

A review of complementary therapies for chemotherapy induced gastrointestinal mucositis

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Summary Administration of chemotherapy often leads to gastrointestinal mucositis (GIM). GIM manifests as nausea, abdominal pain and diarrhoea in recipients of chemotherapy. GIM is a major complication occurring in approximately 80% of patients receiving 5-fluorouracil treatment. These side-effects may become so severe that significant dose reductions are required, ultimately affecting treatment efficacy and patient survival. Complementary and alternative medicine (CAM) is a growing area of public interest. This review will provide an overview of current knowledge of complementary medicinal therapies for chemotherapy induced GIM. An understanding of this evolving literature is useful in discussing these therapies with patients who are considering using them.

Keywords: Mucositis, intestine, complementary medicine

1. Introduction

The fast-renewing intestinal epithelium is vulnerable to the cytotoxicity of chemotherapy. Cytotoxic agents used during chemotherapy are effective at killing cancerous cells however they also indiscriminately target certain healthy tissue. Chemotherapy-induced gastrointestinal mucositis (GIM) is a dose-limiting side effect of many chemoagents. It has been pathologically described as an intestinal inflammation characterized by an early event of epithelia apoptosis (1-3). GIM is a major oncological problem, caused by the treatment of malignant disease with chemotherapeutic agents. It affects the entire gastrointestinal tract and causes pain and ulceration in the mouth as well as abdominal bloating, vomiting and diarrhoea in the small and large intestines (4,5). After treatment with standard dose chemotherapy, approximately 40% of all patients and up to 100% of patients undergoing high-dose chemotherapy with bone marrow or stem cell transplantation reportedly develop GIM (4-6). GIM and its associated complications lead to a dose reduction of chemotherapy and compromises overall survival in cancer patients. GIM adds substantial

burden on the medical care required for cancer patients, increases healthcare costs and reduces patient quality of life (7). Current therapies for treatment of GIM are suboptimal and potentially toxic. There is an acute need for the development of more effective treatment strategies for chemotherapy-induced GIM. Emerging evidence suggests that complementary and alternative medicine (CAM) based therapeutic modalities are highly effective in modulating the immune system, disrupting the proinflammatory cascade and restoring digestive health while improving patient's quality of life. In this context we will review some of the complementary medicine based therapeutics for treatment of GIM. However, before going into these details, it is necessary to understand the architecture of small intestine and current molecular model for pathobiology of GIM.

2. Small intestinal architecture

GIM has been attributed to the high proliferation rate in the intestine that is interrupted and reduced by chemotherapy drugs, and also to direct killing of crypt cells (4-6). The intestinal epithelium is the largest of the body's mucosal surfaces, covering ~400 m² of surface area with a single layer of cells organized into crypts and villi. This surface is continually renewed by pluripotent intestinal epithelial stem cells that reside in the base of crypts, where the pro-liferation, differentiation and functional potential of epithelial cell

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progenitors is regulated by the local stem cell niche (8-10). The epithelial layer of the GI tract is a rapidly renewing tissue with a high cell turnover rate. Stem cells in the lower half of the crypts give rise to daughter cells, thereby producing all the cells of the epithelium. Newly produced cells migrate out of the crypts up to the villus or migrate downward into the base of the crypts and reside under or between the stem cells. During migration up to the villus, most cells differentiate into functional enterocytes (11). It is generally accepted that once epithelial cells complete their migration along the crypt-villous axis they are sloughed off into the lumen. This rapid turnover of cells explains why the GI tract is particularly susceptible to the effects of cytotoxic agents (12). Despite the different sites of action of various chemoagents, the end result is intestinal crypt hyperplasia (4,5). The epithelial stem cells in the small intestine become damaged and no longer divide or differentiate into specific cell lineages following chemotherapy leading to a rapid loss of structure and function (13).

3. Pathobiology of GIM

In the last decade, significant progress has been made in understanding the pathobiology of GIM. It has been postulated that GIM occurs in five overlapping phases: initiation, up-regulation and message generation, signalling and amplification, ulceration and healing (14-16). Upon anti-cancer treatment, both DNA and non-DNA damages occur. DNA strand breaks cause direct injury in cells of the basal epithelium. At the same time, reactive oxygen species (ROS) are formed, starting downstream biological events. Nuclear factor kappa B (NFκB), cyclooxygenase-2 (COX-2) as well as pro-inflammatory cytokines like interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumour-necrosis factor (TNF) have been suggested to play a key role in the development of GIM (17-20). These overlapping steps are thought to be largely driven by the activation of NFκB, subsequently promoting key pro-inflammatory cytokines. Furthermore, apoptosis, pathogenic bacteria, inflammation, matrix metalloproteinases (MMPs), loss of mucosal barrier integrity and toll like receptors (TLRs) have also been reported to play an important role in GIM (21-28).

There is evidence that chemotherapy can induce GIM through various pathways. The timing of histological lesions, peak tissue levels of NFκB and pro-inflammatory cytokines are different according to the chemotherapy agents like irinotecan, methotrexate (MTX) or 5-fluorouracil (5-FU) (29). Commensal bacteria play an important role in intestinal homeostasis, and have some protective effect on the intestinal integrity. Their interactions with TLRs and subsequent activation of NFκB signalling pathway contributes to intestinal homeostasis, maintaining

the barrier function and promoting wound repair and tissue regeneration (30,31). TLRs initiate the innate immune response and the production of pro-inflammatory mediators including IL-1β, nitric oxide and IL-18, whose role in intestinal GIM is well known (32-34). Recently, TLR/MyD88/NF-κB pathway has been implicated in the mechanisms of damage involved in chemotherapy-related GIM (27). It has also been reported that TLR-2 acts as a central regulator of xenobiotic defense and targeting TLR2 may represent a novel therapeutic approach in chemotherapy-induced GIM (26).

Role of platelet-activating factor (PAF) in chemotherapy-induced GIM has also been reported (35). NADPH oxidase 1 (NOX1) has also been suggested to be involved in pathogenesis of GIM (36). Furthermore, role of NOX-2 mediated inflammasome activation and inflammasome-dependent production of IL-1β and IL-18 has also been verified (37). The mucin layer is an integral component of barrier function in the intestine and chemotherapy agents significantly decreased epithelial mucin levels in the jejunum of rats (38). Findings strongly suggest chemotherapy causes tight junction defects which lead to mucosal barrier dysfunction and the development of GIM (23). Thus, it is quite clear that pathobiology of GIM involves many signalling pathways and diverse range of molecules.

Given the literature summarised above, it is clear that some kind of preventative therapy is required for GIM in patients receiving cancer chemotherapy treatment. In many cases of cancer chemotherapy the treatment needs to be discontinued due to high incidence of GIM, risking the life of patients. CAM is widely used, particularly for chronic medical conditions that are difficult to treat. Because only a limited number of treatments are available for GIM, many patients can opt for CAM. Two of the most important CAM therapeutic modalities for chemotherapy induced GIM are based on plants and probiotics.

4. Plant based complementary therapies

Aged garlic extract (AGE) administration has been reported to decrease the severity of jejunal damage against MTX-induced GIM in the small intestine of rats (39,40). MTX induced apoptosis of IEC-6 cells was shown to be depressed by AGE (41). The MTX-induced loss of viable IEC-6 cells was almost completely prevented by the presence of more than 0.1% AGE (41). Grape seed extract (GSE) represent a new therapeutic option to decrease the symptoms of GIM. Compared with 5-FU controls, GSE significantly decreased the histological damage score, increased jejunal crypt depth, attenuated the chemotherapy-induced reduction of mucosal thickness and decreased myeloperoxidase activity in rat models of GIM (42,43). GSE has also been reported to improves epithelial

structure, intestinal epithelial differentiation and suppress inflammation in ileum of IL-10-deficient mice (44,45). Grapes are rich in polyphenols that vary in their distribution inside the grapes with less than 10% in the pulp, 60-70% deposited in seeds, and 20-35% in the skin (46). Proanthocyanidins are believed to be the key bioactive constituents in GSE (47). Administration of proanthocyanidin decreased the jejunal damage and malondialdehyde level, which were caused by MTX treatment and increased superoxide dismutase and glutathione peroxidase levels in rats (48,49).

It has been reported that apricot and beta-carotene treatment may protect the impairment of oxidative stress and ameliorate MTX-induced intestine damage at biochemical and histological levels (50). Single or combined application of apricot and beta-carotene ameliorated the effects like fusion and shortening in the villus, epithelial desquamation, crypt loss, inflammatory cell infiltration in the lamina propria, goblet cell depletion and microvillar damage in the small intestine of rats treated with MTX (50). Administration of Vitamin A is also known to decrease the MTX-induced damage to the small intestine (51). Ellagic acid (EA) and pumpkin seed oil (PSO) have been suggest to protect the small intestine of rats from MTX-induced damage through their antioxidant and anti-inflammatory effects (52). Administration of EA and PSO decreased the intestinal damage, prostaglandin E2 and nitric oxide level (52). Hesperidin, a flavanone glycoside mainly found in citrus fruits has been reported to prevent intestinal epithelial injury resulting from chemotherapy treatment (53). The small intestinal damage score, inducible nitric oxide synthase and interleukin-8 levels were lower MTX plus hesperidin group (53).

Aqueous extract of *Chimonanthus nitens* var. *salicifolius* (CS), a traditional Chinese herb has been found to be beneficial against 5-FU induced GIM in mice (54). CS attenuated the subsequent body weight loss, diarrhea, and faecal blood, reducing the hepatic injury, and maintaining both intestinal length and villus structure (54). Downregulation of apoptotic gene caspase3 in CS group compared with mice treated with 5-FU only was reported (54). CS aqueous extract treatments suppressed the elevation of TNF- α induced by 5-FU challenge. Furthermore, other cytokines, including IL-1 β and IL-12b, were also inhibited by CS treatment, suggesting an anti-inflammatory effect of CS (54). Three flavonoids rutin, quercetin, and kaempferol were identified by HPLC to be abundant in the aqueous extract of CS (54). Rutin is considered as a powerful antioxidant with pharmacological benefits including antitumor, anti-inflammatory, and anti-diarrhoeal effects (55,56).

Curcumin has been reported to significantly reverse chemotherapy-induced weight-loss and damage to intestinal mucosa (57). Curcumin also reduced the expression of pro-apoptotic Bax but stimulated anti-apoptotic Bcl-2 to attenuate 5-FU-induced apoptosis

of intestinal epithelial cells (57). Curcumin decreased the levels of ICAM-1, IL-1 β and TNF- α , but increased the levels of IL-10 and SOD in rat models of GIM (58). Furthermore, mitogen-activated protein kinase phosphatase-1 (MKP-1) was activated but phospho-p38 was inhibited by curcumin (58). Curcumin also repressed I- κ B and interfered with the translocation of NF- κ B into nucleus (58). These findings suggest that curcumin, with anti-inflammatory and anti-oxidant activities may be used as an effective reagent for protecting intestinal mucosa barrier during chemotherapy-induced GIM (58). Administration of beta-glucan following MTX has been reported to attenuate the tissue damage (59). Stimulation index, an indicator of oxidative burst in the neutrophils, was decreased by MTX, while beta-glucan abolished this effect (59). Furthermore, increased leukocyte apoptosis and cell death in MTX-treated animals were inhibited by beta-glucan. These findings suggest that beta-glucan, through its antioxidant and immunoregulatory effects, may be of therapeutic value in alleviating the leukocyte apoptosis, oxidative tissue injury and thereby the intestinal and hepato-renal side effects of MTX treatment (59).

Saireito, a traditional Japanese herbal medicine and a combined formulation of two herbal medicines (shosaikoto and goreisan) reduced 5-FU-induced GIM through reduction of apoptosis in the intestinal crypt *via* suppression of the up-regulation of inflammatory cytokines (60). Administration of saireito significantly suppressed the activation of caspase-3 and reduced 5-FU-increased apoptotic cells, with an inhibition rate of 67.8% (60). Furthermore, it significantly attenuated the up-regulation of both TNF- α and IL-1 β mRNA induced by 5-FU. The inhibition rate was 72.5 and 77.6% for TNF- α and IL-1 β , respectively (60). Iberogast, a mixture of extracts from bitter candytuft (*Iberis amara*), angelica root (*Angelicae radix*), milk thistle fruit (*Silybi mariani fructus*), celandine herb (*Chelidonii herba*), caraway fruit (*Carvi fructus*), liquorice root (*Liquiritiae radix*), peppermint herb (*Menthae piperitae folium*), balm leaf (*Melissae folium*) and chamomile flower (*Matricariae flos*) partially improved the histopathological features of 5-FU induced GIM in rats (61,62). Acteoside, a phenylpropanoid glycoside derived from plant species in the *Scrophularia* genus alleviated MTX-induced small intestinal mucositis possibly by preventing inflammation (63,64). Mucoadhesive formulation of *Bidens pilosa* L. (*Asteraceae*) has recently been reported to reduce intestinal injury from 5-FU induced GIM in mice (65). Plant based complementary medicine therapeutics useful for treatment of chemotherapy induced GIM are summarized in Table 1.

5. Probiotics

Previous studies have indicated that gastrointestinal microflora may be involved in the development

Table 1. Plant based complementary therapies for GIM

Complementary Therapy	Effect	Reference
Aged garlic extract	Apoptosis (Decrease)	39,40,41
Grape seed extract	Inflammation (Decrease), Myeloperoxidase activity (Decrease)	42,43,44,45
Apricot and β -carotene	Inflammatory cell infiltration (Decrease)	50,51
Ellagic acid & pumpkin seed oil	Prostaglandin E2 (Decrease), Nitric oxide (Decrease)	52
Hesperidin	Inducible nitric oxide synthase, Interleukin-8 (Decrease)	53
Aqueous extract of <i>Chimonanthus nitens</i>	Caspase3, TNF- α (Decrease)	54
Curcumin	Bax, IL-1 β , TNF- α , I- κ B (Decrease)	57,58
β -glucan	Leukocyte apoptosis (Decrease)	59
Saireito	Apoptosis, IL-1 β , TNF- α (Decrease)	60
Iberogast	Histopathological features (Improve)	61,62
Acteoside	Inflammation (Decrease)	63, 64
Mucoadhesive formulation of <i>Bidens pilosa</i>	Bax (Decrease), Myeloperoxidase activity (Decrease)	65

of chemotherapy-induced mucositis and diarrhoea (66,67). It has been suggested that intestinal bacteria can attenuate or aggravate GIM by influencing the intestinal inflammatory process, influencing intestinal permeability, influencing the composition of the mucus layer, influencing resistance to harmful stimuli and enhancing epithelial repair, and the activation and release of immune effector molecules (67). Mucins play an important role in maintaining the integrity of the normal intestinal flora by providing attachment sites for intestinal flora and pathogenic bacteria (68). Therefore, maintaining a healthy microbiota and mucin layer during chemotherapy treatment could minimise the complications associated with GIM. In this context, probiotic microorganisms represent a promising therapeutic option in treatment of GIM (69,70). Probiotics are defined as living microorganisms which when administered in adequate amounts, exert desirable health benefits on the host (71). Probiotics are known to exert beneficial effects to the host when ingested, and therefore could be useful in controlling the intestinal microflora during chemotherapy (72,73). Most commonly used probiotic bacteria include genera of *Lactobacillus* and *Bifidobacterium*. However, few strains of *Enterococcus*, *Streptococcus*, *Lactococcus* and certain species of non-pathogenic *Escherichia* strains have also been classified as probiotics (73).

Lactobacillus acidophilus administration concomitantly with 5-FU and alone for two additional days has been reported to significantly reverse the side effects of GIM (74). These include improvement in villus height-crypt depth ratio and decrease in TNF- α and IL-1 β levels after 5-FU treatment (74). Probiotic mixture VSL#3 reduced weight loss, prevented diarrhea and inhibited apoptosis in small and large intestine after treatment with irinotecan (75). *Saccharomyces boulardii* significantly reversed histopathological changes, reduced neutrophil infiltration and reduced concentrations of TNF- α and IL-1 β after 5-FU treatment (76). Cow's milk yoghurt fermented with *Lactobacillus johnsonii* and Sheep's milk yoghurt containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* improved small intestinal barrier functions as determined by

lactulose/mannitol ratio in MTX-induced rat models of GIM (77).

Orally ingested *Streptococcus thermophilus* attenuated MTX-induced small bowel damage in rats as indicated by non-invasive sucrose breath test (78). Probiotic derived supernatant from *Escherichia coli* Nissle 1971 (EcN) partially protected the intestine of dark agouti rats from 5-FU induced GIM and significantly decreased cell death induced in IEC-6 cell lines after treatment with 5-FU (79,80). *Lactobacillus fermentum* BR11 reduced jejunal inflammation as evident from reduced myeloperoxidase activity in 5-FU induced rat models of GIM (81). Pre-treatment EcN and LGG (*Lactobacillus rhamnosus*) supernatants lowered caspase activity, inhibited enterocyte apoptosis and loss of intestinal barrier function induced by 5-FU treatment of IEC-6 cells (82). Oral administration of probiotics *Lactobacillus casei* variety *rhamnosus* (Lcr35) or *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (LaBi) appeared to ameliorate the intestinal mucositis severity by inhibition the expressions of proinflammatory cytokines (83). Various microorganisms used as probiotics for treatment of chemotherapy induced GIM are summarized in Table 2.

6. Miscellaneous complementary therapies

Administration of Emu oil daily via orogastric gavage after intraperitoneally injected single dose of 5-FU improved rate of recovery from GIM (84). Significant decrease in activated neutrophil infiltration, improvement in crypt depth and villus height, decrease in acute inflammation and overall improvement in mucosal architecture in the intestine have been reported after Emu oil supplementation (84,85). Lyprinol, a lipid extract derived from mussels and rich in omega-3 polyunsaturated fatty acids partially improved 5-FU induced GIM in rat models (86). Mice that received dietary supplementation with omega-3 fatty acid were associated with mucosal integrity and a reduced number of apoptotic cells in the ileum mucosa compared to the mice that received the control diet and 5-FU injection (87).

Table 2. Probiotic based complementary therapies for GIM

Complementary Therapy	Effect	Reference
<i>Lactobacillus acidophilus</i>	TNF- α , IL-1 β (Decrease)	74
Probiotic mixture VSL#3	Diarrhea, Apoptosis (Decrease)	75
<i>Saccharomyces boulardii</i>	Neutrophil infiltration (Decrease)	76
<i>Lactobacillus johnsonii</i>	Intestinal barrier functions (Improve)	77
<i>Lactobacillus bulgaricus</i>	Intestinal barrier functions (Improve)	77
<i>Streptococcus thermophilus</i>	Intestinal barrier functions(Improve)	77,78
<i>Escherichia coli</i> Nissle 1971	Apoptosis (Decrease)	79,80
<i>Lactobacillus fermentum</i> BR11	Myeloperoxidase activity (Decrease)	81
<i>Lactobacillus rhamnosus</i> & <i>Escherichia coli</i> Nissle 1971	Caspase activity (Decrease)	82
<i>Lactobacillus acidophilus</i> & <i>Bifidobacterium bifidum</i>	Proinflammatory cytokines (Decrease)	83
<i>Lactobacillus casei</i> variety <i>rhamnosus</i>	Proinflammatory cytokines (Decrease)	83

Table 3. Miscellaneous complementary therapies for GIM

Complementary Therapy	Effect	Reference
Emu oil	Neutrophil infiltration (Decrease)	84,85
Lyprinol	Crypt cell proliferation (Decrease)	86
Omega-3 fatty acid	Mucosal integrity (Improve)	87
L-arginine supplementation	Enterocyte apoptosis (Decrease)	88,89
β -Hydroxy- β -Methylbutyrate, L-Glutamine and L-Arginine	Enterocyte apoptosis (Decrease)	90
Insulin-like growth factor-I	Histopathological parameters (Improve)	91

Dietary L-arginine supplementation decreased enterocyte apoptosis accompanied by decrease in Bax mRNA and protein expression and increased Bcl-2 protein levels in rat model of MTX-induced GIM (88). L-arginine attenuated the histopathological score and myeloperoxidase activity promoting partial mucosal recovery, reducing inflammation and improving intestinal permeability in 5-FU induced GIM (89). Diet containing β -hydroxy- β -methylbutyrate, L-glutamine and L-arginine significantly decreased apoptosis in rats after administration of 5-FU (90). Pre-treatment with insulin-like growth factor-I in rats improved various intestinal parameters and partially attenuated features of intestinal mucositis when assessed 48 h after 5-FU chemotherapy (91). Table 3 summarizes these therapies.

7. Conclusions

Treatment options for patients with GIM are limited, and prevention requires an understanding of the pathophysiological mechanisms underlying GIM development. Despite the debilitating symptoms of chemotherapy-induced GIM, there remains no truly effective treatment strategy capable of preventing the associated intestinal damage. In this context, the role of CAM in treatment of GIM is very critical. Therapies based on CAM have been reported to improve the symptoms of GIM. Enthusiasm for CAM use and research is clearly growing. Medline citations for alternative medicine have steadily increased from 69 citations in the 1970s to 423 citations since 2000 (92). GIM with its varied pathobiology presents many molecular steps that can be targeted with CAM. However, the absence of rigorous scientific testing has

delayed the use of CAM in mainstream medicine. In this review, many studies related to use of CAM in treating GIM have been reported. Further studies are required to optimize the use of these novel agents to alleviate the distressing symptoms of intestinal mucositis. We have much still to learn about CAM treatments in terms of efficacy, safety and cost-effectiveness. For safe treatment of chemotherapy induced GIM, a truly collaborative effort between CAM practitioners, conventional physicians and research scientists is needed.

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