Chemotherapy-Induced Modifications to Gastrointestinal Microflora: Evidence and Implications of Change

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Abstract: Mucositis is a common side effect of chemotherapy which remains poorly understood. Despite advances in the understanding of oral and small intestinal mucositis over recent years, large intestinal mucositis, including diarrhoea, has not been well defined and the underlying mechanisms of the condition are yet to be established. The majority of the literature available concerning large intestinal mucositis is based on clinical observations, with very little basic research existing. However, from the little research conducted, it is likely that the intestinal microflora play a role in the development of chemotherapy-induced mucositis. This review will explore the potentially important relationship between intestinal microflora and the subsequent development of chemotherapy-induced mucositis.

INTRODUCTION

Mucositis is a major oncological problem, caused by the treatment of malignant disease with chemotherapeutic agents [1-5]. The entire gastrointestinal tract (GIT) mucosa is affected by mucositis, with recipients of cancer chemotherapy exhibiting symptoms such as pain, nausea, heartburn, ulceration, abdominal pain, bloating, vomiting, diarrhoea and constipation [2-4]. Major progress has been made in recent years in understanding the mechanisms of oral [6-8] and small intestinal mucositis [9-12], which appears to be more prominent than colonic damage [12]. The large intestine becomes severely damaged following chemotherapy [2, 12], although further research into the mechanisms of large intestinal mucositis is still required. Recent studies have proposed that the mechanisms for the development of mucositis should be similar throughout the alimentary tract, as embryologically it is formed from one structure [13]. Therefore, research for both oral mucositis and gastrointestinal mucositis can be combined into 'alimentary mucositis' [2].

THE NORMAL FUNCTIONING OF THE GASTROINTESTINAL TRACT

The gastrointestinal tract (GIT) is a hollow tube involved in breaking down food for absorption into the body, occurring in five main phases: ingestion, fragmentation, digestion, absorption and elimination of waste [14]. Normal intestinal function is a balance between oral intake, secretions into the GIT, fluid reabsorption and metabolism. Digestion is the main function of the small intestine, the products of which are absorbed. The small intestinal epithelium has a brush border of enzymes on the luminal surface for metabolising ingesta and leading to absorption. Remaining fluid passes to the large intestine to be reabsorbed. The large intestine has the main functions of water recovery from the contents of the small intestine, and the expulsion of faeces to the rectum prior to defaecation [14]. Large intestinal mucosa consists of long intestinal glands (crypts). The crypts contain goblet cells, absorptive cells and a small number of enteroendocrine cells [15]. Water reabsorption is a highly regulated process involving electrolytes and solutes. Sodium is absorbed by epithelial cells, with chloride following due to an electrochemical gradient. An osmotic gradient is also created, allowing water to follow sodium and chloride into the cell [16]. Normal bowel openings occur between three times daily and once every three days. Chemotherapy-induced mucositis affects the absorptive capacity of the small and large intestines, resulting in increased solutes in the lumen. An increase in solutes in the intestinal lumen results in the osmotic movement of water into the lumen, resulting in diarrhoea [17]. Diarrhoea is associated with increased frequency and decreased consistency of bowel motions.

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NORMAL GASTROINTESTINAL FLORA

The microflora of the GIT is a highly complex ecosystem consisting of both aerobic and anaerobic bacteria [18]. Intestinal flora varies with race, sex, age, diet and other factors [19]. The microflora also differs between the stomach, small intestine and large intestine, both in organisms present and numbers of organisms [20, 21]. The "normal" gastrointestinal microflora has a number of key functions including: protection, and metabolism of bilirubin, intestinal mucins, pancreatic enzymes, fatty acids, bile acids, cholesterol and steroid hormones [22-24]. Other functions of gastrointestinal bacteria include nutrient processing, regulation of intestinal angiogenesis, and immune functions [24-26]. Intestinal microflora is kept tightly regulated, although the mechanisms responsible remain poorly understood, although recent studies have suggested toll-like receptor (TLR) signalling may be involved [27-29]. Each region of the GIT has its own distinct microflora.

STOMACH MICROFLORA

The stomach microflora consists predominantly of microorganisms from the oral cavity which are washed down into the stomach with saliva and food particles. The bacterial populations of the stomach are regulated by gastric acidity, generally accommodating acid resistant organisms. Numbers and complexity increases as the pH increases [21, 28]. Oral anaerobes (*Peptostreptococcus spp*., *Fusobacterium spp*., and *Bacteroides spp*.) and *Streptococcus spp*., *Staphylococcus spp*., *Lactobacillus spp*., Veillonellae, *Actinomyces spp*. and a variety of fungi are the main species' found in the stomach [21, 28]. Coliforms, *Clostridium spp*. and *Bacteroides fragilis* are less common in the stomach [30].

SMALL INTESTINE MICROFLORA

The small intestine is a transition zone between the stomach and colon, and contains far fewer organisms than the stomach or large intestine. The microflora found within the duodenum and jejunum are qualitatively similar to those of the stomach. Normal peristaltic motion and the high rate of motility are major host defences against bacterial overgrowth in the small intestine [21]. The ileum shows an increase in coliforms, *Bacteroides spp*., *Bifidobacterium spp*., *Fusobacterium spp*. and *Clostridium spp*., with gram negative bacteria outnumbering gram positive [31].

LARGE INTESTINE MICROFLORA

The number of anaerobes dramatically increases distal to the ileocaecal valve [21]. Colonic flora consists of over 400 different species of bacteria [30]. These include large numbers of anaerobes

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(*Bifidobacterium spp*., *Bacteroides spp*., *Eubacterium spp*. (most common), *Peptotreptococcus spp*. and *Clostridium spp*.), enterococci and Enterobacteriaeceae [28, 30].

FAECAL MICROFLORA

The faecal dry weight is approximately 33% bacteria [19]. Few studies have elucidated exactly which organisms are present in the faecal microflora. A recent study by Stringer and colleagues determined the faecal microflora of the rat by culture methods and found the faeces to have a greater number of bacteria than was seen in the colon, jejunum or stomach. The most prominent cultured bacteria were Enterobacteriaeceae, and included *Lactobacillus spp*., *Enterococcus spp*., *Serratia spp*., *Proteus spp*., and *Staphylococcus spp*.. Anaerobes were also prominent [32]. Another study by Matsuki and colleagues showed human faeces to contain large amounts of *Clostridium spp*., *Bacteroides spp*., and *Bifidobacterium spp.* [33].

CANCER CHEMOTHERAPY AND THE GASTROINTESTINAL TRACT

Cytotoxic chemotherapy is a common treatment for malignancies which has been in use for approximately 50 years [34]. It can cause functional and structural changes to the GIT [1]. Common gastrointestinal symptoms following chemotherapy include heartburn, abdominal pain, diarrhoea (and constipation), bloating and nausea [12]. These symptoms arise as the result of the damage caused by chemotherapy agents [35]. Abdominal pain is caused by the extensive damage occurring in the small intestine. Diarrhoea and constipation are thought to be caused by the alteration in absorptive functions of cells, goblet cell and mucin distribution and composition, and bacterial interactions with these cells and metabolites of the drugs themselves [36, 37]. Cytotoxic drugs are known to act by inducing apoptosis in cancer; apoptosis is also induced in the GIT [3, 11, 38]. There are limited ways the mucosa and underlying layers of the GIT can respond to damage. These are the same ways that chemotherapy causes damage to the GIT, including cell death, which leads to villous atrophy and crypt ablation in the small intestine, and crypt ablation in the large intestine [12].

CHEMOTHERAPY AGENTS AND INTESTINAL FLORA

Some recent studies have now implicated the intestinal microflora in the adverse side effects of chemotherapy. However this is a relatively new area of research and very few papers exist in the literature.

IRINOTECAN

Irinotecan hydrochloride (CPT-11) is a relatively new chemotherapeutic agent used to treat a variety of solid tumours. Its main action on malignant cells is by inhibiting DNA topoisomerase I [37, 39-41]. Irinotecan is converted to 7-ethyl-10-hydroxycamptothecin (SN-38, the active and toxic metabolite) by hepatic and gastrointestinal carboxylesterases [39], responsible for irreversible DNA damage [42]. SN-38 is subsequently conjugated in the liver by glucuronyltransferase to SN-38 glucuronide (SN-38G), a less toxic metabolite, and is excreted into the gastrointestinal tract via bile. SN-38G is susceptible to hydrolysis by bacterial β-glucuronidase to return to SN-38, increasing the presence of SN-38 in the gastrointestinal tract, further contributing to toxicity [37, 43]. Major dose-limiting side effect of irinotecan include severe diarrhoea and leukopenia, with diarrhoea in 60-80% of patients [10, 16, 44-46]. Takasuna and colleagues examined the detoxified form of irinotecan, SN-38G, and reported that the hydrolysis of SN-38G to SN-38 by bacterial β-glucuronidase activity in the intestine, generates intestinal cytotoxicity [37]. The administration of antibiotics to the animals inhibited β-glucuronidase in the intestinal flora and markedly reduced diarrhoea, supporting the argument that irinotecan toxicity is in part due to the intestinal microflora [47]. Stringer and colleagues reported that irinotecan caused qualitative changes to the microflora of the stomach, intestine and faeces of rats [32], and that faecal microflora was significantly changed in a later quantitative study [48]. Of the β-glucuronidase-producing bacteria, *Bacteroides spp.* was shown to decrease after treatment, whereas *Staphylococcus spp.*, *Clostridium spp.* and *E. coli* increased after treatment. Of the 'beneficial' bacteria, *Lactobacillus spp.* and *Bifidobacterium spp.* were both shown to decrease after irinotecan treatment [32]. These changes were not a direct effect of irinotecan, and it was thought that the decreases in the 'beneficial' bacteria allowed proliferation of other potentially pathogenic bacteria in the intestine (unpublished data[1]). Recent studies looking at gene expression analysis following irinotecan treatment showed that members of the TLR signalling pathway (thought to be involved in regulation of microflora) were overwhelmingly upregulated [48, 49]. This may suggest an explanation for the changes to the microflora seen after treatment with irinotecan.

5-FLUOROURACIL (5-FU)

5-Fluorouracil (5-FU) is an antimetabolite that acts as a pyrimidine antagonist [50, 51]. Major side effects of 5-FU include leukopenia, thrombocytopenia and diarrhoea [50, 51]. Although 5-FU is routinely used in the treatment of numerous cancers including colorectal, breast, and liver, a major side effect of administration is diarrhoea and a number of papers have suggested that changes in the intestinal microflora may play a role in the mechanisms underlying the pathophysiology of the diarrhoea. A study conducted by von Bultzingslowen and colleagues determined that levels of anaerobic bacteria increased in the oral cavity of rats, but remained unchanged in both the small intestine and large intestine following treatment with 5-FU. Furthermore, they showed that the proportion of gram negative rods increased in the oral cavity, and in the intestines there was a shift from gram positive to gram negative bacteria [52]. This study also highlighted intestinal bacterial translocation to mesenteric lymph nodes, which may increase the chances of secondary infections following chemotherapy treatment. More recently Stringer and colleagues have demonstrated in rats receiving 5-FU that faecal microflora also altered (unpublished data[1]). However 5-FU induced different changes compared with irinotecan. *Bacteroides spp*., *Lactobacillus spp*. and *Enterococcus spp*. decreased after treatment. *Bifidobacterium spp*. exhibited fluctuations between time points after treatment. *Clostridium spp.*, *E. coli* and *Staphylococcus spp*. increased after treatment. Both of these studies clearly indicate a key relationship between changes in intestinal microflora, 5-FU treatment and gastrointestinal damage. Further studies are now warranted to fully elucidate this relationship.

CAPECITABINE

Capecitabine (N^4-pentyloxycarbonyl-5'-deoxy-5-fluorocytidine) is a tumour-activated oral fluoropyrimidine that is converted to 5-FU by thymidine phosphorylase [53-55]. Capecitabine is an effective chemotherapeutic agent for advanced breast cancer and also for metastatic colorectal cancer [55], however it is commonly associated with the following adverse effects: lymphopenia, anaemia, diarrhoea, hand-and-foot syndrome, nausea, fatigue, hyperbilirubinaemia, dermatitis and vomiting [55]. To the best of our knowledge there has only been one study to date which has implicated interactions between capecitabine and the intestinal microflora. In a single case study a patient receiving bevacizumab, capecitabine and oxaliplatin developed liver abscesses, with pus cultures positive for *Bacteroides fragilis*, an intestinal commensal [56]. This study suggested that it was likely that *B. fragilis* translocated from the intestine, probably due to compromised intestinal wall integrity. With other studies showing alterations in intestinal flora following treatment with other cytotoxic agents [32, 52], it is likely that the intes-

[1] Stringer A, Gibson R, Logan R, Bowen J, Yeoh A, Hamilton J, Keefe D. Gastrointestinal microflora and mucins play a critical role in the development of 5-Fluorouracil-induced gastrointestinal mucositis in DA rats. Submitted to *Exp. Biol. Med.,* Oct.; 2008.

tinal flora changes with capecitabine also. These specific changes are yet to be examined, highlighting the need for further investigations with capecitabine and intestinal flora.

METHOTREXATE

Methotrexate (MTX) belongs to the class of folate antagonists. A study in rabbits demonstrated that administering 5mg/kg MTX for 4 consecutive days induced no change in the intestinal flora [57]. However, no anaerobic bacteria were detected in the intestine in this study. Given the age of this paper (1969), it would be interesting to re-evaluate the findings with more sensitive molecular techniques.

CHLORAMBUCIL

Chlorambucil is an alkylating agent used in the treatment of both solid tumours and haematological malignancies. A report has shown that two patients receiving chlorambucil developed *Clostridium difficile*-associated diarrhoea (CDAD) within 3-4 months of treatment [58]. Neither of the patients were hospitalised at the time of onset of CDAD, nor were antibiotics being administered in the time leading up to the onset. The authors postulated this was due to chemotherapy agents and antimicrobial agents sharing a similar mechanism of action, allowing chemotherapy agents to alter intestinal microflora through an antibiotic-like mechanism, allowing the proliferation of potential pathogenic bacteria (e.g. *C. difficile*, an enteric pathogen) [58]. Another clinical study report also associated chemotherapy agents with the development of CDAD [59]. This study demonstrated the prevalence of CDAD in a haematology-oncology ward, and concluded antibiotic use increased the risk of CDAD. Paclitaxel-carboplatin combination chemotherapy was given to a patient 4 weeks after complete abdominal hysterectomy for endometrial cancer, with antibiotics administered 5 days post-operatively. Stool culture revealed the presence of *C. difficile* at later stages in the duration of severe diarrhoea [60]. Other studies have reported an increased incidence of CDAD in patients with chemotherapy-induced gastrointestinal mucositis [61, 62].

OTHER CHEMOTHERAPY AGENTS

Other chemotherapy agents including mitomycin, etoposide (VP-16), bleomycin, carmustine, cisplatin, cyclophosphamide, cytosine arabinoside, dactarbazine, doxorubicin, thiotepa, vinblastine, and vincristine have been investigated in an *in vitro* study for their antibacterial activity [63]. This study suggests that these antineoplastic drugs have limited effects on bacteria *in vitro* [63]. However, an *in vivo* model may show different results.

IMPLICATIONS OF MICROFLORA CHANGES

Diseased states and/or associated therapies such as chemotherapy-induced mucositis (including diarrhoea and constipation) alter the composition of the intestinal microflora [22, 64-69]. Changes to the microflora have been demonstrated in some cases to have quite detrimental effects, the majority of which are related to the functions performed in the intestine by the microflora, including protection, metabolism of various host- produced biological agents, nutrient processing, intestinal angiogenesis regulation, and immune functions [25, 26, 29]. The variety of changes in the composition of the microflora in such states are seen from the stomach to the colon and faecal flora [65]. The pH of the intestinal environment can also change [67]. As a result of these changes, absorption and other intestinal functions involving the microflora are also altered. Furthermore, the local intestinal microbial environment may also play a key role in the mucosal response to damaging stimuli. It has been reported that mucosal susceptibility to damage is based on a number of tissue specific components, including the local microbial environment [70].

The intestinal microflora in the unaltered state provides a multitude of host-beneficial functions. Protection (of the mucosa, from both overpopulation and the attachment of pathogens), is a key function that is usually achieved with microbial interactions (particularly in the colon), and is important in defining the microflora. There are several mechanisms where bacteria interact with each other to promote or prevent the growth of other bacteria [30]. These mechanisms include depleting substrate materials necessary for another species' growth (e.g. coliforms compete for carbon), creating an environment to stimulate or inhibit growth (e.g. facultative bacteria use available oxygen, maintaining a reduced environment, allowing the growth of strict anaerobes), and production of metabolic by-products (e.g. short chain fatty acids inhibit bacterial proliferation) [31].

Chemotherapy-induced alterations of the intestinal microflora are able to disrupt these mechanisms and allow overgrowth and translocation. Other important host-beneficial functions include the metabolism of bilirubin, intestinal mucins, pancreatic enzymes, fatty acids, bile acids, cholesterol and steroid hormones [23-25]. Gastrointestinal bacteria are also involved in nutrient processing, regulation of intestinal angiogenesis, and immune functions. Disruption of these important gastrointesitnal functions may contribute to the damage caused by chemotherapeutic agents.

SUMMARY

Chemotherapy-induced mucositis is a detrimental side effect of cancer treatment, which may be exacerbated by coexisting changes in the intestinal microflora. Intestinal microflora has been shown to translocate after cancer treatment, causing localised infection and/or bacteraemia. Host-beneficial microflora function may also be compromised during treatment with chemotherapy, contributing to the severe, dose-limiting side effects of cancer chemotherapy.

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