Association of Radiotherapy-Related Intestinal Injury and Cancer-related Fatigue: A Brief Review and Commentary

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Radiotherapy treatment–induced intestinal injury and gut microbial perturbation/dysbiosis have been implicated in the pathobiology of cancer-related fatigue. The objective of this brief review was to explore the available evidence of the relationship between intestinal injury and self-reported fatigue, especially among cancer patients. The scientific evidence—including our own—linking gut mucosal barrier dysfunction and gut microbial perturbation/dysbiosis induced by cancer treatment with worsening of cancer-related fatigue (perhaps through the gut-brain axis) is limited but promising. Emerging data suggest that lifestyle interventions and the administration of specific probiotics may favorably modulate the gut microbiota and potentially mediate beneficial effects leading to improvements in fatigue. [*P R Health Sci J 2021;40:6-11*]

Key words: Radiotherapy-related fatigue, Intestinal injury, Dysbiosis

Radiotherapy-related fatigue (RRF) is an unpleasant and distressing symptom experienced by approximately 67 to 74% of cancer patients (1,2), yet health care providers may under-recognize and undertreat it (3). RRF may also result in unwanted morbidities that have a great impact on health-related quality of life and the physical functioning of cancer survivors (4). A major gap in preventing and managing RRF is that its biological mechanisms have not been identified. One pivotal mechanism that may contribute to RRF development or exacerbation is the gut-brain axis.

Based on the renewed interest in the gut-brain axis and notable advances in high-dimensional sequencing techniques and in data analysis, the possible role of intestinal injury in the pathobiology of RRF is being further explored (5,6). More specifically, it has been proposed that mucosal barrier dysfunction and gut microbial dysbiosis induced by cancer treatment may contribute to the worsening of comorbidities, such as fatigue during a patient's pelvic radiotherapy (RT) (5,6,7,8). This brief review explores the available evidence of the relationship between intestinal injury and fatigue, especially among cancer patients.

Leaky-Gut

Although the mechanisms underlying the structure and the regulation of the epithelial mucosal barrier are complex (9), evidence suggests that acute damage induced by RT treatments can result in gut mucosal barrier dysfunction or leaky gut, which in turn may be related to RRF. For example, evidence suggest that pelvic RT destroys intestinal integrity and increases intestinal permeability, which is greater at the end of RT, producing a leaky gut (10,11). A leaky gut may facilitate the entry of large amounts of intestine-derived toxic factors (e.g., lipopolysaccharides, also called LPSs) into systemic circulation and may induce the aberrant activation of the immune system, resulting, for example, in cytokine-induced inflammatory reactions, which can affect brain function and increase symptom severity (5,7,8).

Serum levels of proteins released by dying mature enterocytes, such as fatty acid-binding protein (I-FABP) (12), as well as serum levels of citrulline (indicator of small intestine epithelial cell damage) (9) are among the proposed biomarkers of intestinal permeability (13). Additional information on the potential biomarkers of intestinal mucosal barrier injury can be found in recent extensive reviews (9,13). In fact, significant decreases in serum levels of citrulline and concentrations of I-FABP have been reported after myeloablative therapy

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(including chemotherapy and total body irradiation) in hematopoietic stem-cell transplant recipients (12). Plasma citrulline has also been linked to RT-induced small bowel mucosal atrophy and RRF (5,14). Wang et al. (2015) reported a significant negative correlation between citrulline concentration and fatigue scores at weeks 3 and 5 of pelvic RT. Jakobsson et al. (2010) reported similar findings. Although these findings suggest that the intestinal epithelial atrophy related to pelvic RT may contribute to RRF development or intensification, further exploration/understanding of the potential mechanisms by which leaky gut contributes to RRF is needed.

Endotoxemia

As previously highlighted, continuous damage to the gut mucosa by endogenous or exogenous events, such as RT treatment, often permit the translocation of bacteria and bacterial products (11,15). It has been suggested that in the event of the loss of the intestinal barrier, the commensal bacteria, such as indigenous microbiota, are no longer contained by the intestinal barrier (16). However, since the gut lumen is then predominantly exposed to gram-negative bacteria (containing elevated levels of endotoxins called LPSs), the commensal bacteria may enter the mesenteric lymph node and blood stream as a result of the barrier breach, causing endotoxemia (17). The increased translocation of LPSs from gram-negative bacteria may lead to autoantibody production, disruption of tight junctions, and both local gastrointestinal inflammation and systemic inflammation (17). Further, there is also evidence that the systemic increase of LPSs also contributes to central neuro-inflammation manifested by increased brain tumor necrosis factor alpha (TNFα) and by marked elevation of pro-inflammatory mediators (18). As a consequence, plasma levels of LPS and LPS-binding protein (LBP), as well as the LPS/LBP receptors CD14, are suggested endotoxemia markers (15).

Increased serum levels of LPS in pelvic cancer patients undergoing RT have been reported (14); however, there is evidence from an animal study linking LPS to fatigue (19). In one study, mice given LPSs (3 mg/kg) combined with swim stress (forced exercise to induce physical fatigue) showed decreased locomotor activity, decreased fall-off time in a rotarod test, and increased serum levels of interleukin-1β and interleukin-6 (IL-6), compared with untreated mice (19). A number of investigators have attempted to show a relationship between endotoxemia markers and increased fatigue severity in other fatiguing conditions (e.g., chronic fatigue syndrome). For example, one study reported a significant positive correlation between IgA responses to LPSs of enterobacteria and self-reported fatigue in chronic fatigue syndrome patients (17). Future mechanistic studies exploring a causal link between endotoxemia and RRF, as well as mediation factors, may strengthen our proposition regarding the interplay of the gut-brain axis in the pathobiology of RRF.

Intestinal Dysbiosis

The gut is one of the organs of the body with the largest concentrations of bacteria, which range from 101 to 103 per gram in the upper intestine and from 1011 to 1012 per gram in the colon (9,20,21). Six major gut microbial phyla, namely Firmicutes (e.g., Clostridium genus), Bacteroidetes (e.g., Prevotella and Bacteroides genera), Actinobacteria (e.g., Bifidobacterium genus), Proteobacteria (e.g., Escherichia and Shigella genera), Fusobacteria, and Verrucomicrobia, compose the gut bacterial microbiome of healthy individuals (21). Furthermore, perturbation of the composition of the gut bacteria (e.g., caused by various factors, such as RT and/or chemotherapy, stress, exercise, dietary pattern, antibiotic treatment) may result in gut microbial dysbiosis (6,9,11,22). Gut microbial dysbiosis is often characterized by lower species diversity, fewer beneficial microbes, the expansion of pathogenic microbes, and the production of factors toxic or harmful to host cells (22).

Culture-based and 16S ribosomal RNA (rRNA) gene sequencing studies of stool bacteria have revealed some evidence of a general decrease in the biomass of intestinal microbiota and an imbalance of the intestine bacterial community towards decreased numbers of commensal bacteria associated to RT (11,14,23). For example, a small study (n = 10) of abdominal cancer patients, using DNA fingerprinting and cloning-sequencing techniques, found that patients with diarrhea had significantly modified bacterial profiles during pelvic RT compared both to healthy controls and to patients with no diarrhea but who received RT (23). Additionally, patients with diarrhea exhibited increased levels of *Actinobacteria*, increased levels of *Bacilli*, and decreased levels of *Clostridia* compared to those patients with no diarrhea. Indeed, the overgrowth of gram-negative *Bacilli* has been linked to the pathogenesis of intestinal radiation toxicity (23). Similarly, another small study, this one of gynecological cancer patients undergoing RT and using pyrosequencing analysis of the 16S rRNA gene, showed a significant decrease in phylum *Firmicutes* and an increase in phylum *Fusobacterium* in the participants compared to healthy volunteers (24). At the family level, *Clostridiaceae* and *Eubacteriaceae* were more common and *Prevotellaceae*, *Oscillospiraceae*, and *Fusobacteriaceae* less common in cancer patients compared to healthy individuals. Importantly, a reduction in the diversity of the *Firmicutes* phylum has been linked to some systemic inflammatory diseases (e.g., bowel disorders) (22,23,25). However, the commensal intestinal microbiota might also have beneficial effects. For example, in one animal study, the administration of *Prevotella histicola*, native to the human gut, was linked to immunomodulatory and anti-inflammatory capabilities (26). In addition, a significant decrease in the Shannon diversity index (bacterial richness and evenness) during pelvic RT has been reported (14). Nonetheless, these findings need to be interpreted with caution, as the previously mentioned studies included several important limitations (e.g., small sample size, limited number

of sequences analyzed, exclusion of additional changes in other groups of bacteria). It is also possible that these changes in the microbiota profile may be attributed to the diagnosed cancer (11). For example, *Fusobacterium nucleatum* infection has been found to be associated with colorectal cancer development, with an increased risk for metastasis, and with a potential colorectal cancer biomarker (27).

Interestingly, although there was a paucity of studies linking imbalance of the intestinal microbiota, known as dysbiosis, with RRF, one of the reviewed studies suggested a potential relationship between dysbiosis and fatigue interference during pelvic radiation therapy (14). Wang et al.'s 2015 study did not report any direct associations between gut microbial dysbiosis and fatigue scores using the Multidimensional Fatigue Inventory. However, the latter study found that microbial diversity and richness and the *Firmicutes/Bacteroides* ratio were significantly altered prior to pelvic radiation therapy in patients who later developed diarrhea and reported significant worsening in fatigue. Although bacterial communities have been the primary focus of gut dysbiosis research, recently it has been suggested that fungal dysbiosis (28) may have a role in the experience of fatigue (29). One small study using 18S rRNA sequencing found that healthy controls (n = 17) had a slightly lower average relative abundance of fungi compared to patients with myalgic encephalomyelitis or chronic fatigue syndrome (n = 17) (30.6% vs. 33.6%) (29). The latter study also reported reduced gut eukaryotic diversity (eukaryotes in the gut include protozoa and fungi), although non-statistically significant, among myalgic encephalomyelitis/chronic fatigue syndrome patients. In addition, the authors of the latter study highlighted limitations in eukaryotic research, including the low abundance and diversity of eukaryotes in the human gut microbiome, the limited number of high-quality eukaryotic reads, and the possibility that 18S databases were incomplete at that moment (29). Despite this evidence, not enough comprehensive 16S rRNA and/or 18S rRNA gene sequencing data exists to determine whether RRF is associated with RT-induced changes of the gut microbiota. Such findings could broaden our understanding of the pathobiology linking intestinal dysbiosis and RRF.

Metabolites

Recent interest in the possibility that physiological levels of numerous bioactive metabolic products (metabolites) formed by the gut microbiota may be associated with cancer-related symptoms has emerged. Indeed, pre-clinical studies have shown significant increases of metabolites, such as valine, leucine, isoleucine, and 2-oxoisopentanoate, in plasma, as well as significant decreases in citrulline and hydroxyproline in a rat model of fatigue compared to the control and food-restricted groups (30). Additionally, one pilot clinical study of breast cancer patients undergoing chemotherapy showed that increased fatigue, post-chemotherapy, was associated with decreased and/or increased levels of metabolites from the tryptophan pathway (31). Increased fatigue was associated with decreased blood levels of multiple bile-related compounds (e.g., taurochenodeoxycholic acid, taurocholic acid) and with increased levels of other classes of metabolites, such as diazines, monosaccharides, and fatty acids (31). The latter study also found significant associations between other metabolites and other symptoms, including pain and depression. There is also evidence of increased d-lactic acid (a product of the microbial fermentation of carbohydrate) and urine metabolites (e.g., amino-hydroxy-N-methyl-pyrrolidine) in patients with other fatiguing and cognitive-impairing conditions, such as chronic fatigue syndrome (32,33). Moving forward, metabolomic studies may enable the identification of metabolites or metabolic pathways that may contribute to the understanding of the pathobiology of fatigue and identify potential targets to develop interventions for RRF (30,31).

Inflammation

Cancer and its treatments affect many biological networks, including those related to the immune and inflammation responses. Immune activation is frequently characterized by an elevation of pro-inflammatory cytokines, an increased expression of T lymphocyte, and the decreased function of natural killer (NK) cells. For example, RT was found to induce the elevation of circulating levels of pro-inflammatory cytokines (TNFα, IL-6, CD8+ T lymphocytes, and NK cells in patients with prostate cancer [34]). Pro-inflammatory cytokines, such as interleukin-1, interleukin-1 receptor antagonist, and IL-6, have been associated with fatigue severity in cancer survivors (35). High levels of c-reactive protein and haptoglobin have been shown to significantly correlate with fatigue (5). Immune dysregulation can also be triggered by the translocation of microbes from an inflamed and/or leaky-gut (15) as a side effect of cancer therapy.

There is also evidence that the gut microbiome regulates important processes, including inflammation and immunity, at both the local gastrointestinal and the systemic inflammation levels (22). Evidence from a number of animal studies suggests that signals from commensal bacteria deliver tonic immune stimulation, which in turn results in the activation threshold of innate antiviral immunity (36). Additionally, commensal microorganisms may regulate gut inflammatory responses. Some *Bifidobacterium* strains and *Bacteroides thetaiotaomicron* strains are able to attenuate inflammation via the suppression of nuclear factor kappa B, resulting in the decrease of pro-inflammatory cytokines and cyclooxygenase (24,37). Similarly, microorganisms in *Clostridium* cluster XIVa have been reported to play a role in decreasing the inflammation of the gut epithelium (24,38). Furthermore, there is also evidence that chemotherapy induced increases in *Clostridium* cluster XI and *Enterobacteriaceae* abundance to potentially pathogenic levels may be associated with diarrhea (e.g., *Clostridium difficile*–associated diarrhea) (39). Notably, further comprehensive molecular analysis studies are needed to confirm and determine

both the role of a certain group of bacteria in the etiology of RT-induced dysbiosis and the side effects of RT (e.g., mucositis and diarrhea).

Implications for practice

RRF continues to be a prevalent, debilitating, and complex symptom experienced by many cancer patients. Since the findings of the current brief review suggest that cancer treatments have significant consequences on intestinal permeability and gut dysbiosis, which consequences may include fatigue, it is plausible that pharmacological (e.g., probiotics) and lifestyle interventions targeting the modulation of the gut microbiota may provide insight into the prevention or amelioration of fatigue (40,41). For example, one study found that 12 weeks of probiotic supplementation (a commercial combination of *Lactobacillus rhamnosus* and *Lactobacillus helveticus*) improved the fatigue, depression, mood, and quality of life of colorectal cancer survivors, with such improvements not being observed in the members of the placebo group (42). Another study examined associations between changes in gut microbiota composition and changes in cardiorespiratory fitness, fatigue, anxiety, depression, and sleep dysfunction in breast cancer survivors before and 3 months after participating in a physical activity trial (43). This latter study found that increased fatigue interference mean scores are associated with an increased mean Shannon diversity index (43). In addition, that study showed that the magnitude of change in fatigue interference was associated with the frequency of *Faecalibacterium* and *Prevotella* genera. This is important because there is increasing evidence of the potential anti-inflammatory roles of *Prevotella histicola* and *Faecalibacterium prausnitzii* (26,44).

It is also possible that strategies aimed at restoring and maintaining a healthy intestinal barrier (e.g., avoiding a high fat diet and processed food, eating meals rich in fiber and fruits) (9) could be introduced into a nutritional intervention study that might, upon completion, provide insight into the prevention or amelioration of fatigue. Clinical trials designed to explore the efficacy of diets rich in fruits, vegetables, whole grains, and omega-3 fatty acid-rich foods in reducing fatigue have reported improvements in fatigue and sleep among breast cancer survivors (45). Additionally, better-quality diets (as measured by the Healthy Eating Index 2010) have been found to be associated with lower fatigue during cancer survivorship (46). In contrast, one study of prostate cancer patients undergoing RT found that reducing the intake of insoluble dietary fibers and lactose had no effect on either fatigue or other aspects of quality of life (47). Much research exploring the clinical relevance of promising pharmacological and lifestyle interventions is needed.

Research implications, including our preliminary gut microbiome work

Pilot studies are underway, including our own, to explore whether RT-induced dysbiosis is associated with the symptom experience of rectal cancer patients undergoing pelvic RT. Using Illumina MiSeq technology, we recently conducted a 16S ribosomal RNA (rRNA) gene sequencing pilot study from stool samples of rectal cancer patients undergoing chemoradiation. This was done to provide initial evidence of the biological/gut microbial processes related to the associations of chemoradiation and dysbiosis with the symptom experience in this population. In fact, we recently reported the association between self-reported fatigue (48) or sleep-disturbance (49) and gut microbial alpha diversity during CRT, as well as significant correlations between the abundances of *Bacteroides* and *Blautia2* and co-occurring symptoms at the end of chemoradiation (50). However, because of the limited number of participants and lack of a control/comparison group, our results were exploratory in nature and should be interpreted with caution. Small sample sizes present additional challenges for statistical analyses in terms of significance testing after multiple corrections and the inability to control for multiple important covariates (e.g., age, sex assigned at birth, body mass index). Future studies using metagenomic and metatranscriptomic approaches, and/or using real-time PCR to quantify certain bacteria beyond the genus level, may further validate our findings. Studies examining the strength of the associations among patient (host) characteristics, gut dysbiosis, intestinal injury, and host inflammatory markers with fatigue might be part of our future research. Gaining this knowledge may strengthen our proposition regarding the interplay of the gut–brain axis in the pathobiology of RRF.

Moving forward, despite notable milestones already having been reached in the field of microbiome research, the following important challenges remain: the development of novel bioinformatic and novel molecular tools, the development of methods for integrating new and current data, and the generation of data that are directly proportional to their biological significance, among others (51). Addressing these challenges will allow for the transformation of a chaotic plethora of metagenomic information and physiologically relevant material into manageable bites of biological data, thereby advancing the understanding of both symptom science and personalized cancer care.

Conclusion

The pathobiology of RRF is not completely understood but is considered multifactorial, with the gut–brain axis, mucosal barrier dysfunction, and gut microbial dysbiosis thought to play roles. Identifying patients at a greater risk of developing RRF may promote the optimized delivery of anticancer therapy and the provision of tailored supportive care and aid all involved in making well-informed, evidence-based decisions regarding treatment. Recent evidence suggests that gut microbiota–targeted interventions (e.g., probiotics) may potentially modulate pelvic radiation–induced side effects (52). However, because microbiota differentiation can be observed in each individual, personalized plans of care and therapeutic strategies should be the focus of future personalized cancer care research

(53,54). Larger-scale longitudinal studies are needed to further investigate the roles of other factors (e.g., health status, diet, body mass index, lifestyle, social factors, environmental factors, pain, stress, diarrhea) that may affect the variability of gut microbiota measurements and/or fatigue symptoms in patients with cancer who are receiving RT.

Resumen

La lesión intestinal inducida por el tratamiento de radioterapia y la perturbación microbiana intestinal/disbiosis se han implicado en la patobiología de la fatiga relacionada al cáncer. El objetivo de esta breve revisión fue explorar la evidencia disponible de la relación entre la lesión intestinal y el auto reporte de la fatiga, especialmente entre los pacientes con cáncer. La evidencia científica, incluyendo la nuestra, que vincula la disfunción de la barrera mucosa intestinal y la perturbación/disbiosis microbiana intestinal inducida por el tratamiento del cáncer con el empeoramiento de la fatiga, tal vez a través del eje intestino-cerebro, es limitada, pero prometedora. Los datos emergentes sugieren que intervenciones en el estilo de vida y la administración de probióticos específicos podrían modular de manera favorable la microbiota intestinal y potencialmente mediar efectos beneficiosos, resultando en mejoras en la fatiga.

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