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Probiotic Therapy in Radiation-Induced Intestinal Injury and Repair

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Abstract

Intestinal injury from ionizing radiation is a clinically important entity, as enteritis symptoms occur commonly after radiotherapy for pelvic malignancies. Preventative or therapeutic options for radiation enteritis are mostly unsatisfactory; however, available data suggests that probiotic bacteria—those which confer health benefit—may have therapeutic value. Previous reports from both human trials and animal models have evaluated various end points for probiotic usage in limiting radiation-associated intestinal damage. Newer data suggests that particular probiotics and/or their secreted or derived bacterial products may have unique radioprotective properties. We will review the area with a focus on new developments surrounding probiotic therapy in radiation-induced intestinal injury and repair.

Keywords

probiotics; radiation; enteritis; lactobacillus rhamnosus GG; LGG; COX-2

Background

Intestinal injury commonly occurs after radiotherapy for pelvic or abdominal malignancies. Side effects associated with acute radiation enteritis occur in up to 75% of patients and differ both clinically and pathophysiologically from the sequelae associated with chronic radiation enteritis.[1] Acute symptoms relate to radiation-induced malabsorption and include bloating and diarrhea. In addition to adversely effecting quality of life, symptoms occasionally lead to significant dehydration requiring hospitalization. The acute side effects typically resolve within 2 to 3 weeks after radiation therapy is complete. The high prevalence of acute radiation enteritis, coupled with the paucity of adequate preventative or therapeutic strategies, underscores the importance of further investigation in this field.

There is a clear link between gastrointestinal health and the gut's resident bacteria. Over the last decade we have come to recognize that commensal bacteria, largely via toll-like receptors (TLRs), influence the intestinal epithelial response to injury in a complex manner.

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Conflicts of Interest
The authors declare no conflicts of interest.

These interactions are best highlighted in work on animal models of inflammatory bowel disease;[2,3] these interactions may also hold true for the intestinal epithelial response to radiation-induced injury. Crawford and Gordon demonstrated that germ-free mice are markedly resistant to lethal radiation injury and exhibit significantly fewer apoptotic endothelial cells in the mesenchymal cores of their small intestinal villi when compared to conventionally raised animals.[4] Interestingly, we found that mice deficient in MyD88 (thus lacking the major downstream signaling pathway for most TLRs) demonstrate increased epithelial apoptosis in response to radiation.[5] Clearly these potentially disparate findings underscore the complexities of the dynamic interactions between the gut and its flora. While the mechanistic underpinnings of this relationship are just beginning to be explored, clinical studies have long noted the benefits of certain probiotic bacteria in the symptomatic response to intestinal injury.

Probiotics are live microbial organisms that, when ingested, can confer benefit to the host via direct bacterial-host interaction or secreted factors. Delivery of probiotics to the GI tract can be as a supplement or as a food component such as with yogurt. These organisms have been proposed to benefit a variety of conditions both gastrointestinal in origin and extraintestinal. The best evidence supporting probiotic use is in treatment of infectious diarrhea and pouchitis; however, there is also data supporting a potential role for these beneficial bacteria in the treatment or prevention of radiation enteritis.[1,6] Over the last decade sporadic investigations have furthered this hypothesis, and recent studies have now elucidated more specifically how individual probiotic bacteria strains and their bacterial products may be most effective for this condition.

In light of our laboratory's recent findings describing the radioprotective effects of the probiotic Lactobacillus rhamnosus GG, the following sections will highlight both human trials and model-derived data covering the use of probiotics in radiation-induced intestinal injury. New findings providing insight into potential probiotic mechanisms of action will be discussed in greater detail.

Human Trials

Among other therapeutic agents, various probiotics have been investigated for their potential to reduce the significant GI side effects associated with radiation exposure. One group of investigators performed a large study with the commercially available probiotic mixture VSL#3. In a double-blind placebo-controlled study of nearly 500 patients undergoing pelvic radiotherapy, Delia and colleagues showed that when compared to the placebo group, patients taking VSL#3 experienced radiation-induced diarrhea less frequently and at a lower degree of severity.[7] Other smaller studies have been conducted with different Lactobacillus species as single agents. Both L. Acidophilus and L. Casei failed to meet meaningful endpoints in patient groups undergoing pelvic radiation for gynecologic malignancies.[8,9] Lactobacillus rhamnonus GG (LGG) has been evaluated in two different investigations where radiotherapy was utilized. Osterlund found less severe diarrhea in colorectal cancer patients who took LGG while receiving chemotherapy with 5-FU.[10] Notably, more than a third of those subjects were also undergoing pelvic radiation therapy. Hungarian researchers also showed that LGG was modestly effective in improving stool consistency and delaying

the need for other antidiarrheal therapy in patients undergoing pelvic radiotherapy.[11] It is possible that this second study would have found more favorable outcomes had the LGG administration been started prior to the initiation of radiation, rather than offered as a rescue therapy after symptoms had begun. Overall, these clinical trials have shown a trend, albeit a modest one, toward a benefit for probiotics in limiting the severity of GI symptoms associated with pelvic radiation. Additionally, it is reassuring that none of these studies reported any adverse infectious events linked to the probiotic usage.

Animal Models

Earlier studies from animal models provide supporting evidence for a GI protective role of probiotics in radiation injury. However, the published investigations have used different doses of radiation, various animal models, and a range of assessed end points.[12-14] Together the results suggest that probiotics can reduce radiation-associated small intestinal mucosal ulcerations, gram-negative bacteremia, endotoxemia, and early deaths. While supportive of the theory, the studies fell short on analytic rigor and the provision of meaningful mechanistic insight.

Animal studies have led to numerous proposed mechanisms for the pathology and symptoms that result from acute gastrointestinal radiation injury. Intestinal epithelial cell death, disruption of the interepithelial tight junctions, and changes induced in the microvascular endothelium are among proposed mechanisms.[15-19] The small intestinal epithelium is typified by a state of continuous and rapid cell turnover within the crypt. Multipotent epithelial stem cells produce rapidly dividing transit cells, which ultimately produce differentiated mature epithelial cells. These mature cells then migrate onto the villus. Noxious stimuli, including radiation, can kill epithelial cells and result in disruption of the epithelial membrane. After this injury, the stem cells proliferate, giving rise to an increased number of transit cells, which in turn form regenerative crypts to repopulate the mucosa. Evaluation of both the regenerative crypt number and positional apoptosis data is used to quantify radiation injury to epithelial stem cells.

Recent studies have provided both clinical support for and new insight into the signaling mechanisms by which bacteria may exhibit their radioprotective effects. We have found that the probiotic Lactobacillus rhamnosus GG has radioprotective capabilities. This unique probiotic, originally cultured by Gorbach and Goldin, demonstrates the ability to both resist bile and gastric acid as well as to successfully adhere to small intestinal mucosa.[20] Yan *et al*. showed *in vitro* that this bacterium, as well as its secreted protein, P40, can inhibit TNF-alpha induced epithelial cell apoptosis via activation of the antiapoptotic AKT pathway and inhibition of the proapoptotic P38/MAPK pathway.[21,22] Given our previous finding that PGE2 reduces radiation-induced epithelial apoptosis via a mechanism involving AKT activation,[23] we sought to determine whether LGG could exhibit intestinal radio-protection. Oral administration of LGG or its culture media supernatant prior to radiation increased the number of regenerative epithelial crypts and reduced epithelial cell apoptosis, particularly in the putative stem cell domain.[24] In experiments with mice deficient in MyD88 or cyclooxygenase-2 (COX-2), we found that the radio-protective effects of LGG are independent of TLR-mediated, MyD88-dependent signaling, yet require COX-2 (an enzyme

upstream of PGE2). Recent corroborative reports indicate that LGG can also improve intestinal epithelial barrier function via tight junction modifications.[25-27] Our future studies will be directed at further delineating the involved signaling pathways and identifying the effective soluble secreted factor(s).

Demonstrating that bacterial interactions can offer radioprotection via a different signaling pathway, Burdelya and colleagues used stimulation of the innate immune system to exploit pharmacologic mimicry of an antiapoptotic mechanism frequently acquired by tumor cells (constitutive activation of the nuclear factor–kB (NF-kB).[28] The investigators showed that a polypeptide drug derived from the flagellin of a Salmonella species could initiate an NF-KB dependent pathway to protect intestinal epithelial cells from radiation induced damage. The polypeptide's signaling occurred via TLR5 activation, and when administered prior to radiation exposure, it limited both histologic damage and radiation-associated lethality. Testing was successful in both mice and rhesus monkeys. The results of this study also underscored the necessity of administering a radioprotective agent as prophylaxis rather than postinsult.

Summary and Future Directions

This is an exciting time for the physiologic study of probiotics in health. Radiation enteritis should be considered as a potential model to study the interaction of probiotic bacteria with a disease state for several reasons. First, radiation-induced intestinal injury is a clinically prevalent condition for which few therapeutic options exists. Second, we now have both experimental and clinical trial data to suggest a potential benefit for probiotics. Finally, well-characterized animal and cell culture models of radiation injury exist and can be used in further studies to delineate both the optimal therapeutic conditions and the mechanistic pathways of specific probiotics, probiotic mixtures, or probiotic-derived products. In support of this, recent advances in radiation injury models suggest that isolation of specific TLR agonists or other soluble secreted proteins from probiotics may be equally or perhaps even more efficacious than the live bacteria.[24,28] Further characterization and testing of these novel findings will advance understanding of bacterial-host interaction, disease mechanism, and likely therapy.

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References

1. Zimmerer T, et al. Medical prevention and treatment of acute and chronic radiation induced enteritis – is there any proven therapy? A short review. Z Gastroenterol. 2008; 46:441–448. [PubMed: 18461520]
2. Fukata M, et al. Cox-2 is regulated by Toll-like receptor-4 (TLR4) signaling: Role in proliferation and apoptosis in the intestine. Gastroenterology. 2006; 131:862–877. [PubMed: 16952555]
3. Stenson WF. Toll-like receptors and intestinal epithelial repair. Curr Opin Gastroenterol. 2008; 24:103–107. [PubMed: 18301257]
4. Crawford PA, Gordon JI. Microbial regulation of intestinal radiosensitivity. Proc Natl Acad Sci USA. 2005; 102:13254–13259. [PubMed: 16129828]

5. Riehl T, et al. Radiation-induced Apoptosis is increased in MyD88-/- mice and the increase is reversed by PGE2 [abstract]. Gastroenterology. 2007; 132:A-566.
6. Floch MH, et al. Recommendations for probiotic use. J Clin Gastroenterol. 2006; 40:275–278. [PubMed: 16633136]
7. Delia P, et al. Use of probiotics for prevention of radiation-induced diarrhea. World J Gastroenterol. 2007; 13:912–915. [PubMed: 17352022]
8. Salminen E, et al. Preservation of intestinal integrity during radiotherapy using live Lactobacillus acidophilus cultures. Clin Radiol. 1988; 39:435–437. [PubMed: 3141101]
9. Giralt J, et al. Effects of probiotic lactobacillus casei DN-114 001 in prevention of radiation-induced diarrhea: results from multicenter, randomized, placebo-controlled nutritional trial. Int J Radiat Oncol Biol Phys. 2008
10. Osterlund P, et al. Lactobacillus supplementation for diarrhoea related to chemotherapy of colorectal cancer: a randomised study. Br J Cancer. 2007; 97:1028–1034. [PubMed: 17895895]
11. Urbancsek H, et al. Results of a double-blind, randomized study to evaluate the efficacy and safety of Antibiophilus in patients with radiation-induced diarrhoea. Eur J Gastroenterol Hepatol. 2001; 13:391–396. [PubMed: 11338068]
12. Demirer S, et al. Effects of probiotics on radiation-induced intestinal injury in rats. Nutrition. 2006; 22:179–186. [PubMed: 16459231]
13. Dong MY, Chang TW, Gorbach SL. Effects of feeding lactobacillus GG on lethal irradiation in mice. Diagn Microbiol Infect Dis. 1987; 7:1–7. [PubMed: 3319370]
14. Seal M, et al. Experimental radiotherapy-induced enteritis: a probiotic interventional study. J Dig Dis. 2007; 8:143–147. [PubMed: 17650226]
15. Paris F, et al. Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. Science. 2001; 293:293–297. [PubMed: 11452123]
16. MacNaughton WK. Review article: new insights into the pathogenesis of radiation-induced intestinal dysfunction. Aliment Pharmacol Ther. 2000; 14:523–528. [PubMed: 10792113]
17. Potten CS. A comprehensive study of the radiobiological response of the murine (BDF1) small intestine. Int J Radiat Biol. 1990; 58:925–973. [PubMed: 1978853]
18. Dublineau I, et al. Effects of chronic 137Cs ingestion on barrier properties of jejunal epithelium in rats. J Toxicol Environ Health A. 2007; 70:810–819. [PubMed: 17454557]
19. Dublineau I, et al. Functional and structural alterations of epithelial barrier properties of rat ileum following X-irradiation. Can J Physiol Pharmacol. 2004; 82:84–93. [PubMed: 15052289]
20. Conway PL, Gorbach SL, Goldin BR. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. J Dairy Sci. 1987; 70:1–12. [PubMed: 3106442]
21. Yan F, et al. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. Gastroent. 2007; 132:562–575.
22. Yan F, Polk DB. Probiotic bacterium prevents cytokine-induced apoptosis in intestinal epithelial cells. J Biol Chem. 2002; 277:50959–50965. [PubMed: 12393915]
23. Tessner TG, et al. Prostaglandin E2 reduces radiation-induced epithelial apoptosis through a mechanism involving AKT activation and bax translocation. J Clin Invest. 2004; 114:1676–1685. [PubMed: 15578100]
24. Ciorba MA, et al. Lactobacillus rhamnosus GG prevents radiation induced small intestinal injury in a MyD88 independent, but COX2 dependent manner [abstract]. Gastroent. 2008; 134(4 Supp 1):M1198.
25. Johnson-Henry KC, et al. Lactobacillus rhamnosus strain GG prevents enterohemorrhagic Escherichia coli O157:H7-induced changes in epithelial barrier function. Infect Immun. 2008; 76:1340–1348. [PubMed: 18227169]
26. Chang S, et al. Lactobacillus GG prevent impaired barrier function in intestinal epithelial cells [abstract]. Gastroent. 2008; 134(4 Supp 1):M1737.
27. Seth A, et al. Probiotics ameliorate the hydrogen peroxide-induced epithelial barrier disruption by a PKC- and MAP kinase-dependent mechanism. Am J Physiol Gastrointest Liver Physiol. 2008; 294:G1060–1069. [PubMed: 18292183]

28. Burdelya LG, et al. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. Science. 2008; 320:226–230. [PubMed: 18403709]