• **Title:** Microbiota and radiation-induced bowel toxicity – lessons from inflammatory bowel disease for the radiation oncologist.

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ABSTRACT

New gastrointestinal symptoms are frequent after pelvic radiotherapy and can impact significantly on the quality of life of cancer survivors. The intestine harbours an important organ: the microbiota. The impact of radiation on the flora and the clinical implications of a modified microbial balance after radiotherapy are now beginning to emerge. In this review, we highlight the importance of the microbiota for intestinal homeostasis and discuss the similarity between inflammatory bowel disease, which has been extensively researched, and radiation-induced gastrointestinal toxicity. Using microbiota profiles for risk assessment, as well as manipulating the intestinal flora for prevention and treatment of radiation enteropathy may become a reality and would be of considerable relevance to the increasing numbers of long term cancer survivors.

INTRODUCTION

The discovery of ionizing radiation by William Roentgen in 1895 started a new era in cancer research and therapeutics. The first attempt to use radiotherapy in cancer management occurred in 1896, with damaging effects to the intestine being described one year later. This makes gastro-intestinal (GI) radiation-induced toxicity one of the first reported challenges to radiation therapy.

The intestine is within the treatment field for all intra-abdominal, retroperitoneal and pelvic tumours. Radiation enteropathy can be defined as a progressive, ischaemic, profibrotic process occurring after irradiation of the pelvis and/or the abdomen, which is driven by pathophysiological processes which are yet inadequately defined. In clinical practice and human studies, the presence of significant GI toxicity is defined by the development of any new GI symptoms during or after radiotherapy. This definition has significant shortcomings, but is currently the only realistic definition pending the development of objective biomarkers of toxicity. In the UK, about 17000 patients receive pelvic radiation as part of their cancer treatment a year, with 90% of patients reporting a change in their bowel function. This results in a negative impact on daily activity in up to 50%.

Intestinal toxicity is a multifactorial problem, related not only to the way and dose of radiation delivered, but also to an intrinsic process within tissues responding to cellular injury which is independent of the radiation dose. Individual genetic background and expression pattern, pre-morbid conditions such as diet and smoking, as well as the cellular microenvironment, may be important, although their exact contribution to the development of toxicity is not known.

The human gastrointestinal tract contains 1-2 kg of bacteria and their role in initially driving or controlling intestinal inflammation has become increasingly evident. The intestinal microbiota, a term which collectively refers to the micro-organisms living in...
the gut, may play an important role in the development and perpetuation of intestinal radiation injury. This review explores mechanisms that may be altered in the host-microbiota relationship following pelvic irradiation and draws parallels with findings in inflammatory bowel disease (IBD).

SEARCH STRATEGY AND SELECTION CRITERIA

In order to review the existing literature on the microbiota-host relationship during and after abdomino-pelvic radiotherapy and to draw parallels with IBD, a search of original published studies and reviews until 2013 was done by use of MEDLINE and EMBASE databases. Search terms included: “radiation enteritis OR enteropathy”, “radiation proctitis OR proctopathy”, “radiation colitis OR colopathy”, “radiotherapy”, “IMRT”, “late toxicity”, “gastrointestinal symptoms”, “quality of life”, “gastrointestinal motility”, “prostate”, “gynaecological”, “microbiota OR microbiome”, “intestinal bacteria”, “dysbiosis”, “antibiotics”, “16S rRNA”, “metabolomics”, “metabonomics”, “IBD”, “Inflammatory Bowel Disease”, “Crohn’s disease” and “Ulcerative Colitis”. Search results were manually screened for relevance and potentially relevant papers retrieved. Reference lists in every paper were scrutinised to identify other possible relevant studies. Where appropriate, relevant chapters in reference books in the subject were analyzed and are referenced.

THE GUT AND THE MICROBIOTA: A BALANCING ACT

Composition of the microbiota, orthobiosis and dysbiosis

A healthy adult's intestine hosts up to $10^{14}$ microbes, with 300 to 500 species of bacteria, dominated by two phyla: Firmicutes and Bacteroidetes. Proteobacteria, Actinobacteria, Fusobacteria, Cyanobacteria and Verrucomicrobia, are present in much smaller numbers. Other organisms present in our gut include eukaryotes (0.5%), archae (0.8%) and viruses (5.8%).

A healthy microbiota is difficult to define, as the intestinal flora is unique to each individual at the species and strain levels. However, there are communities that are associated with gut health at the family and class levels – this means that even if species slightly differ, an overall homology in intestinal microbial groups defines a healthy gut flora. Furthermore, microbial composition in health remains stable for 60% of the species over time in a single individual. Recently, three clusters of microbial populations, referred to as enterotypes, have been described, defining three different “standard” microbial populations: enterotype 1, enriched with Bacteroides; enterotype 2, enriched with Prevotella; and enterotype 3, the most frequent of all, enriched with Ruminococcus. However this classification remains controversial.

The human intestine is colonised by microbes immediately after birth. Environmental factors such as diet and genetic background of the host then stochastically shape the microbiota into a stable community of symbionts. Its resilience to environmental factors is clearly shown by stability over time within an individual.
“orthobiosis” is used for the balanced composition of the indigenous microbiota which produces a beneficial influence of human health. In contrast, the global imbalance of bacterial populations in the gut is termed “dysbiosis” and its association with a number of disease processes including IBD is clearly established.\textsuperscript{16} The “orthobiotic” microbiota is species-specific, as demonstrated in a study of reciprocal transplanted microbiota between mice and zebrafish where a shift in the microbial community back towards the conventional profile of their respective species was seen.\textsuperscript{17} Orthobiosis is, therefore, actively sought by the healthy organism.

**Host-microbiota symbiosis**

Microbes obtain nutrients from the host, as well as protection from competing species by the immune system. However, they benefit the host through different interactions.

*Microbiota and the intestinal epithelium*

The microbiota plays a central role in the development of the epithelium. Germ-free animal models have decreased epithelial proliferation in their crypts of Lieberkühn when compared to conventionally-raised (reared normally) and conventionalized (born germ-free and later colonized with a normal microbiota) animals.\textsuperscript{18} This is an evolutionarily-conserved pattern.\textsuperscript{19}

A molecule produced by the Gram-positive *Bacillus subtilis* interacts with epithelial cells, activating survival pathways and enhancing antioxidant capacity.\textsuperscript{20} *B. subtilis* seems to counteract cytotoxic effects of both radiotherapy and chemotherapeutic agents\textsuperscript{21} and hints at an inherent property of the microbiota that could be exploited for patient benefit during cancer therapy.

*Microbiota and the immune system*

The enteric flora “educates” the immune system from early life, while the immune system shapes and closely interacts with the microbiota.\textsuperscript{22} Germ-free animals have a rudimentary mucosal-associated lymphoid tissue and defects of cell-mediated immunity.\textsuperscript{7}

The immune system has to distinguish between beneficial commensals and harmful pathogens within a heterogeneous gut microbial community.\textsuperscript{11,23} Some bacterial species directly contact epithelial cells or dendritic cells (DCs). Antigens ingested by M cells in Peyer’s patches may also be sampled by DCs.

To sense the microbiota, the host uses pattern recognition receptors: these are the Toll-like receptors (TLRs) and the nucleotide-oligomerization domains (NODs), found in the intestinal epithelium and in immune cells. These receptors recognize bacterial components that may be microbe-associated molecular patterns (MAMP) or pathogen-associated molecular patterns (PAMP). TLRs are transmembrane proteins and may be located at the surface of cells or intracellularly, within the endosomal membranes. They recognize different components of bacteria. PAMP-TLR binding
leads to an immune response to eradicate potentially harmful pathogens. MAMP-TLR binding tends to activate pathways involved in gut homeostasis (see fig.1).\textsuperscript{11,24}

The relevance of TLRs for patients undergoing radiotherapy has been highlighted by CBLB502, a derivative of the bacterial component flagellin, which possibly promotes radioresistance in normal tissues. Flagellin leads to NFkB activation by binding to TLR5, inhibiting p53 function and inducing factors contributing to cell protection and tissue regeneration, including reactive oxygen species scavengers, apoptosis inhibitors and cytokines. In addition, flagellin is immuno-stimulatory, thereby promoting a tumouricidal effect.\textsuperscript{24, 25}

Formylated peptide receptors (FPRs) are expressed in neutrophils, macrophages and intestinal epithelial cells. They recognize bacterial cell wall products tagged with an N-formyl group, leading to radical oxygen species formation, which, at the high levels elicited by pathogens, are microbicidal. They also lead to chemotaxis and upregulate pro-inflammatory pathways. Interestingly, by eliciting small increases in reactive oxygen species through FPR activation, gut commensals enhance antioxidant capacity, cytoprotection and epithelial restoration.\textsuperscript{26}

\textbf{Microbiota and metabolism}

The human gut microbial genome, collectively known as the intestinal microbiome, is enriched with metabolic genes involved in the degradation and processing of diet-derived substrates, producing energy sources usable by both the host and the symbiont. It also produces vitamins and amino acids.\textsuperscript{9,11}

A dysbiotic milieu may have a metabolic impact and provoke increased inflammation. Short chain fatty acids (SCFA), are known to have immuno-modulatory properties and play a role in radiation-induced bowel toxicity. Their metabolism is affected by dysbiosis.\textsuperscript{27,28} Moreover, microbial imbalances contribute to carcinogenesis in the colon through chronic inflammation, immune evasion or suppression and by modifying the metabolism of carcinogenic substances.\textsuperscript{29}
Figure 1: Interactions between the microbiota and the intestinal epithelium

Orthobiosis, through different interactions with intestinal tissues, protects them from radiation effects. Inversely, dysbiosis potentially contributes to deleterious effects provoked by radiation to the healthy gut.
THE MICROBIOTA AND RADIATION-INDUCED BOWEL TOXICITY

Pelvic radiation disease has been defined as transient or longer term problems, ranging from mild to very severe, arising in healthy tissues resulting from radiotherapy to a tumour of pelvic origin.\textsuperscript{30} Radiation enteropathy (see fig. 2), one of its components, has been arbitrarily divided in acute (until 90 days after radiotherapy) and late enteropathy (occurring thereafter). Acute changes in the gastrointestinal tract are mediated by the cytotoxic effect of radiation to the rapidly proliferating epithelium, which is amplified by inflammation. Both determine increases in free radicals, damaging the DNA and producing functional effects.\textsuperscript{31} Eosinophilic crypt abscesses are the most characteristic change seen acutely with radiotherapy.\textsuperscript{4} Activation of the coagulation system leads to ischemia and further necrosis. Increased formation of thrombin, a serine protease, is important in promoting thrombus formation and platelet activation, but also in regulating inflammation by enhancing epithelial permeability and recruiting neutrophils, and monocytes.\textsuperscript{32}

This mechanism may lead to ulceration, exposing underlying tissues to bacteria, changing the host-microbial interaction and increasing inflammation, as the immune system struggles to contain bacterial translocation. The ulcer may then progress to fibrosis, a process initially driven by a TGFβ1-mediated phenotypic switch turning fibroblasts into collagen matrix-producing myofibroblasts. However, ulceration is not necessary for a fibrotic process to occur, as microvascular lesions and inflammation are sufficient to initiate fibrosis, which progresses independently of inflammation.\textsuperscript{33} Furthermore, intestinal tissues with a delayed radiation response may become necrotic, eliciting a sustained inflammatory response.\textsuperscript{34}

Radiation toxicity has been viewed as a consequence of the cytotoxic effects on target cells, dividing early and late reacting tissues according to their radiosensitivity, repair capacity, proliferation rate and tissue organization. While radiation kills cells by direct or indirect effects on the DNA,\textsuperscript{21} toxicity is also mediated by other mechanisms: indirect effects, such as inflammation or bystander effects; and functional effects, such as modification of gene expression.\textsuperscript{3} In addition to the repetitive and cumulative nature of damage to normal tissue induced by fractionated radiotherapy, a non-healing acute response may progress into a late effect. This is termed a consequential effect, defined as late effects which in their frequency or severity are influenced by the severity of the acute changes in the same tissue, including grade or duration.\textsuperscript{34}

Dysbiosis may play a role in radiation enteropathy. The gut flora is heterogeneous, with different microorganisms having different radiosensitivities. Furthermore, commensals are in close interaction with an environment that dramatically changes during pelvic radiotherapy. Therefore, dysbiosis is not only likely to develop, but also contribute to toxicity.
Figure 2: Mechanisms of radiation-induced gastrointestinal symptoms

Several mechanisms act concurrently to provoke radiotherapy-induced gastrointestinal symptoms, both in the acute and late settings.
Preclinical studies

In the 1960s, it was discovered that irradiated germ-free mice developed fewer gastrointestinal symptoms, leading to studies using antibiotics as radiation response modifiers, which had conflicting results. In 1981 it was observed that there was a significant increase in the number of bacteria on the intestinal villi of rats after irradiation, suggesting that radiation-induced changes to the intestine are associated with changes in the microbiota.

Lipopolysaccharide (LPS), a component of the external membrane of Gram-negative bacteria, is a radioprotectant in mice by a mechanism involving the TNF receptor 1 and apobec-1, which binds and stabilizes cyclooxygenase-2 RNA in subepithelial fibroblasts, leading to an increase in prostaglandin E2. Tumour necrosis factor alpha (TNFα) is likely to be the relevant ligand involved in this pathway. LPS also promotes the gene expression of the radioprotectant IL-1α in mice, an effect which is dependent of the murine P-glycoprotein.

LPS also promotes the gene expression of the radioprotectant IL-1α in mice, an effect which is dependent of the murine P-glycoprotein.

Germ-free mice are radioresistant to total body irradiation with 10-22 Gy followed by a bone marrow transplant (to avoid marrow failure). Animals colonized only with B. thetaiomicron and E. coli have similar outcomes. In contrast, conventionally-raised and conventionalized animals have radiosensitive phenotypes. As germ-free animals are known to have an impaired epithelial turnover, this eliminates differences due to germ-free rearing. A protein called fasting-induced adipose factor, also known as angiopoietin-like protein 4, is involved in this difference. The parallel with IBD is noteworthy: colonization with a microbiota is necessary for the development of colitis in mouse models, while germ-free mice are resistant.

Probiotics are live organisms that, when consumed in an adequate amount, confer a health effect on the host, whereas prebiotics are non-digestible foods that promote the growth or activity of specific micro-organisms, promoting a health effect. Synbiotics are mixtures of prebiotics and probiotics. The use of these three types bacterial supplementation has been explored pre-clinically using single fraction irradiation. Results were encouraging, leading to less radiation-induced diarrhoea and histological damage, but none of these studies used a probiotic specifically designed to reduce toxicity.

The medical relevance of these observations, produced by a small number of studies, is nevertheless questionable. Single-fraction radiotherapy does not adequately reproduce the conditions of fractionated therapy. Moreover, radiosensitivity differs between animal models and the microbiota is also known to vary between species. Finally, these studies focus only on acute radiation-induced changes.
Clinical studies

**Bacterial overgrowth**

Small intestinal bacterial overgrowth (SIBO) is a manifestation of radiation enteropathy, both in the acute and late settings.\(^4\) A study of 41 patients with late intestinal toxicity reported the relationship between migrating motor complex patterns and overgrowth of gram negative bacilli (GNB), concluding that impaired motility is a cause of gastrointestinal GNB colonization.\(^{45}\) However, this associative study did not have baseline data, making it difficult to discern cause and effect. A recent study, in a cohort of 39 patients undergoing pelvic radiotherapy, used glucose hydrogen breath testing, a test with suboptimal specificity and sensitivity, performed at baseline and at 4-5 weeks of treatment. The incidence of SIBO was observed to be 26%.\(^{46}\)

**Dysbiosis and radiation**

A further study\(^{47}\) evaluated faecal microbial populations of ten patients undergoing pelvic radiotherapy by DNA fingerprinting and sequencing of the 16S rRNA gene (unique to prokaryotes) at baseline, during and after treatment. The clinical endpoint was the onset of diarrhoea. The six symptomatic patients showed progressively increasing dysbiotic pattern, which was maintained after radiotherapy. In a subgroup analysis (two patients with and two without diarrhoea) comparing microbial profiles, a clustering of patients with diarrhoea is reported, with an overall increase in Bacilli and Actinobacteria and a decrease in Clostridia. However, this study had a small number of patients and it only addressed acute diarrhoea, which is not the sole symptom of acute radiation enteropathy. The authors did not record if dysbiosis started after or before the onset of diarrhoea, so any relationship with causality was lost.

**Surgery and gastrointestinal toxicity**

There are several reports on the predictive value of previous abdominal surgery for the development of gastrointestinal toxicity. A higher incidence of rectal bleeding after radiotherapy has been reported to be associated with previous abdominal surgery (namely cholecystectomy and appendectomy)\(^{48,\text{(appendix3)}}\) in some but not all studies.\(^{49,\text{(appendix2)}}\) We should note that, even though appendectomy has been suggested as a risk factor for late radiation-induced rectal bleeding, there is considerable evidence that it is protective against the development of ulcerative colitis.\(^{48}\) Valdagni et al. suggest a possible mechanism for this potential increase in susceptibility in rectal toxicity after appendectomy, as, by removing an important reservoir of gut-protecting bifidobacteria, this surgical procedure may be removing important protectors of intestinal homeostasis, but this needs further corroborative research. Even though they are present in the appendix, bifidobacteria are present in higher densities in the distal colon\(^{51}\) and appendectomy has not been associated with an increased toxicity in other areas of the bowel.
Interestingly, the microbiota is known to be altered not only structurally but also functionally after bariatric surgery, with a shift towards a Proteobacteria-dominated flora. It should be noted that Proteobacteria and other Gram-negative bacilli are known to be increased in the advent of radiation-induced late intestinal toxicity. Even though these observations are intriguing, available studies reporting on bacterial profile and/or function modification after surgery are scarce. It is also difficult to dissociate the influence of the microbiota from alterations in gut immunity and inflammation after surgery. Moreover, these patients are commonly treated with large doses of antibiotics, which may be a cause for long-term imbalance of the microbiota and pathogen-derived colitis. Further studies in this area are therefore necessary to assess the impact of surgery on the structure and function of gut commensals and its possible role in the predisposition to radiotherapy-induced gastrointestinal toxicity.

**Microbiota interventions**

Clinical studies addressing probiotic, prebiotic or synbiotic interventions are summarized in table 1. Although each study claims significant benefits in either preventing or treating radiotherapy-induced gastrointestinal symptoms, the evidence is not compelling. Very few of the studies used widely accepted toxicity endpoints and none has been reproduced in large cohorts. Nevertheless, they show that attempts at bacteriotherapy appear safe in the context of radiation enteropathy.
<table>
<thead>
<tr>
<th>Authors &amp; Year</th>
<th>Type of Study</th>
<th>Preparation</th>
<th>Number of Patients randomized analyzed</th>
<th>Type of Malignancy</th>
<th>Type of Treatment</th>
<th>Claimed effect of intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salamon et al. 1988</td>
<td>Preventative, RCT</td>
<td><em>Lactobacillus acidophilus</em> 2x10^9 CFU/4 x 3 weeks + lactulose</td>
<td>24/21</td>
<td>Gynaecological tumours</td>
<td>50 Gy to pelvis and 80 Gy to femoral (radiotherapy boost)</td>
<td>Reduction of diarrhoea (p&lt;0.01)</td>
</tr>
<tr>
<td>Urbanke et al. 2001</td>
<td>Therapeutic, Double-blind RCT with placebo</td>
<td><em>Lactobacillus rhamnosus</em> 1.5x10^6 CFU/1.1.d + lactulose</td>
<td>205/205</td>
<td>Several lower abdominal and pelvic tumours</td>
<td>50 Gy</td>
<td>No significant differences in the need for rescue anti-diarrhoeal therapy</td>
</tr>
<tr>
<td>Delan et al. 2007</td>
<td>Preventative, Double-blind RCT with placebo</td>
<td><em>VSL#3</em> (L. casei, L. plantarum, L. acidophilus, L. delbrueckii subsp. bulgaricus, R. homaei, B. bifidum, Streptococcus thermophilus subsp. thermophilus) 450x10^6 CFU/kg 1,1.d</td>
<td>460/462</td>
<td>Post-operative cervical, sigmoid and rectal tumours</td>
<td>Adjuvant radiotherapy, 68-70 Gy</td>
<td>Reduction in diarrhoea incidence and severity (p&lt;0.001), reduction in need of rescue anti-diarrhoeal therapy (p&lt;0.03)</td>
</tr>
<tr>
<td>Giral et al. 2008</td>
<td>Preventative, Double-blind RCT with placebo</td>
<td><em>Lactobacillus casei</em> DM 214 007 [10^9 CFU 1,1.d</td>
<td>118/82</td>
<td>Cervical and endocervical tumours</td>
<td>Adjuvant radiotherapy, 45-50.4 Gy and weekly cisplatin (40 mg/m²)</td>
<td>Improved stool consistency (p&lt;0.05), no significant differences in need of rescue anti-diarrhoeal therapy and GI toxicity</td>
</tr>
<tr>
<td>Chitrapunyav et al. 2010</td>
<td>Preventative, Double-blind RCT with placebo</td>
<td><em>Lactobacillus acidophilus</em> plus <em>Bifidobacterium bifidum</em> 2x10^9 CFU/kg b.i.d</td>
<td>63/63</td>
<td>Cervical tumours</td>
<td>50 Gy to the tumour and pelvis, 50 Gy to the para-aorta and radiotherapy boost (59.7 Gy), plus weekly cisplatin (40 mg/m²)</td>
<td>Reduction in diarrhoea severity (p=0.002), reduction in need of rescue anti-diarrhoeal therapy (p&lt;0.05), Improved stool consistency (p&lt;0.001)</td>
</tr>
</tbody>
</table>

Table 1: Main bacteriotherapy studies for the treatment and/or prevention of radiation enteropathy
RADIATION-INDUCED GASTROINTESTINAL TOXICITY AND INFLAMMATORY BOWEL DISEASE: SOME SIMILARITIES

Several mechanisms implicating the microbiota seem to be common between IBD and radiation enteropathy. IBD involves the colon and small intestine, with the two main forms being Crohn’s disease (CD) and ulcerative colitis (UC).

Clinical comparison

Clinically, IBD and radiation enteropathy are often characterized by symptoms such as diarrhoea, rectal bleeding and malabsorption. Another common feature is pronounced infiltration of innate and adaptive immune cells into the lamina propria, accompanied by epithelial destruction and mucosal ulceration. While UC tends to be confined to the mucosa, CD and radiation enteropathy tend to be transmural. Submucosal fibrosis, which seems to be triggered in part by oxidative stress, may feature. Furthermore, barrier function is impaired in these diseases, as defects in the epithelial tight junctions lead to an increase in paracellular permeability.

Studies suggest that patients with Crohn’s disease and ulcerative colitis have a 29%-46% increased risk of severe acute or chronic complications after radiation treatment (Table 2). The risk of high rates of significant acute toxicity has led some oncologists to conclude that patients with IBD should not receive pelvic radiotherapy. These studies have methodological issues and have included limited patient cohorts.

Inflammatory mechanisms

Radiation enteropathy, UC and CD share some inflammatory mechanisms: ionizing radiation induces the gene expression and secretion of cytokines including IL-1β, TNFα, and TGFβ, which are also upregulated in IBD. The mechanism of fibrosis in late radiation enteropathy has been linked to TGF-β1 and its downstream effectors and may progress independently of inflammation, as observed in IBD. Furthermore, an imbalance in the ratio between the IL-1 receptor antagonist and IL-1β has been shown in all these conditions. However, while IBD is idiopathic, radiation toxicity has a defined stimulus, and the inflammatory component is less prominent. Involved antioxidant pathways also differ and the apoptotic component is more prominent in radiation enteropathy.

Gene associations

Associations between IBD and polymorphisms of the NOD2 gene, Th17 pathway, MHC and epithelial barrier genes have been well characterized. In contrast, few susceptibility genes have been associated with late radiation enteropathy and a recent study failed to validate any clinical effect of published single nucleotide polymorphisms associated with late toxicity.
Table 2 - Studies reporting the risk of toxicity from pelvic radiotherapy in patients with Inflammatory Bowel Disease (IBD).

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Type of radiotherapy</th>
<th>Type of case series</th>
<th>Number of patients</th>
<th>IBD type</th>
<th>Primary cancer</th>
<th>Follow up (months)</th>
<th>Acute toxicity (≥Grade3)</th>
<th>Chronic toxicity (≥Grade3)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiersten et al.</td>
<td>1996</td>
<td>EBRT</td>
<td>Retrospective</td>
<td>5</td>
<td>Not specified</td>
<td>GI</td>
<td>Not specified</td>
<td>0%</td>
<td>N/A</td>
<td>Not specified</td>
</tr>
<tr>
<td>Gramm et al.</td>
<td>1998</td>
<td>BT, BT+EBRT</td>
<td>Retrospective</td>
<td>5</td>
<td>Not specified</td>
<td>3 UC, 3 CD</td>
<td>Prostate</td>
<td>42</td>
<td>0% (100%)</td>
<td>0%</td>
</tr>
<tr>
<td>Green et al.</td>
<td>1999</td>
<td>EBRT</td>
<td>Retrospective</td>
<td>15</td>
<td>N/A</td>
<td>Rectum</td>
<td>60</td>
<td>7%</td>
<td>13%</td>
<td>Not specified</td>
</tr>
<tr>
<td>Willett et al.</td>
<td>2000</td>
<td>EBRT</td>
<td>Retrospective</td>
<td>28</td>
<td>UC, CD</td>
<td>Various</td>
<td>32</td>
<td>21%</td>
<td>29%</td>
<td>42% active IBD at start. Chronic severe toxicity more common in UC</td>
</tr>
<tr>
<td>Song et al.</td>
<td>2001</td>
<td>Not specified</td>
<td>Retrospective</td>
<td>24</td>
<td>UC, CD</td>
<td>Various</td>
<td>Median: 11 Range:1-137</td>
<td>19%</td>
<td>13%</td>
<td>42% active disease at start. Severe toxicity associated with concurrent chemotherapy.</td>
</tr>
<tr>
<td>Peters et al.</td>
<td>2006</td>
<td>BT, BT+EBRT</td>
<td>Retrospective</td>
<td>11(?), 13(?)</td>
<td>17 UC, CD</td>
<td>Prostate, Prostate</td>
<td>48.5</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Barnett et al.</td>
<td>2011</td>
<td>EBRT</td>
<td>Prospective</td>
<td>35</td>
<td>Not specified</td>
<td>Prostate</td>
<td>Up to 5 years</td>
<td>Not specified</td>
<td>Not specified</td>
<td>Increased acute &amp; late toxicity</td>
</tr>
<tr>
<td>Pal et al.</td>
<td>2012</td>
<td>BT</td>
<td>Retrospective</td>
<td>13</td>
<td>UC, CD</td>
<td>Prostate</td>
<td>4-2 years</td>
<td>23%</td>
<td>15%</td>
<td>Endoscopic biopsies -&gt; ulceration</td>
</tr>
</tbody>
</table>

EBRT = External beam radiotherapy. BT = Brachytherapy. UC = Ulcerative colitis. CD = Crohn’s disease. GI = gastrointestinal. N/A = Not applicable.

Please see references 4-10 in the appendix and reference 49 in the text.
INFLAMMATORY BOWEL DISEASE: A BLUEPRINT FOR RADIATION-INDUCED BOWEL TOXICITY?

Dysbiosis

Patients with IBD have depletion and reduced diversity of the Firmicutes and Bacteroidetes phyla, resembling what is observed after radiotherapy.\(^{47,53}\) They also have an increase in Proteobacteria,\(^{27}\) as in radiation enteropathy. The Proteobacteria phylum includes pathogens such as \(E.\) \(coli\) or the \(Shigella\) and \(Klebsiella\) genera.\(^{29,45}\) Dysbiosis is therefore a common feature, with a decrease in species involved in intestinal homeostasis and an increase in pathogens. When dysbiosis is established in a predisposed individual at the onset of radiotherapy, it may provoke increased severity of intestinal toxicity, but also secondary injury to exposed tissues.

An alternative hypothesis is that alterations of microbial composition and function in the gut are a consequence and not a cause of inflammation, supported by the facts that no particular microorganism has been implicated as a pathogen in IBD and that the pathogenic mechanisms are still obscure.\(^{16}\) A study performed on a colitis mouse model showed that inflammation drove dysbiosis, with the latter abating with anti-TNF\(\alpha\) treatment.\(^{61}\) Moreover, antibiotics do not seem to have an effect in UC, even though they sometimes provide benefit in CD. Additionally, probiotics seem to be of some value in UC and pouchitis, the most common and clinically relevant complication of ileal pouch-anal anastomosis after colectomy.\(^{62}\) Their effects in CD are far less evident.\(^{63}\)

Nevertheless, recent evidence suggests that the overall microbiota profile is important. Transmission of a colitogenic enteric flora was shown to be necessary for transmission of the disease to progeny of a transgenic colitis mouse model.\(^{64}\) The authors propose that rising TNF\(\alpha\) leads to increased intestinal permeability and apoptosis, provoking ulceration and contact of the bacteria with TNF\(\alpha\), shifting bacterial populations and/or enhancing their virulence. TNF\(\alpha\) is known to be involved in both late radiation enteropathy and IBD.\(^{2,53}\) Microbial gene expression patterns may be altered in a colitic environment, and in turn, may contribute to disease. This should be highlighted when looking at the role of microbes, as they turn from friend to foe.

Disease mechanisms

Recent research has clarified how the microbiota interacts with host homeostasis. These mechanisms may be important in GI symptoms observed after abdomino-pelvic radiotherapy.

Changes in profile and function

A study involving 231 subjects revealed 12% of microbial metabolic pathways to be altered in IBD patients, compared to only a 2% change in the microbial genera profile.\(^{27}\) There is a shift towards a phenotype that allows the microbiota to cope with oxidative stress. Glutathione, an antioxidant synthesized by Proteobacteria and a few
streptococci and enterococci, is functionally enhanced. Moreover, there is an increase in riboflavin biosynthesis and in genes of the pentose-phosphate pathway in patients with CD. Riboflavin is necessary for regenerating oxidized glutathione, as is nicotinamide adenine dinucleotide phosphate (NADPH), a product of the pentose-phosphate pathway. This reflects that dysbiosis associated with intestinal inflammation promotes a selection of bacteria capable of withstanding a highly oxidative environment, also present after radiotherapy. Additionally, there is an increase in genes involved in pathogenesis and virulence, which is consistent with a model where inflammation, by provoking tissue damage, releases metabolites, thereby modifying selective pressures. Similar processes may be a part of the mechanism of radiation enteropathy.

Bile acids

Anaerobic bacteria convert primary into secondary bile acids (BAs). Secondary BAs are anti-inflammatory: they inhibit TNFα, IL1α, IL1β and IL6 through the BA-specific receptor TGR5. Dysbiosis, in the context of IBD, modifies this balance, as it decreases the production of secondary BAs, creating a pro-inflammatory environment. Imbalances in the microbiota produced after intestinal radiation seem to recapitulate observations made in the context of IBD, so this mechanism may also be of importance in radiation enteropathy, but further research is needed.

Short-chain fatty acids

Propionate, butyrate and acetate are SCFA with anti-inflammatory properties, suppressing pro-inflammatory cytokines such as NFκB, TNFα, IL-1α, or IL-6. Butyrate also has a role in maintaining the stability of the intestinal epithelial barrier. The microbiota partly determines the availability and production of these molecules. Fibrolytic bacteria degrade large polysaccharides into smaller carbohydrates, which are then fermented into SCFAs. Roseburia is an acetate consumer and butyrate producer, while Phascolarctobacterium is a propionate producer and they are both reduced in IBD. Faecalibacterium prausnitzii is a major butyrate producer and is reduced in CD. These effects are clinically relevant in IBD and in acute radiation proctitis. They may also be relevant in radiation enteropathy.

FUTURE PROSPECTS – TREATMENT AND RISK ASSESSMENT OF RADIATION ENTEROPATHY

Research and characterization of the microbiota is enhanced by new technologies which give genomic and metabolomic profiles. However, clinical studies in this area are hampered by the lack of an objective biomarker of toxicity. Clinical scores are inadequate for precise measurement of radiation effects. Additionally, future work will need to account for the complex dose-volume distributions achieved by modern radiotherapy and their potential impact on the gut microbiota, which are, however, beyond the scope of this paper.
The development of next-generation sequencing technologies and metabolic phenotyping may make stratification of patient risk for radiotherapy-induced GI toxicity a realistic possibility. This is supported by reports of common bacterial traits in patients with radiation-induced GI symptoms. A recent pilot study employed an electronic nose and field asymmetric ion mobility spectrometry to detect selected metabolites in the stool of 23 patients before and four weeks after pelvic radiotherapy, with promising results in risk prediction. It is attractive to attribute this difference to changes in the microbiota. Nevertheless, these results should be carefully interpreted, since faecal metabolomes do not currently stratify populations according to their gut bacteria.

Faecal microbiota transplantation (FMT) has been recently reintroduced as a treatment for C. difficile-induced colitis and evidence of its benefit is steadily growing. This technique involves the transplantation of a faecal sample from a donor to a patient. The initial approach with patient appointed donors has evolved to volunteer donors, by banking frozen processed faecal material. Modification of a colitogenic microbial profile by introducing a healthy donor’s microbiota may result in a clinical benefit for the patient. Even though its potential in IBD has been studied, evidence comes mainly from case reports and small trials, mostly showing a benefit. There are still concerns about the safety of this technique, since available evidence is limited. Therefore, larger, randomised trials are necessary. FMT has never been tried in the context of radiation intestinal toxicity. Its clinical potential seems nevertheless exciting, as this may be an inexpensive, potentially efficacious radiation-response modifier, possibly allowing for increases in the therapeutic index of pelvic radiotherapy.

Overall, the potential of the microbiota as a risk assessment and treatment tool for radiation-induced gut toxicity seems promising. If confirmed, it may be an important step forward in oncology, allowing for inexpensive, patient-tailored treatment to modulate toxicity.

REFERENCES


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APPENDIX

Supplementary Reading

Microbiota and radiation-induced bowel toxicity – lessons from inflammatory bowel disease for the radiation oncologist.


