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Newly Developed Synbiotics and the Chemotherapy-Damaged Gut

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Abstract

Mucositis is a common side-effect of cancer chemotherapy and radiotherapy. Features of mucositis include erythema, ulceration, and inflammation of the gastrointestinal tract accompanied by clinical symptoms of abdominal pain and digestive disturbances. New treatment strategies are required. Experimental evidence is accumulating showing therapeutic promise for new nutraceutical agents including probiotic bacteria, probiotic-derived factors, prebiotics, and plant extracts. However, the targeted development of new combinations of these agents (synbiotics) to combat mucositis remains largely unexplored. The current review addresses the potential for these nutraceutical agents to reduce the severity of chemotherapy-damaged mucositis by strategically aligning their underlying mechanism of action with features of mucositis pathogenesis. The potential for certain plant extracts to act as prebiotics, in combination with probiotics or their derived factors, is further investigated. These unique synbiotic formulations could form the basis of a new naturally sourced adjunctive approach to cancer chemotherapy.

Keywords

mucositis, probiotics, probiotic-derived factors, plant extracts, chemotherapy, cancer

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Cancer and Mucositis

Cancer is medically known as a malignant neoplasm that results from abnormal unregulated cell growth.[1] These transformed cells can metastasize to the surrounding uninvolved tissues via the lymphatic system or bloodstream.[2] Cancer types include bladder cancer,[3] lung cancer,[4] breast cancer,[5] colon and rectal cancer,[6] non-Hodgkin lymphoma,[7] endometrial cancer,[8] pancreatic cancer,[9] kidney cancer,[10] prostate cancer,[11] leukemia,[12] and thyroid cancer.[13]

Treatment approaches for malignancies include surgery, chemotherapy, radiation therapy, immunotherapy, and monoclonal antibody therapy.[14-16] Chemotherapy is employed widely in cancer management.[17] However, its effectiveness is often limited by its toxicity to other normal, healthy cells and tissues.[17] The toxicity to the digestive tract is termed mucositis.

Mucositis

Chemotherapy drugs are used to limit the proliferation of tumor cells. However, these drugs coincidentally impair populations of normal cells such as enterocytes.[18] Mucositis is a common side-effect of cancer chemotherapy. It causes painful inflammation and ulceration of the mucous membranes lining the digestive tract.[19] The term *alimentary mucositis* describes the damage that occurs along the entire gastrointestinal tract. Mucositis can also affect the mouth, referred to as oral mucositis.[19,20] The current review focuses mainly on gastrointestinal mucositis.

A significant number of patients undergoing chemotherapy (40% to 100%) experience different stages of mucositis, depending on the dose and types of chemotherapeutic agent employed.[21,22]

Intestinal damage caused by chemotherapy can manifest in several ways. For example, chemotherapy has been reported to trigger the death of normal rapidly dividing cells, especially those lining the gastrointestinal tract.[23] Chemotherapy has also been demonstrated to change the gut microbiota communities in human individuals and intestinal mucin levels in rats.[23,24] This damage is responsible for breaches to the intestinal barrier, accompanied by increases in intestinal permeability in cancer patients undergoing chemotherapy.[25] Subsequently,

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chemotherapy-induced intestinal impairment provides a conduit for entry of pathogenic bacteria into the systemic circulation, with pro-inflammatory cytokine release and associated inflammation.[20] In addition, oxidative stress contributes to further intestinal injury.[26] Considered together, damage induced by chemotherapy may lead to intestinal dysfunction, inflammation, and an impaired immune system.[22,23]

Cancer patients undergoing chemotherapy usually experience symptoms that include abdominal distension, nausea, dysphagia, loss of taste, reduced oral intake, weight loss, and malnutrition.[22,27] Moreover, patients can also experience diarrhea caused by electrolyte imbalance, bleeding from intestinal ulcerations, and bacterial sepsis.[22,28] Indeed, death may even occur.[22]

Pathogenesis of Mucositis

To reduce the severity of mucositis, it is important to understand the pathophysiology of this condition. Sonis[29] in 2004 summarized the 5 stages of chemotherapy-induced mucositis. Briefly, the first stage is an ''initiation phase,'' associated with an increased level of reactive oxygen species and DNA damage to intestinal cells and tissues. The second stage is the ''upregulation and message generation phase'' characterized by the production of cell transcription factors such as nuclear factor κ-light-chain-enhancer of activated B cells. Together with apoptosis, the mucosa becomes more friable and the patient begins to feel pain. The third stage is the ''signaling and amplification phase.'' During this stage, levels of tumor necrosis factor-α are increased, together with increased levels of nuclear factor κ-light-chain-enhancer of activated B cells, resulting in further cell apoptosis and tissue injury beneath the mucosal surface. This is followed by the ''ulceration and inflammation phase,'' which is associated with bacterial colonization and the production of pro-inflammatory cytokines such as interleukin-1 and tumor necrosis factor-α. Together this can result in bacteremia, sepsis, and breached mucosa integrity. The final stage is a healing phase, whereby the intestinal epithelium begins to recover and the normal homeostasis is restored in the gut microbiota.[29]

Treatment of Mucositis

Interest in studying oral mucositis has grown steadily[27,30]; however, in contrast, research into treatments for gastrointestinal mucositis has received less attention. To date, treatment approaches for gastrointestinal mucositis have mainly focused on the identification of agents with the potential to protect the mucosa and promote the repair process, without compromising the cytotoxic effects of chemotherapy. Several growth factors such as milk growth factors and insulin-like growth factor-I[28,31,32] have exhibited the potential to promote differentiation and proliferation of epithelial cells affected by chemotherapy. Moreover, new nutraceuticals have recently been tested in experimental models for their effects against mucositis. These include probiotic preparations such as VSL#3,[33] *Streptococcus thermophilus*,[34] *Lactobacillus* spp., and *Escherichia coli* Nissle 1917[35] together with prebiotics such as fructo-oligosaccharide.[36] Moreover, recently, probiotic-derived factors have begun to show promise at preventing mucositis in vivo and in vitro.[35,37] In addition, there are now early indications that antioxidant and anti-inflammatory constituents in plant extracts, such as Iberogast[38] and grape seed extract,[39] and animal-sourced oils, such as Lyprinol[40] and Emu oil,[41] could have utility in preventing mucositis.

In this review, certain probiotic strains, probiotic-derived factors, and plant extracts will be explored for their potential to ameliorate mucositis. The different nature and origins of these agents would suggest that the severity of mucositis could be reduced through different pathways. Thus, the strategy of using strategically defined combinations of these nutraceuticals, such as combinations of probiotics/probiotic-derived factors and plant extracts, could be more effective than the individual agents in protection from mucositis.

Probiotics and Mucositis

Probiotics are living microorganisms that, when administered in a sufficient amount, confer health benefits to the host.[42] *Lactobacillus* and *bifidobacterium* genera have been found to be the most effective probiotics. *Lactococcus*, *streptococcus*, and *enterococcus* species, as well as some nonpathogenic strains of *Escherichia coli* and some bacilli and yeast strains, may also meet this definition.[43]

The beneficial properties of probiotics have been studied over decades. It is believed that probiotics have the potential to maintain a healthy gut microbiota, and most importantly reduce the severity of certain disorders, which include gastrointestinal diseases such as inflammatory bowel disease, diabetes, and atopic diseases.[44-46] Indeed, *Escherichia coli* Nissle 1917 has been prescribed widely for the treatment of inflammatory bowel disease in some countries, including Germany.[47]

The mechanisms of probiotic action have been summarized by Howarth in 2010.[48] Briefly, certain probiotics have the ability to adhere to the intestinal–lumen interface, compete with pathogens for nutrients and binding sites, enhance mucosal barrier function, modulate cell kinetics by adjusting the proliferation to apoptosis ratio, and promote innate and adaptive immune responses. Based on these features, it is possible to speculate that probiotics, especially some newfound probiotic strains, could hold the potential to reduce gut damage from chemotherapy administration.

Prisciandaro et al reviewed evidence supporting the use of probiotics for the potential prevention and treatment of chemotherapy-induced mucositis.[49] The current review extends this strategy to include recently identified probiotics and the added potential for these bacteria to be more effective when administered in combination with certain antioxidant plant extracts.

Competition With Pathogens for Binding Sites

It has been reported that mucin binding proteins can be identified from probiotics such as *Bifidobacteria bifidium* species

and *Lactococcus lactis* ssp. lactis BGKP1, contributing to binding properties in vivo and in vitro.[50,51] In addition, a few *Lactobacillus* spp. and *Bifidobacterium* spp. were found to inhibit the growth of certain intestinal pathogens, including *Escherichia coli* and *Salmonella* spp., in laboratory experiments.[52,53] Probiotics may therefore compete with pathogens for binding sites and nutrients, potentially decreasing the likelihood of pathogen infection and secondary infections associated with chemotherapy.

Probiotic Versus Microbiota

Chemotherapy tends to alter the composition of the microbiota toward a more pathogenic community. For example, decreases of *Clostridium* spp., *Lactobacillus* spp., and *Streptococcus* spp. and an increase in *Escherichia* spp. were found in the jejunum of 5-fluorouracil-injected rats.[24] Administration of probiotics (*Bifidobacteria* and *Lactobacillus*), for 7 days after elective laparoscopic radical surgery for colorectal cancer, significantly modified the fecal microbiota by increasing numbers of *Bifidobacterium*, *Lactobacillus*, and *Enterococcus* and decreasing *Escherichia coli* and *Staphylococcus aureus*.[54] Thus, certain probiotics could potentially restore and normalize the chemotherapy-affected gut microbiota.

Maintenance of the Gut Barrier

Breach of the intestinal barrier has always been a serious issue for cancer patients undergoing chemotherapy.[25] Disturbed mucin secretion, goblet cell production, and enterocyte tight junction proteins amplify the risk of bacterial infection and translocation into surrounding tissues.[24,29] Some probiotic strains such as *Lactobacillus plantarum* spp., and the probiotic mixture VSL#3, have demonstrated capacities to modulate tight junction proteins such as occludin, zonula, occludens-1, claudin-1, claudin-2, claudin-4, junction adhesion molecule-A, and F-actin, which are important in building the physical connections between epithelial cells for normal gut permeability.[55-57] VSL#3 increased expression of the epithelial tight junction protein, occludin, and downregulated expression of claudin-2, which attenuated increased gut permeability in mice with experimentally induced Crohn's disease.[55] Moreover, certain probiotics have the ability to maintain intestinal mucin levels, which may be influenced by chemotherapy.

The capacity for probiotic strains such as *Lactobacillus* spp. and *Clostridium tyrobutyricum* to adjust mucin secretion could therefore play a pivotal role in restoring microbiota composition and mucosal immunity in chemotherapy-treated patients.[58-60] Furthermore, certain probiotics have the potential to modulate cell kinetics by adjusting homeostasis between apoptosis and proliferation. For example, *Lactobacillus rhamnosus* regulated cellular proliferation and migration and mitogen-activated protein kinase pathways, responsible for cell proliferation, differentiation, and cytoprotection.[61,62] In contrast, some other probiotic mixtures such as kefir were found to reduce the incidence of apoptosis in heart tissues by controlling the activities of pro-apoptotic proteins such as Bax, bad, cytochrome c, and caspase-3 in ovalbumin-treated rats.[63] Considered together, an imbalance in the ratio of enterocyte apoptosis to proliferation, induced by chemotherapy, could potentially be restored by the intake of certain probiotics.

Probiotics and Immunity

Administration of certain probiotics can enhance innate immunity by increasing numbers and activities of immune cells and mediating inflammation.[45] In addition, probiotics may improve adaptive immune function by modulation of antigen-specific antibodies.[54] For example, numbers of circulating natural killer cells and immature T cell subsets increased in elderly individuals ingesting *Lactobacillus delbrueckii* subsp. *bulgaricus* 8481 for 6 months.[45] Moreover, the immune risk phenotype, characterized by an inverted CD4/CD8 ratio, an increase of $CD8+CD28^{null}$ T cells, and cytomegalovirus infection was also amplified in the same study.[45,64] Furthermore, administration of *Clostridium tyrobutyricum* depressed expression of pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-18 in the descending colon of dextran sodium sulfate–treated mice.[59] Similar results have also been described in cytokine-mediated gastrointestinal diseases,[65] alcohol-induced inflammation,[66,67] and even chemotherapy-induced mucositis.[34] Besides, administration of probiotics (Jinshuangqi tablets) for 7 days significantly improved levels of IgA, IgG, IgM, interelukin-2, and CD4+ in the serum of colorectal cancer patients, demonstrating the capacity for probiotics to modulate adaptive immunity.[54]

Probiotic-Derived Factors

Probiotic-derived factors are proteins or other molecules released from living probiotics, with the potential to confer health benefits to the host. Investigation of these factors could be used to achieve therapeutic benefits while avoiding risks related to the administration of live bacteria.[48] Identification of specific probiotic-derived factors also provides an opportunity to better understand mechanisms of probiotic action.

Competition With Pathogens

Bacteriocins, such as reuterin and other proteinaceous molecules, have been shown to inhibit the adhesion and viability of known enteric pathogens.[48,68] *Lactobacillus acidophilus* ATCC 4356 releases a proteinaceous molecule that inhibited the activity of 8 of the human *Campylobacter jejuni* strains affecting Caco-2 cells.[69] Moreover, other strains of *Lactobacillus* spp. produced non–lactic acid molecules in their cell-free cultured supernatants, which were responsible for killing activity against pathogenic *Salmonella enterica* serovar Typhimurium SL1344.[70,71] Together, these probiotic-derived factors both restricted the activity and killed certain pathogens, thereby decreasing the likelihood of infection, potentially reducing bacterial invasion caused by chemotherapy.

Maintenance of the Gut Barrier

Modulation of the mucus layer, tight junctions, and gut permeability by probiotic-derived factors play an important role in the maintenance of gut integrity. These factors could therefore potentially ameliorate the gut damage induced by chemotherapy.[48] Administration of *Lactobacillus rhamnosus* supernatants to alcohol-treated mice altered intestinal tight junction proteins by increasing ileum mRNA levels of claudin-1.[67] Moreover, mRNA levels of intestinal trefoil factor, P-glycoprotein, and cathelin-related antimicrobial peptide were also restored by *Lactobacillus rhamnosus* supernatant pretreatment.[67] In addition, *Lactobacillus rhamnosus* supernatants prevented barrier dysfunction of Caco-2 cell monolayers, induced by alcohol.[72] Certain proteinaceous factors extracted from *Clostridium butyridium* CGMCC0313-1 have been reported to augment the expression of A20 in HT-29 cells, which is important in the maintenance of barrier function.[73] Together, these in vitro results demonstrate the potential for probiotic-derived factors to maintain the intestinal barrier following chemotherapy-induced gut damage in vivo and in vitro.

Cell Kinetics

Two novel proteins, p75 (75 kDa) and p40 (40 kDa), released from *Lactobacillus rhamnosus*, have been reported to promote the propagation of epithelial cells and prevent apoptosis of epithelial cells induced by tumor necrosis factor-α.[65] Moreover, a recent study by Lebeer et al found that p75, renamed as Msp1, secreted by *Lactobacillus rhamnosus*, is an *O*-glycosylated protein.[74] Glycosylation of Msp1 plays an important role in communication between the microbe and the host.[74] In addition, Prisciandaro et al recently reported that supernatants from *Lactobacillus rhamnosus* and *Escherichia coli* Nissle 1917 significantly decreased caspase 3/7 activity after a challenge by the chemotherapy antimetabolite, 5-fluorouracil, in the IEC-6 cell line, showing their potential ability to prevent or inhibit enterocyte apoptosis induced by chemotherapy.[35]

Immunity

Probiotic-derived factors have been shown to influence pathogen-induced or oxidative stimuli–induced inflammation. Several metabolites (<3000 Da), released from *Bifidobacterium breve* and *Streptococcus thermophilus*, have demonstrated the ability to inhibit tumor necrosis factor-α secretion from lipopolysaccharide-affected peripheral blood mononuclear cells or the THP-1 cell line.[75] In addition, these factors (metabolites) were reported to suppress lipopolysaccharide-FITC (a fluorescent marker by flow cytometry) binding to THP-1 cells and also to inhibit nuclear factor-κB activation. Moreover, *Lactobacillus reuteri*–formed biofilms have been shown to decrease tumor necrosis factor production in lipopolysaccharide-activated monocytoid cells.[76] Caco-2 cells pretreated with spent culture supernatants of *Lactobacillus plantarum* 2142 for 1 hour decreased interleukin-8 synthesis and, in addition, induced expression of Heat-shock protein (Hsp) 70.[77] More recently, probiotic *Lactobacillus paracasei* CNCM I-4034 cell-free supernatants reduced pro-inflammatory tumor necrosis factor-α and chemokine MCP-1 in human dendritic cells challenged with enteropathogenic *Salmonella*.[78] Interestingly, these supernatants produced similar beneficial effects to their living "parent" probiotic, *Lactobacillus paracasei* CNCM I-4034.

Probiotic-derived factors from *Bifidobacterium bifidum* LMG13195 could be a potential immunoregulator in vitro. These soluble factors, after being previously cocultured with HT29 cells, enhanced numbers of $CD4^{+}CD25^{high}$ cells expressing chemokine receptor Treg markers in human peripheral blood mononuclear cells.[79] Probiotic-derived factors could therefore exert similar effects to their parent probiotic counterparts by modulating immune cells to reduce inflammation and restore immunity affected by chemotherapy.

Prebiotics

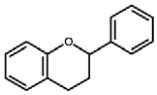
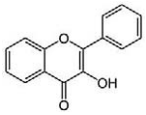
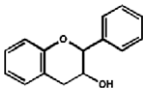
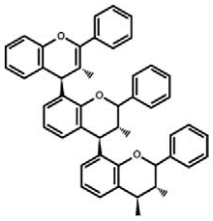
Prebiotics are indigestible compounds that "selectively stimulate the growth and/or activity of microbial species in the gut microbiota and confer health benefits to the host."[80] Prebiotics must be neither hydrolyzed nor absorbed in the proximal gastrointestinal tract, must be selectively fermented by one or a limited number of beneficial bacteria in the intestine, and be able to alter the colonic microbiota toward a healthier composition.[80,81] Prebiotic compounds include inulin,[82] a plant derived fructan; lactulose[83]; galacto-oligosaccharide[84]; and fructo-oligosaccharide.[85] Other compounds studied for their prebiotic potential include arabinoxylan-oligosaccharides,[86] chito-oligosaccharides,[87] epilactose,[88] germinated barley foodstuff,[89] mannan-oligosaccharides,[90] sialyl-oligosaccharides,[91] xylo-oligosaccharides,[92] and β-1,4-mannobiose.[93]

The potential role of prebiotics in mucositis treatment has recently (2012) been reviewed comprehensively by Wang et al.[94] Briefly, prebiotics have the ability to modulate probiotic bacteria by selective colonization of the intestinal microbiota, thereby decreasing chemotherapy-induced intestinal dysbiosis. Prebiotics could therefore maintain homeostasis of the gut microbiota following induction of mucositis. Moreover, certain prebiotics play an important role in the digestion and absorption of nutrients in the gut[95] and modulate intestinal barrier function through effects on mucin expression[96] and modification of mucosal immune responses.[97] Prebiotics could therefore become new nutraceuticals in terms of the amelioration of mucositis. Howarth recently proposed the importance of inflammasomes in mediating inflammation by prebiotics. However, further studies are required to determine the underlying mechanism of prebiotic action in the prevention and/or treatment of mucositis.[98]

Plant Extracts and Their Active Constituents

Many natural plant extracts have demonstrated medicinal uses at inhibiting the viability of pathogens.[99] These include Cat's

Table 1. Classification and Chemical Structure of Phenolic Compounds.

Classification	Chemical Structure
Plant	Example
Phenolic compounds	 (Simple phenols)
Flavonoids and many others	 (Flavonoids)
Flavonols (ketone group)	 (Flavonols; ketone group)
Flavanols (non-ketone group)	 (Flavanols; non-ketone group)
Proanthocyanidins (high-molecular-weight polymers)	 (Proanthocyannidins)
Free radical scavenging Antioxidant Antibacterial and antiviral Anti-inflammatory Anti-carcinogenic Anti-allergenic Vasodilatory	

Adapted from Fine AM. Oligomeric proanthocyanidin complexes: history, structure, and phytopharmaceutical applications. *Altern Med Rev.* 2000;5:144-151.

claw (*Uncaria tomentosa*), Maca (*Lepidium meyenii*), and Dragon's blood (*Croton lechleri*). However, in this review, we focus on the potential of readily accessible plant extracts, such as grape seed extract and Iberogast (and their commonly shared active constituents), to reduce the severity of gastrointestinal mucositis.

Phenolic compounds or polyphenols are widely distributed in the plant regime.[100] They are characterized by the presence of multiple phenol structures. Flavonoids are one type of phenolic compounds, which can be classified into flavonols, flavanols, and many others. Flavonol comprises a ketone group (RC(=O)R′); flavanol or flavan-3-ols (catechins) belong to the flavonoid classification and are structurally similar to flavonol, but they are in the non-ketone group (Table 1).[101]

In chemical structure, single-molecule flavan-3-ol adds hydroxyls (–OH) becoming catechin or its isomer, epicatechin. Procyanidins, members of the proanthocyanidins, are concentrated polymers formed from the monomers catechin and epicatechin.[102] Therefore, catechin trimers become oligomeric proanthocyanidin complexes and higher order polymers become anthocyanidins (Table 1).[102]

The most significant effect of procyanidins/proanthocyanidins is that they possess reactive oxygen species free radical scavenging ability. This results from antioxidant activities in many plant-sourced extracts including grape seed extract, Cat's Claw, Maca, and Dragon's Blood.[103-106] The antioxidant ability of plant extracts against reactive oxygen species is elucidated in the following sections.

Effects of Active Constituents of Plant Extracts on Reactive Oxygen Species

Reactive oxygen species are chemically reactive molecules (free radicals) containing oxygen such as oxygen ions and peroxides. Oxidative stress plays an important role in the pathogenesis of disorders, which include rheumatoid arthritis, asthma, psoriasis, and contact dermatitis.[105] Chemotherapy generates reactive oxygen species, which can initiate a series of biological actions.[29] For example, reactive oxygen species induce neutrophil infiltration and pro-inflammatory cytokine production, such as tumor necrosis factor-α. Increased tumor necrosis factor-α in the submucosa activates and amplifies transcription factors such as nuclear factor-κB. This is thought to induce cell apoptosis, leading to breakdown of the intestinal epithelial monolayer, intestinal barrier damage, pathogen invasion, and clinical manifestations of mucositis.[29,107,108] Furthermore, reactive oxygen species potentially reacts with macromolecules of the gut mucosa, such as lipids, proteins, and nucleic acid, and causes lipid peroxidation, which has a role in destruction of the intestinal epithelium.[39,109] Therefore, overproduction of reactive oxygen species plays an important role in the pathogenesis of chemotherapy-induced gut damage.

Many studies have reported the antioxidative properties of phenolic compounds. Phenolic substances such as procyanidins interact with the polar head group of phospholipids in cell membranes to exert their antioxidant effects.[110] Verstraeten et al[111] showed that dimer and trimer procyanidins protected the bilayer from oxidant-induced stress by interacting with membrane phospholipids. Facino et al[112] reported that a procyanidin-enriched diet fed to rats was associated with increased antioxidant activity in the plasma and reduced cardiac damage. Similar results were obtained by Busserolles et al,[113] who fed rats procyanidin-rich extracts for 8 weeks. Plasma antioxidant capacity was higher in rats than those fed the control diet, indicating the antioxidant effect of plasma procyanidins in vivo.[113]

Malondialdehyde level is a presumptive marker for lipid peroxidation that results from lipid-free radical interaction.[106] Kalin et al reported the ability of proanthocyanidins to decrease malondialdehyde levels.[106] This has also been demonstrated by Gulgun et al,[109] who showed that proanthocyanidins decreased mucosal damage and oxidant stress from the chemotherapy drug methotrexate through decreasing lipid peroxidation. Additionally, proanthocyanidins significantly inhibited toxicity from cancer chemotherapeutic drugs (Idarubicin and 4-hydroxyperoxycyclophosphamide) through increased expression of the anti-apoptotic protein, Bcl-2.[114] Furthermore, Kalin et al[106] concluded that proanthocyanidins decreased the induction of adhesion molecules such as ICAM-1, VCAM-1, and E-selectin, which are responsible for damage to the vascular endothelium.

Procyanidins/proanthocyanidins also exert anti-inflammatory and anti-ulcerogenic effects.[39,109] Bak et al[115] showed that procyanidins from wild grape seeds exhibit a chemopreventive character, due to an increase in nuclear factor E2–related factor expression. Nuclear factor E2–related factor is related to the antioxidant response element, which mediates expression of phase II detoxifying/antioxidant enzymes, such as NAD(P)H: quinone oxidoreductase-1 (NQ01) and heme oxygenase-1 (HO-1). These enzymes play a vital role in cell protection and cancer prevention. Furthermore, it is believed that proanthocyanidins exert an antithrombotic effect and can improve mucosal blood flow, which may contribute to antiulcer effects.[109] In an in vivo study, proanthocyanidins exerted antiulcer effects on the gastric mucosa, through significantly inhibiting myeloperoxidase activities (as an inflammatory indicator) and stimulating superoxide dismutase activities (as an antioxidative indicator) in rats.[116] It was reported that proanthocyanidin decreased leukocyte infiltration and tumor necrosis factor-α and interleukin-1β (pro-inflammatory factors), CINC-1 (an important mediator that promotes migration of neutrophils), and nitrite levels in the pleural exudation, which occurred after a carrageen-induced acute inflammatory reaction in rats.[117] These results further supported the anti-inflammatory effects of proanthocyandins.

In summary, phenolic compounds possess antioxidant, anti-inflammatory, and anti-ulcerogenic properties. These properties of procyanidins/proanthocyanidins are partly contributed by their free-radical scavenging characteristics, modulating apoptotic regulatory genes such as bcl-2 and p53, thereby influencing the production of pro-inflammatory factors such as tumor necrosis factor-α and interleukin-1β.[109,114] Plant extracts, which contain these active constituents, could therefore be of benefit in treating or preventing mucositis.

Plant Extracts and Intestinal Mucositis

Very few studies have tested the effects of plant extracts on mucositis. Cheah et al[39] studied the potential for grape seed extract to protect the rat intestine from 5-fluorouracil-induced intestinal damage in vitro and in vivo. These investigators reported that grape seed extract had the ability to restore IEC-6 cell viability following damage by 5-fluorouracil, indicating that grape seed extract was partially effective against this chemotherapy drug in vitro. Additionally, grape seed extract showed the ability to reduce 5-fluorouracil-induced intestinal myeloperoxidase activity, an indicator of inflammation. Furthermore, grape seed extract was found to remain active in the large bowel.[39] Moreover, Gulgun et al showed promising results for proanthocyanidins in improving methotrexate-induced intestinal damage in rats.[109] Proanthocyanidins reduced methotrexate-induced inflammation and associated ulceration and normalized the activities of superoxide dismutase and glutathione peroxidase. This indicated that proanthocyanidins amplified the defense system against oxidative stress and also decreased lipid peroxidation following methotrexate-induced damage.[109]

Iberogast, also known as STW5, is a mixture of 9 different herbal extracts containing bitter candytuft (*Iberis amara*), angelica root (*Angelicae radix*), milk thistle fruit (*Cardui mariae 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*).[118] Iberogast modulates gastrointestinal motility and restricts gastric acid production. It also possesses anti-inflammatory, antioxidative, and free radical–inhibiting properties. The active compounds in Iberogast, especially flavonoids, are believed to contribute to its pharmacological properties in the therapy of several gastrointestinal conditions such as functional dyspepsia and irritable bowel syndrome.[118] Indeed, recently, Iberogast has been tested for its therapeutic efficacies on 5-fluorouracil-induced mucositis in rats.[38] Iberogast improved the histopathological features of mucosa, which were damaged by 5-fluorouracil injection. Villus height and crypt depth were increased significantly in Iberogast-fed rats treated with 5-fluorouracil.[38] However, other indicators of mucositis were unaffected by Iberogast in 5-fluorouracil-treated rats.[38] These results indicate that Iberogast may possess the potential to reduce the severity of chemotherapy-induced mucositis; however, further studies on dose and frequency of administration are warranted.

Other plant extracts such as glycolipid extracts from spinach, which comprise high levels of glycoglycerolipids, have revealed antioxidative and anti-inflammatory effects in the 5-fluorouracil setting in vivo and in vitro. Mucosal injury, such as villous atrophy and misaligned crypts in the jejunum of rats affected by 5-fluorouracil, were ameliorated significantly in rats fed glycolipid extracts.[119] Moreover, glycolipid extracts reduced mRNA expression of inflammatory cytokines such as interleukin-1α and tumor necrosis factor-α caused by 5-fluorouracil injection in the same group of rats. In addition, the active constituents of spinach extract, such as monogalactosyl-diacylglycerol and diglactosyl-diacylglycerol, inhibited the production of reactive oxygen species in phorbol ester challenged Caco-2 cells.[119] Furthermore, following 5-fluorouracil administration, hamsters fed *Eriobotrya japonica* seed extract showed no epithelial tissue defects or bacterial infections at the local site of mucositis in the oral cavity.[120] Plasma lipid peroxide levels were also decreased significantly in the same

group.[120] Moreover, in a clinical trial, the severity of radiation-induced mucositis was attenuated in patients, as measured by significantly decreased serum interleukin-6 levels, following gargling and ingestion of indigowood root (*Isatis indigotica* Fort.).[121]

In summary, certain plant extracts such as grape seed extract and Iberogast have revealed anti-inflammatory and antioxidant effects on chemotherapy-induced mucositis. These plant extracts could be a potential new prophylactic treatment strategy for intestinal mucositis,[39] although further research is required. Further studies should focus on identifying the bioactive constituents involved together with the identification of further, as yet untested plant extracts.

Combinations of Probiotics/Probiotic-Derived Factors and Plant Extracts

Synbiotics are defined as strategically-identified symbiotic combinations of probiotics and prebiotics. Synbiotics may be exhibit greater potency compared to the sum of each individual agent. Shimizu et al recently elucidated the importance of synbiotics in the maintenance of gut health and treatment of critical illnesses such as septic complications.[122] Fermented milk supplemented with 2 probiotic strains, *Bifidobacterium lactis* Bi-07 and *Lactobacillus acidophilus* NCFM, and a prebiotic, isomaltooligosaccharide, administered to healthy adults and mice improved intestinal health as indicated by increases in fecal bifidobacteria and lactobacilli and decreases in enterobacilli.[123] Humoral and cell-mediated immunity have also been improved by the same tested synbiotics in mice.[123] The combination of *Lactobacillus acidophilus* and ginger extract improved ulcer index, mucus secretion, oxidative stress, and histopathological parameters, when compared with the individual agents, in a gastric ulcer setting in rats.[124]

Very few studies have described the effects of synbiotics on reducing the severity of mucositis. Smith et al tested the combination of *Lactobacillus fermentum* BR11 and the prebiotic, fructo-oligosaccharide, in 5-fluorouracil-injected rats.[36] These investigators found that *Lactobacillus fermentum* BR11 partially reduced the inflammation caused by 5-fluorouracil in the small intestine in rats. However, the combination did not provide additional therapeutic benefit for mucositis. Further studies are required to test the effects of combinations of different probiotics and prebiotics on reducing the severity of mucositis.

The prebiotic potential of virtually all plant extracts identified to date remains essentially unexplored. New nutraceuticals, including probiotics, probiotic factors, prebiotics, and plant extracts, hold the potential to decrease gut damage through different mechanisms, thereby increasing the likelihood of achieving clinically measurable net benefit. For example, certain probiotics could combat mucositis through effects on bacterial composition in the bowel whereas certain plant extracts would likely be more potent at counteracting oxidative stress created by chemotherapy. Therefore, indicated combinations of probiotics (or probiotic factors) and plant extracts could potentially reduce the severity of mucositis to a greater extent than each of the agents used independently, thereby potentially achieving measurable clinical improvement.

As the majority of prebiotics are derived from plants, future studies could focus on determining the prebiotic properties of a wider range of plant extracts. Future challenges will include mechanisms to identify specific combinations of probiotics and prebiotics that exert synergistic benefits in mucositis treatment. Determining dosing regimens, frequency of administration, the specific bioactive factors involved, and their associated pharmacodynamics will represent further challenges before these promising new nutraceutical formulations can be recommended for mucositis treatment.

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References

1. Queensland Government. Cancer. http://access.health.qld.gov.au/hid/Cancer/WhatisCancer/cancer_fs.asp. Accessed November 26, 2012.
2. Cancer Research UK. What cancer is. http://cancerhelp.cancerresearchuk.org/about-cancer/what-is-cancer/cells/what-cancer-is. Accessed November 26, 2012.
3. Lima L, Dinis-Ribeiro M, Longatto-Filho A, Santos L. Predictive biomarkers of bacillus calmette-guerin immunotherapy response in bladder cancer: where are we now? *Adv Urol*. 2012;2012: 232609.
4. Narayan C, Kumar A. Constitutive over expression of IL-1β, IL-6, NF-κB, and Stat3 is a potential cause of lung tumorgenesis in urethane (ethyl carbamate) induced Balb/c mice. *J Carcinog*. 2012;11:9.
5. Villarreal-Garza C, Shaw-Dulin R, Lara-Medina F, et al. Impact of diabetes and hyperglycemia on survival in advanced breast cancer patients. *Exp Diabetes Res*. 2012;2012:732027.
6. Kahlert C, Gaitzsch E, Steinert G, et al. Expression analysis of aldehyde dehydrogenase 1A1 (ALDH1A1) in colon and rectal cancer in association with prognosis and response to chemotherapy. *Ann Surg Oncol*. 2012;19:4193-4201.
7. Teras LR, Patel AV, Hildebrand JS, Gapstur SM. Postmenopausal unopposed estrogen and estrogen plus progestin use and risk of non-Hodgkin lymphoma in the American Cancer Society Cancer

Prevention Study-II Cohort [published online September 14, 2012]. *Leuk Lymphoma*. doi:10.3109/10428194.2012.722216.
8. Colas E, Pedrola N, Devis L, et al. The EMT signaling pathways in endometrial carcinoma. *Clin Transl Oncol*. 2012;14: 715-720.
9. Pazienza V, Tavano F, Benegiamo G, et al. Correlations among PPARγ, DNMT1, and DNMT3B expression levels and pancreatic cancer. *PPAR Res*. 2012;2012:461784.
10. Lou S, Ren L, Xiao J, Ding Q, Zhang W. Expression profiling based graph-clustering approach to determine renal carcinoma related pathway in response to kidney cancer. *Eur Rev Med Pharmacol Sci*. 2012;16:775-780.
11. Andreotti G, Koutros S, Berndt SI, et al. The interaction between pesticide use and genetic variants involved in lipid metabolism on prostate cancer risk. *J Cancer Epidemiol*. 2012;2012:358076.
12. Witt O, Milde T, Deubzer HE, et al. Phase I/II intra-patient dose escalation study of vorinostat in children with relapsed solid tumor, lymphoma or leukemia. *Klin Padiatr*. 2012;224:398-403.
13. Bellelli R, Castellone MD, Garcia-Rostan G, et al. FOXM1 is a molecular determinant of the mitogenic and invasive phenotype of anaplastic thyroid carcinoma. *Endocr Relat Cancer*. 2012;19: 695-710.
14. Lorusso D, Mancini M, Di Rocco R, Fontanelli R, Raspagliesi F. The role of secondary surgery in recurrent ovarian cancer. *Int J Surg Oncol*. 2012;2012:613980.
15. Wietek BM, Kratt T. Current MRI staging of rectal cancer. *Rofo*. 2012;184:992-1001.
16. Waldmann TA. Immunotherapy: past, present and future. *Nat Med*. 2003;9:269-277.
17. Gibson E, Monje M. Effect of cancer therapy on neural stem cells: implications for cognitive function. *Curr Opin Oncol*. 2012;24: 672-678.
18. Decker-Baumann C, Buhl K, Frohmüller S, von Herbay A, Dueck M, Schlag PM. Reduction of chemotherapy-induced side-effects by parenteral glutamine supplementation in patients with metastatic colorectal cancer. *Eur J Cancer*. 1999;35:202-207.
19. Sonis ST. Oral mucositis in cancer therapy. *J Support Oncol*. 2004;2:3-8.
20. Sonis ST, Elting LS, Keefe D, et al. Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. *Cancer*. 2004;100: 1995-2025.
21. Sonis ST. A biological approach to mucositis. *J Support Oncol*. 2004;2:21-32.
22. Keefe DM, Schubert MM, Elting LS, et al. Updated clinical practice guidelines for the prevention and treatment of mucositis. *Cancer*. 2007;109:820-831.
23. Duncan M, Grant G. Oral and intestinal mucositis—causes and possible treatments. *Aliment Pharmacol Ther*. 2003;18:853-874.
24. Stringer AM, Gibson RJ, Logan RM, et al. Gastrointestinal microflora and mucins may play a critical role in the development of 5-fluorouracil-induced gastrointestinal mucositis. *Exp Biol Med (Maywood)*. 2009;234:430-441.
25. Keefe DM, Brealey J, Goland GJ, Cummins AG. Chemotherapy for cancer causes apoptosis that precedes hypoplasia in crypts of the small intestine in humans. *Gut*. 2000;47:632-637.
26. Sonis S. Mucositis as a biological process: a new hypothesis for the development of chemotherapy-induced stomatotoxicity. *Oral Oncol*. 1998;34:39-43.
27. Lalla RV, Peterson DE. Treatment of mucositis, including new medications. *Cancer J*. 2006;12:348-354.
28. Howarth GS, Cool JC, Bourne AJ, Ballard FJ, Read LC. Insulin-like growth factor-I (IGF-I) stimulates regrowth of the damaged intestine in rats, when administered following, but not concurrent with, methotrexate. *Growth Factors*. 1998;15: 279-292.
29. Sonis ST. Pathobiology of mucositis. *Semin Oncol Nurs*. 2004;20: 11-15.
30. Rodríguez-Caballero A, Torres-Lagares D, Robles-García M, Pachón-Ibáñez J, González-Padilla D, Gutiérrez-Pérez JL. Cancer treatment-induced oral mucositis: a critical review. *Int J Oral Maxillofac Surg*. 2012;41:225-238.
31. Howarth GS, Francis GL, Cool JC, Xu X, Byard RW, Read LC. Milk growth factors enriched from cheese whey ameliorate intestinal damage by methotrexate when administered orally to rats. *J Nutr*. 1996;126:2519-2530.
32. Howarth GS. Insulin-like growth factor-I and the gastrointestinal system: therapeutic indications and safety implications. *J Nutr*. 2003;133:2109-2112.
33. Bowen JM, Stringer AM, Gibson RJ, Yeoh AS, Hannam S, Keefe DM. VSL#3 probiotic treatment reduces chemotherapy-induced diarrhea and weight loss. *Cancer Biol Ther*. 2007;6: 1449-1454.
34. Tooley KL, Howarth GS, Lymn KA, Lawrence A, Butler RN. Oral ingestion of streptococcus thermophilus diminishes severity of small intestinal mucositis in methotrexate treated rats. *Cancer Biol Ther*. 2006;5:593-600.
35. Prisciandaro LD, Geier MS, Chua AE, et al. Probiotic factors partially prevent changes to caspases 3 and 7 activation and transepithelial electrical resistance in a model of 5-fluorouracil-induced epithelial cell damage. *Support Care Cancer*. 2012;20: 3205-3210.
36. Smith CL, Geier MS, Yazbeck R, Torres DM, Butler RN, Howarth GS. *Lactobacillus fermentum* BR11 and fructo-oligosaccharide partially reduce jejunal inflammation in a model of intestinal mucositis in rats. *Nutr Cancer*. 2008;60:757-767.
37. Prisciandaro LD, Geier MS, Butler RN, Cummins AG, Howarth GS. Probiotic factors partially improve parameters of 5-fluorour acil-induced intestinal mucositis in rats. *Cancer Biol Ther*. 2011;11:671-677.
38. Wright TH, Yazbeck R, Lymn KA, et al. The herbal extract, Iberogast, improves jejunal integrity in rats with 5-fluorouracil (5-FU)-induced mucositis. *Cancer Biol Ther*. 2009;8:923-929.
39. Cheah KY, Howarth GS, Yazbeck R, et al. Grape seed extract protects IEC-6 cells from chemotherapy-induced cytotoxicity and improves parameters of small intestinal mucositis in rats with experimentally-induced mucositis. *Cancer Biol Ther*. 2009;8: 382-390.
40. Torres DM, Tooley KL, Butler RN, Smith CL, Geier MS, Howarth GS. Lyprinol only partially improves indicators of small intestinal integrity in a rat model of 5-fluorouracil-induced mucositis. *Cancer Biol Ther*. 2008;7:295-302.

41. Abimosleh SM, Tran CD, Howarth GS. Emu oil: a novel therapeutic for disorders of the gastrointestinal tract? *J Gastroenterol Hepatol*. 2012;27:857-861.
42. Reid G. The importance of guidelines in the development and application of probiotics. *Curr Pharm Des*. 2005;11:11-16.
43. Borchers AT, Selmi C, Meyers FJ, Keen CL, Gershwin ME. Probiotics and immunity. *J Gastroenterol*. 2009;44:26-46.
44. Yang CM, Cao GT, Ferket PR, et al. Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function, and cecal microflora in broiler chickens. *Poult Sci*. 2012;91: 2121-2129.
45. Moro-Garcia MA, Alonso-Arias R, Baltadjieva M, et al. Oral supplementation with *Lactobacillus delbrueckii* subsp. bulgaricus 8481 enhances systemic immunity in elderly subjects [published online May 30, 2012]. *Age (Dordr)*. doi:10.1007/s11357-012-9434-6.
46. Tanriover MD, Aksoy DY, Unal S. Use of probiotics in various diseases: evidence and promises. *Pol Arch Med Wewn*. 2012; 122(suppl 1):72-77.
47. Schultz M. Clinical use of *E. coli* Nissle 1917 in inflammatory bowel disease. *Inflamm Bowel Dis*. 2008;14:1012-1018.
48. Howarth GS. Probiotic-derived factors: probiotaceuticals? *J Nutr*. 2010;140:229-230.
49. Prisciandaro LD, Geier MS, Butler RN, Cummins AG, Howarth GS. Evidence supporting the use of probiotics for the prevention and treatment of chemotherapy-induced intestinal mucositis. *Crit Rev Food Sci Nutr*. 2011;51:239-247.
50. Lukic J, Strahinic I, Jovcic B, et al. Different roles of lactococcal aggregation factor and mucin binding protein in adhesion to gastrointestinal mucosa. *Appl Environ Microbiol*. 2012;78: 7993-8000.
51. Gonzalez-Rodriguez I, Sanchez B, Ruiz L, et al. Role of extracellular transaldolase from *Bifidobacterium bifidum* in mucin adhesion and aggregation. *Appl Environ Microbiol*. 2012;78: 3992-3998.
52. Bujnakova D, Kmet V. Functional properties of *Lactobacillus* strains isolated from dairy products. *Folia Microbiol (Praha)*. 2012;57:263-267.
53. Aloisio I, Santini C, Biavati B, et al. Characterization of *Bifidobacterium* spp. strains for the treatment of enteric disorders in newborns. *Appl Microbiol Biotechnol*. 2012;96:1561-1576.
54. Zhu DJ, Chen XW, Wu JH, et al. Effect of perioperative intestinal probiotics on intestinal flora and immune function in patients with colorectal cancer. *Nan Fang Yi Ke Da Xue Xue Bao*. 2012;32: 1190-1193.
55. Corridoni D, Pastorelli L, Mattioli B, et al. Probiotic bacteria regulate intestinal epithelial permeability in experimental ileitis by a TNF-dependent mechanism. *PLoS One*. 2012;7:e42067.
56. Harhaj NS, Antonetti DA. Regulation of tight junctions and loss of barrier function in pathophysiology. *Int J Biochem Cell Biol*. 2004;36:1206-1237.
57. Zhou YK, Qin HL, Zhang M, et al. Effects of *Lactobacillus plantarum* on gut barrier function in experimental obstructive jaundice. *World J Gastroenterol*. 2012;18:3977-3991.
58. O'Callaghan J, Buttó LF, MacSharry J, Nally K, O'Toole PW. Influence of adhesion and bacteriocin production by *Lactobacillus salivarius* on the intestinal epithelial cell transcriptional response. *Appl Environ Microbiol*. 2012;78:5196-5203.
59. Hudcovic T, Kolinska J, Klepetar J, et al. Protective effect of *Clostridium tyrobutyricum* in acute dextran sodium sulphate-induced colitis: differential regulation of tumour necrosis factor-alpha and interleukin-18 in BALB/c and severe combined immunodeficiency mice. *Clin Exp Immunol*. 2012;167:356-365.
60. Duary RK, Bhausaheb MA, Batish VK, Grover S. Anti-inflammatory and immunomodulatory efficacy of indigenous probiotic Lactobacillus plantarum Lp91 in colitis mouse model. *Mol Biol Rep*. 2012;39:4765-4775.
61. Lin PW, Nasr TR, Berardinelli AJ, Kumar A, Neish AS. The probiotic *Lactobacillus* GG may augment intestinal host defense by regulating apoptosis and promoting cytoprotective responses in the developing murine gut. *Pediatr Res*. 2008; 64:511-516.
62. Guma M, Firestein GS. c-Jun N-terminal kinase in inflammation and rheumatic diseases. *Open Rheumatol J*. 2012;6:220-231.
63. Wang HF, Tseng CY, Chang MH, et al. Anti-inflammatory effects of probiotic *Lactobacillus paracasi* on ventricles of BALB/C mice treated with ovalbumin. *Chin J Physiol*. 2012; 55:37-46.
64. Hadrup SR, Strindhall J, Kollgaard T, et al. Longitudinal studies of clonally expanded CD8 T cells reveal a repertoire shrinkage predicting mortality and an increased number of dysfunctional cytomegalovirus-specific T cells in the very elderly. *J Immunol*. 2006;176:2645-2653.
65. Yan F, Cao H, Cover TL, Whitehead R, Washington MK, Polk DB. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology*. 2007;132:562-575.
66. Forsyth CB, Farhadi A, Jakate SM, Tang Y, Shaikh M, Keshavarzian A. *Lactobacillus* GG treatment ameliorates alcohol-induced intestinal oxidative stress, gut leakiness, and liver injury in a rat model of alcoholic steatohepatitis. *Alcohol*. 2009;43: 163-172.
67. Wang Y, Liu Y, Sidhu A, Ma Z, McClain C, Feng W. *Lactobacillus* rhamnosus GG culture supernatant ameliorates acute alcohol-induced intestinal permeability and liver injury. *Am J Physiol Gastrointest Liver Physiol*. 2012;303:G32-G41.
68. Talarico TL, Dobrogosz WJ. Chemical characterization of an antimicrobial substance produced by *Lactobacillus reuteri*. *Antimicrob Agents Chemother*. 1989;33:674-679.
69. Campana R, Federici S, Ciandrini E, Baffone W. Antagonistic activity of *Lactobacillus acidophilus* ATCC 4356 on the growth and adhesion/invasion characteristics of human *Campylobacter jejuni*. *Curr Microbiol*. 2012;64:371-378.
70. Fayol-Messaoudi D, Berger CN, Coconnier-Polter MH, Liévin-Le Moal V, Servin AL. pH-, lactic acid-, and non-lactic acid-dependent activities of probiotic *Lactobacilli* against *Salmonella enterica* Serovar Typhimurium. *Appl Environ Microbiol*. 2005; 71:6008-6013.
71. Pridmore RD, Pittet AC, Praplan F, Cavadini C. Hydrogen peroxide production by *Lactobacillus johnsonii* NCC 533 and its role in anti-Salmonella activity. *FEMS Microbiol Lett*. 2008;283: 210-215.

72. Wang Y, Kirpich I, Liu Y, et al. *Lactobacillus rhamnosus* GG treatment potentiates intestinal hypoxia-inducible factor, promotes intestinal integrity and ameliorates alcohol-induced liver injury. *Am J Pathol*. 2011;179:2866-2875.
73. Song CH, Liu ZQ, Huang S, Zheng PY, Yang PC. Probiotics promote endocytic allergen degradation in gut epithelial cells. *Biochem Biophys Res Commun*. 2012;426:135-140.
74. Lebeer S, Claes IJ, Balog CI, et al. The major secreted protein Msp1/p75 is O-glycosylated in *Lactobacillus rhamnosus* GG. *Microb Cell Fact*. 2012;11:15.
75. Ménard S, Candalh C, Bambou JC, Terpend K, Cerf-Bensussan N, Heyman M. Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. *Gut*. 2004; 53:821-828.
76. Jones SE, Versalovic J. Probiotic *Lactobacillus reuteri* biofilms produce antimicrobial and anti-inflammatory factors. *BMC Microbiol*. 2009;9:35.
77. Nemeth E, Fajdiga S, Malago J, Koninkx J, Tooten P, van Dijk J. Inhibition of *Salmonella*-induced IL-8 synthesis and expression of Hsp70 in enterocyte-like Caco-2 cells after exposure to non-starter lactobacilli. *Int J Food Microbiol*. 2006;112:266-274.
78. Bermudez-Brito M, Munoz-Quezada S, Gomez-Llorente C, et al. Human intestinal dendritic cells decrease cytokine release against *Salmonella* infection in the presence of *Lactobacillus paracasei* upon TLR activation. *PLoS One*. 2012;7:e43197.
79. Lopez P, Gonzalez-Rodriguez I, Sanchez B, et al. Interaction of *Bifidobacterium bifidum* LMG13195 with HT29 cells influences regulatory-T-cell-associated chemokine receptor expression. *Appl Environ Microbiol*. 2012;78:2850-2857.
80. Roberfroid M, Gibson GR, Hoyles L, et al. Prebiotic effects: metabolic and health benefits. *Br J Nutr*. 2010;104(suppl 2): S1-63.
81. Looijer-van Langen MA, Dieleman LA. Prebiotics in chronic intestinal inflammation. *Inflamm Bowel Dis*. 2009;15:454-462.
82. Abrams SA, Griffin IJ, Hawthorne KM, et al. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. *Am J Clin Nutr*. 2005;82:471-476.
83. Bovee-Oudenhoven IM, ten Bruggencate SJ, Lettink-Wissink ML, van der Meer R. Dietary fructo-oligosaccharides and lactulose inhibit intestinal colonisation but stimulate translocation of salmonella in rats. *Gut*. 2003;52:1572-1578.
84. Alliet P, Scholtens P, Raes M, et al. Effect of prebiotic galacto-oligosaccharide, long-chain fructo-oligosaccharide infant formula on serum cholesterol and triacylglycerol levels. *Nutrition*. 2007; 23:719-723.
85. Cherbut C, Michel C, Lecannu G. The prebiotic characteristics of fructooligosaccharides are necessary for reduction of TNBS-induced colitis in rats. *J Nutr*. 2003;133:21-27.
86. Maki KC, Gibson GR, Dickmann RS, et al. Digestive and physiologic effects of a wheat bran extract, arabino-xylan-oligosac-charide, in breakfast cereal. *Nutrition*. 2012;28:1115-1121.
87. Liu P, Piao XS, Kim SW, et al. Effects of chito-oligosaccharide supplementation on the growth performance, nutrient digestibility, intestinal morphology, and fecal shedding of *Escherichia coli* and *Lactobacillus* in weaning pigs. *J Anim Sci*. 2008;86: 2609-2618.
88. Nishimukai M, Watanabe J, Taguchi H, et al. Effects of epilactose on calcium absorption and serum lipid metabolism in rats. *J Agric Food Chem*. 2008;56:10340-10345.
89. Kanauchi O, Oshima T, Andoh A, Shioya M, Mitsuyama K. Germinated barley foodstuff ameliorates inflammation in mice with colitis through modulation of mucosal immune system. *Scand J Gastroenterol*. 2008;43:1346-1352.
90. Yang Y, Iji PA, Kocher A, Thomson E, Mikkelsen LL, Choct M. Effects of mannanoligosaccharide in broiler chicken diets on growth performance, energy utilisation, nutrient digestibility and intestinal microflora. *Br Poult Sci*. 2008;49:186-194.
91. Sinclair HR, Smejkal CW, Glister C, et al. Sialyloligosaccharides inhibit cholera toxin binding to the GM1 receptor. *Carbohydr Res*. 2008;343:2589-2594.
92. Xu B, Wang Y, Li J, Lin Q. Effect of prebiotic xylooligosaccharides on growth performances and digestive enzyme activities of allogynogenetic crucian carp (Carassius auratus gibelio). *Fish Physiol Biochem*. 2009;35:351-357.
93. Ibuki M, Kovacs-Nolan J, Fukui K, Kanatani H, Mine Y. Analysis of gut immune-modulating activity of beta-1,4-mannobiose using microarray and real-time reverse transcription polymerase chain reaction. *Poult Sci*. 2010;89:1894-1904.
94. Wang H, Geier MS, Howarth GS. Prebiotics: a potential treatment strategy for the chemotherapy-damaged gut? *Crit Rev Food Sci Nutr*. In press.
95. Bogusławska-Tryk M, Piotrowska A, Burlikowska K. Dietary fructans and their potential beneficial influence on health and performance parameters in broiler chickens. *J Cent Eur Agric*. 2012;13:272-291.
96. Leforestier G, Blais A, Blachier F, et al. Effects of galacto-oligosaccharide ingestion on the mucosa-associated mucins and sucrase activity in the small intestine of mice. *Eur J Nutr*. 2009; 48:457-464.
97. Abdelouhab K, Rafa H, Toumi R, Bouaziz S, Medjeber O, Touil-Boukoffa C. Mucosal intestinal alteration in experimental colitis correlates with nitric oxide production by peritoneal macrophages: effect of probiotics and prebiotics. *Immunopharmacol Immunotoxicol*. 2012;34:590-597.
98. Howarth GS. Commentary on prebiotic utility in colitis: will inflammasomics hold the key? *J Nutr*. 2012;142:1189-1190.
99. Gonzales GF, Valerio LG Jr. Medicinal plants from Peru: a review of plants as potential agents against cancer. *Anticancer Agents Med Chem*. 2006;6:429-444.
100. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr Rev*. 1998;56:317-333.
101. Fine AM. Oligomeric proanthocyanidin complexes: history, structure, and phytopharmaceutical applications. *Altern Med Rev*. 2000;5:144-151.
102. Schwitters B. *OPC in Practice: The Hidden Story of Proanthocyanidins*. 2nd ed. Rome, Italy: Alfa Omega Editrice; 1993.
103. Bagchi D, Sen CK, Ray SD, et al. Molecular mechanisms of cardioprotection by a novel grape seed proanthocyanidin extract. *Mutat Res*. 2003;523-524:87-97.

104. Erlejman AG, Fraga CG, Oteiza PI. Procyanidins protect Caco-2 cells from bile acid- and oxidant-induced damage. *Free Radic Biol Med*. 2006;41:1247-1256.
105. Geronikaki AA, Gavalas AM. Antioxidants and inflammatory disease: synthetic and natural antioxidants with anti-inflammatory activity. *Comb Chem High Throughput Screen*. 2006;9:425-442.
106. Kalin R, Righi A, Del Rosso A, et al. Activin, a grape seed-derived proanthocyanidin extract, reduces plasma levels of oxidative stress and adhesion molecules (ICAM-1, VCAM-1 and E-selectin) in systemic sclerosis. *Free Radic Res*. 2002;36: 819-825.
107. Banan A, Fields JZ, Zhang LJ, Shaikh M, Farhadi A, Keshavarzian A. Zeta isoform of protein kinase C prevents oxidant-induced nuclear factor-kappaB activation and I-kappaBalpha degradation: a fundamental mechanism for epidermal growth factor protection of the microtubule cytoskeleton and intestinal barrier integrity. *J Pharmacol Exp Ther*. 2003;307:53-66.
108. Keshavarzian A, Banan A, Farhadi A, et al. Increases in free radicals and cytoskeletal protein oxidation and nitration in the colon of patients with inflammatory bowel disease. *Gut*. 2003; 52:720-728.
109. Gulgun M, Erdem O, Oztas E, et al. Proanthocyanidin prevents methotrexate-induced intestinal damage and oxidative stress. *Exp Toxicol Pathol*. 2010;62:109-115.
110. Erlejman AG, Verstraeten SV, Fraga CG, Oteiza PI. The interaction of flavonoids with membranes: potential determinant of flavonoid antioxidant effects. *Free Radic Res*. 2004;38: 1311-1320.
111. Verstraeten SV, Hammerstone JF, Keen CL, Fraga CG, Oteiza PI. Antioxidant and membrane effects of procyanidin dimers and trimers isolated from peanut and cocoa. *J Agric Food Chem*. 2005;53:5041-5048.
112. Facino RM, Carini M, Aldini G, et al. Diet enriched with procyanidins enhances antioxidant activity and reduces myocardial post-ischaemic damage in rats. *Life Sci*. 1999;64:627-642.
113. Busserolles J, Gueux E, Balasinska B, et al. In vivo antioxidant activity of procyanidin-rich extracts from grape seed and pine (*Pinus maritima*) bark in rats. *Int J Vitam Nutr Res*. 2006;76: 22-27.
114. Joshi SS, Kuszynski CA, Benner EJ, Bagchi M, Bagchi D. Amelioration of the cytotoxic effects of chemotherapeutic agents by grape seed proanthocyanidin extract. *Antioxid Redox Signal*. 1999;1:563-570.
115. Bak MJ, Jun M, Jeong WS. Procyanidins from wild grape (*Vitis amurensis*) seeds regulate ARE-mediated enzyme expression via Nrf2 coupled with p38 and PI3K/Akt pathway in HepG2 Cells. *Int J Mol Sci*. 2012;13:801-818.
116. Iwasaki Y, Matsui T, Arakawa Y. The protective and hormonal effects of proanthocyanidin against gastric mucosal injury in Wistar rats. *J Gastroenterol*. 2004;39:831-837.
117. Garbacki N, Tits M, Angenot L, Damas J. Inhibitory effects of proanthocyanidins from *Ribes nigrum* leaves on carrageenin acute inflammatory reactions induced in rats. *BMC Pharmacol*. 2004;4:25.
118. Wegener T, Wagner H. The active components and the pharmacological multi-target principle of STW 5 (Iberogast). *Phytomedicine*. 2006;13(suppl 5):20-35.
119. Shiota A, Hada T, Baba T, et al. Protective effects of glycoglycerolipids extracted from spinach on 5-fluorouracil induced intestinal mucosal injury. *J Med Invest*. 2010;57:314-320.
120. Takuma D, Guangchen S, Yokota J, et al. Effect of *Eriobotrya japonica* seed extract on 5-fluorouracil-induced mucositis in hamsters. *Biol Pharm Bull*. 2008;31:250-254.
121. You WC, Hsieh CC, Huang JT. Effect of extracts from indigowood root (*Isatis indigotica* Fort.) on immune responses in radiation-induced mucositis. *J Altern Complement Med*. 2009; 15:771-778.
122. Shimizu K, Ogura H, Asahara T, et al. Probiotic/synbiotic therapy for treating critically ill patients from a gut microbiota perspective [published online August 19, 2012]. *Dig Dis Sci*. doi:10.1007/s10620-10012-12334-x.
123. Wang S, Zhu H, Lu C, et al. Fermented milk supplemented with probiotics and prebiotics can effectively alter the intestinal microbiota and immunity of host animals. *J Dairy Sci*. 2012; 95:4813-4822.
124. Singh PK, Kaur IP. Synbiotic (probiotic and ginger extract) loaded floating beads: a novel therapeutic option in an experimental paradigm of gastric ulcer. *J Pharm Pharmacol*. 2012; 64:207-217.