosal thickness ($P = 0.04$), intestinal wall thickness ($P = 0.03$), and collagen III accumulation ($P = 0.003$).

Conversely, multivariate analysis of the data from the experimental groups treated with SR140333 (NK-1 antagonist), vehicle, and GR73632 (substance P agonist) revealed a borderline significant difference in RIS ($P = 0.04$; Fig. 4) and loss of mucosal surface area ($P = 0.04$), with SR140333 ameliorating injury and GR73632 enhancing injury. Moreover, SR140333 ameliorated and GR73632 exacerbated deposition of both collagen I and collagen III ($P = 0.004$ and 0.02 , respectively; Fig. 5). The number of PCNA-positive smooth muscle cells and reactive intestinal wall thickening did not reach statistical significance (data not shown). Again, on univariate analysis, receptor blockade with SR140333 had a greater influence on the level of injury than administration of substance P agonist, with SR140333-treated animals exhibiting

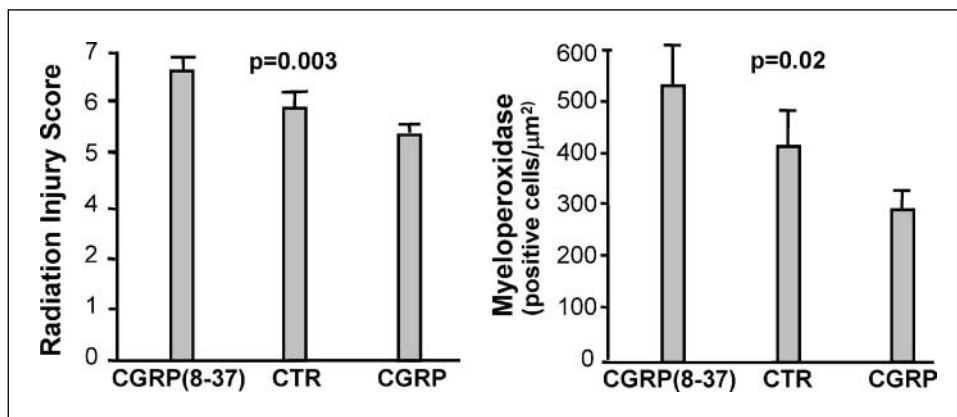
lower RIS ($P = 0.02$) and less pronounced loss of mucosal surface area ($P = 0.005$), collagen III deposition ($P = 0.02$), and infiltration of myeloperoxidase-positive cells ($P = 0.004$).

Discussion

Sensory neurons are required for normal mucosal homeostasis and an appropriate response to injury. Ablation of sensory nerves before irradiation significantly exacerbates intestinal radiation toxicity (3). Sensory neurons release many biologically active peptide mediators. Among these mediators, substance P, an 11-amino acid peptide of the tachykinin peptide family, and CGRP, a 37-amino acid peptide, are particularly important in gastrointestinal pathology and pathophysiology. The present study shows that CGRP and substance P are both overexpressed in small bowel after localized exposure to ionizing radiation and that CGRP ameliorates, whereas substance P enhances, the intestinal radiation response.

Consistent with the findings from the present study, substance P exacerbates and CGRP ameliorates bowel pathology in other intestinal inflammation models. For example, SR140333 protects against colitis induced by 2,4,6-trinitrobenzene sulfonic acid (10) and NK-1 receptor knockout mice are protected against intestinal inflammation (22). Mice deficient in neutral endopeptidase (the enzyme that degrades substance P) sustain increased intestinal injury

Fig. 2. CGRP modulation: structural radiation injury and mucosal inflammation. Overall structural injury (RIS) and level of mucositis (myeloperoxidase) in irradiated small intestine 2 weeks after irradiation (17 Gy single dose). CGRP(8-37), CGRP antagonist; CTR, vehicle control; CGRP, CGRP agonist.



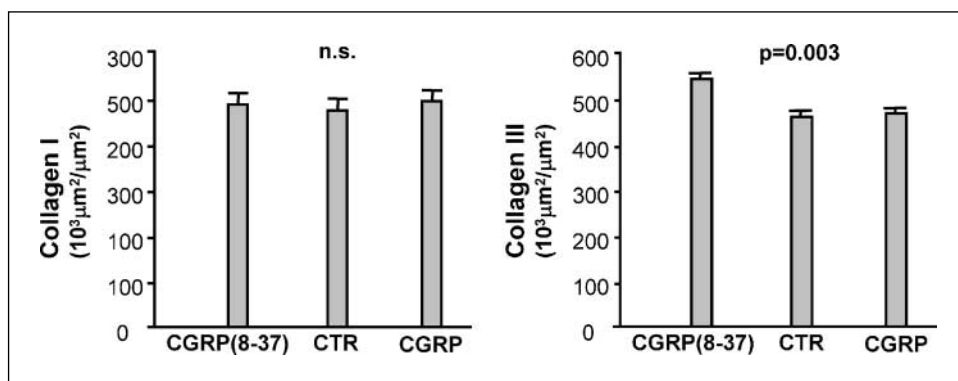


Fig. 3. CGRP modulation: influence on collagen deposition in irradiated intestine. Deposition of collagen types I and III, measured by quantitative immunohistochemistry, in irradiated small intestine 2 weeks after irradiation (17 Gy single dose).

(23). Conversely, CGRP ameliorates, whereas CGRP₈₋₃₇ and CGRP-blocking antibodies exacerbate, 2,4,6-trinitrobenzene sulfonic acid – induced colitis (8).

Although the roles of substance P and CGRP in inflammatory bowel disease models are well established, the roles of these neuropeptides in intestinal radiation toxicity are less clear, and the few published studies are mostly descriptive and inconclusive. Correlative clinical and animal studies show increased levels of substance P after intestinal irradiation (24, 25). Moreover, NK-1 receptor antagonists modulate changes in gut motility and plasma protein extravasation after whole-body irradiation (26, 27). To our knowledge, the influence of CGRP modulation on radiation toxicity has not yet been systematically investigated.

The present study showed that SR140333 and CGRP₈₋₃₇ were more effective radiation response modifiers than the corresponding neuropeptide agonists. This observation has translational implications. Hence, in the postradiation situation, when both substance P and CGRP are increased, blocking an adverse biological effect at the receptor level may be more effective than attempting to increase the beneficial effects of a neuropeptide that is already overexpressed. Moreover, NK-1 receptor antagonists have shown antiemetic efficacy. Therefore, if developed as a strategy to minimize intestinal radiation toxicity, NK-1 receptor antagonists might also provide symptomatic relief from radiation-associated nausea and vomiting.

Based on previous studies done in our laboratory, it is tempting to speculate that the mechanisms by which substance P exacerbates and CGRP ameliorates intestinal radiation toxicity involve interactions between sensory enteric nerves

and intestinal mast cells. Sensory nerves exhibit close (often referred to as “synaptic”) associations with mast cells, with 90% of intestinal mucosal mast cells in direct contact with or within 2 μm of neurons (28). Although all substance P-containing neurons also contain CGRP, a subpopulation of cells contains only CGRP (29). A recent study from our laboratory, focused on the role of capsaicin-sensitive (sensory) nerves in intestinal radiation toxicity, further supports a role for interactions between sensory nerves and mast cells. Hence, although the intestinal radiation response was exacerbated in sensory nerve-ablated rats, the effect of sensory nerve ablation was severely blunted in mast cell-deficient animals (3).

Similar to the situation with other types of intestinal injury, there are prominent interactions between the microvasculature and the epithelium after intestinal radiation exposure. For example, maintaining postradiation microvascular integrity, such as in genetically modified mice where the endothelium is apoptosis resistant, is associated with decreased radiation-induced crypt cell death and less mucosal injury than in what is seen in wild-type littermate control mice (4). The enteric nervous system interacts directly with both microvascular endothelium (30, 31) and intestinal epithelium (32, 33). However, because enteric denervation is generally assumed to have a trophic effect on the epithelium (32), the observations in the present study can more easily be explained in terms of interactions with the microvasculature and/or cells of the immune system than by a primary epithelial effect.

The mechanisms by which substance P exacerbates and CGRP ameliorates radiation toxicity remain to be elucidated. The NK-1 receptor is expressed on a wide variety of cells,

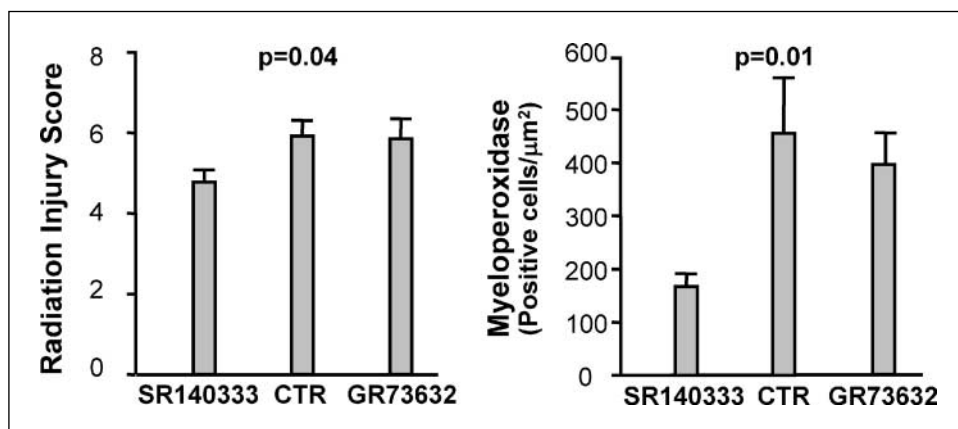
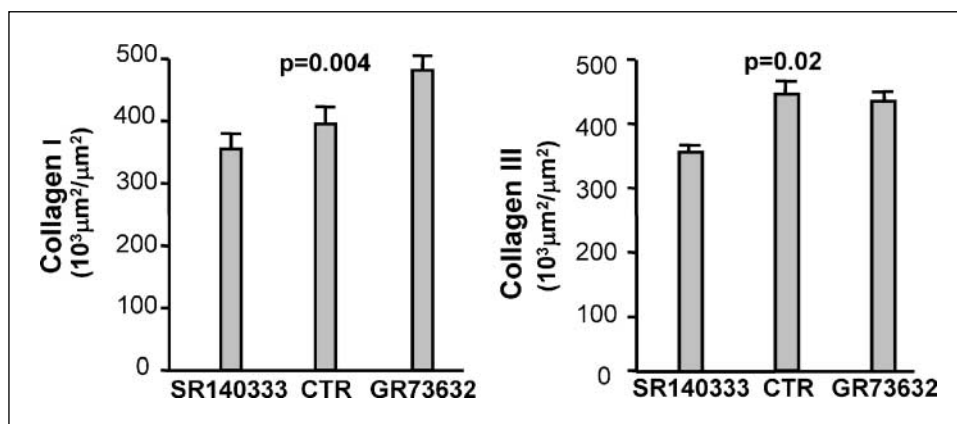


Fig. 4. Substance P modulation: influence on structural radiation injury and mucosal inflammation. Overall structural injury (RIS) and level of mucositis (myeloperoxidase) in irradiated small intestine 2 weeks after irradiation (17 Gy single dose). SR140333, NK-1 receptor antagonist; GR73632, substance P agonist.

Fig. 5. Substance P modulation: influence on radiation-induced intestinal wall collagen deposition. Deposition of collagen types I and III was measured by quantitative immunohistochemistry (see text for details) in irradiated small intestine 2 weeks after irradiation (17 Gy single dose).



including mast cells. Substance P released by enteric neurons activates NK-1 receptors on mast cells and is a major mediator of neuron-mast cell interactions (34). After injury, substance P also exacerbates gastrointestinal inflammation by increasing superoxide production by neutrophils (35), by increasing plasma protein extravasation, and by increasing mast cell production of tumor necrosis factor- α (36), thus impairing the hyperemic response to injury (37). A translational implication of the effect on mucosal blood flow is that it would be important to administer NK-1 blockers to patients after, rather than before, each dose of radiation to avoid hyperemia-associated radiosensitization.

CGRP is a potent vasodilator, protects the gut from a variety of insults, and accelerates recovery by increasing intestinal blood flow (38). CGRP inhibits the edema-promoting actions of several inflammatory mediators, including histamine, leukotriene B₄, and serotonin (39), and many of the cytoprotective and anti-inflammatory properties of CGRP are mimicked by sensory nerve stimulation (39). This supports our findings in the capsaicin-treated rat model (3) and the observation from the present study that CGRP modulation influences postradiation neutrophil accumulation. Moreover, CGRP also suppresses tumor necrosis factor- α production (40) and counteracts substance P-induced production of reactive oxygen species by neutrophils and macrophages (35). It is also conceivable that CGRP exerts some of its protective effects by modulating mast cell function. Mucosal mast cells express the CGRP receptor (41), and CGRP is involved in the recruitment and activation of intestinal mucosal mast cells (42). The molecular underpinnings of the qualitatively different mast cell activation by CGRP and substance P remain to be elucidated. For example, substance P stimulates, whereas CGRP inhibits, the release of histamine (43).

Interestingly, CGRP potently inhibits and CGRP₈₋₃₇ stimulates platelet aggregation (44, 45). Although platelet aggregation was not the focus of the present study, it is tempting to speculate that platelet aggregation may be partly responsible for

the effects of CGRP/CGRP₈₋₃₇ seen in the present study. Hence, we and others have shown a significant protective effect of platelet aggregation inhibitors on radiation toxicity in intestine and other organs (46, 47).

Considering the colocalization of substance P and CGRP in sensory neurons, interactions between these neuropeptides may also influence the development of postradiation intestinal toxicity. For example, although both neuropeptides are degraded by neutral endopeptidase, substance P is degraded 88-fold faster than CGRP (48). Because neutral endopeptidase is down-regulated in the mucosa and smooth muscle cell layers of inflamed intestine (49), the delicate balance between CGRP and substance P in the physiologic situation is severely perturbed in the postradiation situation, leading to accumulation of substance P. Subsequent substance P-evoked release of proteolytic enzymes from mast cells may further accelerate the degradation of CGRP (50). Taken together, these studies support the notion that the local balance of inflammatory and anti-inflammatory neuropeptides is key to tissue homeostasis in the gastrointestinal tract and, conversely, imbalance of this system may contribute substantially to the pathophysiologic manifestations of intestinal radiation injury.

Conclusion

CGRP and substance P, the two major neuropeptides released by enteric sensory neurons, are overexpressed after irradiation. Modulation of CGRP and substance P using specific agonists and receptor blockers shows that the two neuropeptides have opposing effects on the development of intestinal radiation toxicity: CGRP ameliorates injury whereas substance P exacerbates injury. Pharmacologic modulators of neuropeptides or neutral endopeptidase should be explored as potential strategies to mitigate or treat intestinal injury in the therapeutic application of radiation as well as in the ongoing efforts to develop medical countermeasures against radiation accidents or radiological terrorism.

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