TOPIC HIGHLIGHT

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Gastro-intestinal toxicity of chemotherapeutics in colorectal cancer: The role of inflammation

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Abstract

Chemotherapy-induced diarrhea (CID) is a common and often severe side effect experienced by colorectal cancer (CRC) patients during their treatment. As chemotherapy regimens evolve to include more efficacious agents, CID is increasingly becoming a major cause of dose limiting toxicity and merits further investigation. Inflammation is a key factor behind gastrointestinal (GI) toxicity of chemotherapy. Different chemotherapeutic agents activate a diverse range of pro-inflammatory pathways culminating in distinct histopathological changes in the small intestine and colonic mucosa. Here we review the current understanding of the mechanisms behind GI toxicity and the mucositis associated with systemic treatment of CRC. Insights into the inflammatory response activated during this process gained from various models of GI toxicity are discussed. The inflammatory processes contributing to the GI toxicity of chemotherapeutic agents are increasingly being recognised as having an important role in the development of anti-tumor immunity, thus conferring added benefit against tumor recurrence and improving patient survival. We review the basic mechanisms involved in the promotion of immunogenic cell death and its relevance in the treatment of colorectal cancer. Finally, the impact of CID on patient outcomes and therapeutic strategies to prevent or minimise the effect of GI toxicity and mucositis are discussed.

Core tip: Many new drugs are available for use in the treatment of colorectal cancer, resulting in improved prognosis, but also more frequent and severe side-effects. In order to implement complex chemotherapy regimens most effectively, a greater understanding of the underlying mechanisms of associated toxicities are required. Different chemotherapeutic agents activate a diverse range of pro-inflammatory pathways culminating in distinct histopathological changes in intestinal mucosa. However, inflammation also has beneficial effects; enhancing anti-tumor immunity. A better understanding of how to manage the gastrointestinal side-effects of chemotherapy allowing for optimal dosing and induction of immunity will further improve outcomes in colorectal cancer.

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INTRODUCTION

Colorectal cancer (CRC) is the one of the most common forms of cancer worldwide and is the fourth most common cause for cancer related death[1]. While its incidence is continuing to rise in developing countries, developed countries such as the United States are observing a falling trend in CRC, likely secondary to screening[2]. Prognosis of CRC in the developed countries has also improved, with CRC specific mortality falling over the past 20 years[3]. The reasons behind this are multi-factorial and include earlier diagnosis and increased access to better oncological care. Advancements made in systemic chemotherapy for CRC and the development of novel biological agents have contributed to increased patient longevity; by preventing recurrence of disease in non-metastatic cases, and by down-staging or preventing disease progression in metastatic cases[4]. However, with this progress comes an increased incidence of drug toxicities and side-effects. To obtain the maximum benefit of new combination chemotherapy regimens, a better understanding of side effects and patient management is required.

Gastrointestinal toxicity is one of the most commonly encountered side effects experienced during systemic therapy for CRC[5]. Chemotherapy induced diarrhea (CID) has been reported to affect 50% of CRC patients receiving 5-fluorouracil (5-FU) as single agent and severe CID can develop in up to 40% of patients receiving combination chemotherapy[6]. CID is one of the major causes of dose limiting serious toxicity in chemotherapy regimens containing 5-FU. Chemotherapy agents exert toxic damage on the gastrointestinal (GI) epithelium which is at least partly mediated by activating the inflammatory cascade. Herein we review the mechanisms that are involved in GI toxicity during chemotherapy for CRC and their potential effect on cancer cells; by triggering immunogenic cell death, which in turn may have an impact on cancer relapse and survival.

CHEMOTHERAPY USE AND ASSOCIATED TOXICITY

5-FU is the main backbone agent used in systemic chemotherapy for CRC. When used as a single agent or as combination therapy with Oxaliplatin or Irinotecan in adjuvant chemotherapy there is evidence to show that a reduction in relapse by up to 33% can be achieved[7]. 5-FU based combination therapies have also shown efficacy in advanced CRC by improving progression free survival[8]. CID is a common side effect encountered during 5-FU based chemotherapy. It has been reported that 50%-80% of patients receiving 5-FU based adjuvant therapy for CRC develop CID of any grade; while grade 3 or 4 CID occurred in up to 30% of patients in clinical trials (Table 1). GI toxicity from 5-FU is influenced by several factors with different chemotherapy regimens generating varying incidences of CID. 5-FU given as a short infusion appeared to be better tolerated with less GI side effects

as compared to 5-FU given as a bolus[9]. Capecitabine, an oral fluoropyrimidine, has shown similar efficacy to intravenous 5-FU in clinical trials with better safety profile and less diarrhea though there was no difference in the number of reported cases of severe (Grade III or IV) CID[10].

Combination therapy has shown better efficacy and survival compared to single agent 5-FU therapy[11]. However such combinations enhance treatment related toxicities, including CID. This is especially evident in combination therapy of intravenous 5-FU and Irinotecan; as both 5-FU and Irinotecan have been shown to have direct toxic effects on the intestinal mucosa[12]. In trials where bolus Irinotecan were given with weekly bolus 5-FU and Leucovorin (IFL regimen) for CRC, an unacceptably high rate of GI toxicity and mortality were observed[13,14]. This toxicity is ameliorated somewhat with another regimen, whereby short term infusional 5-FU is administered together with Irinotecan every other week (FOLFIRI regimen); with reported grade 3 or 4 diarrhea incidence of around 14 percent[15].

Similarly Oxaliplatin combined with intravenous 5-FU has shown increased rates of GI toxicity. Short infusional 5-FU in combination with Oxalipatin (*e.g.*, FOLFOX regimen) was noted to be better tolerated than combination therapy with weekly bolus 5-FU (*e.g.*, FLOX regimen) in terms of CID; highlighting the importance of drug scheduling of 5-FU in the development of GI toxicity[16]. The mode of fluoropyrimidine administration also seems to have an impact on the toxicity profile in combination therapy. Capecitabine combined with Oxaliplatin (XELOX regimen) for treatment of metastatic CRC has shown similar efficacy but reduced incidence of severe diarrhea was noted compared to FOLFOX (14% *vs* 24%)[17]. In contrast, Capecitabine combined with Irinotecan (XELIRI) resulted in higher rates of severe CID compared to FOLIRI during treatment for metastatic CRC; indicating that toxicity profiles between different forms of fluoropyrimidine administration cannot be automatically assumed when combined with other drugs[15].

There is now increasing use of targeted therapies in the management of metastatic CRC[18]. While these agents seldom cause severe CID alone; they could further potentiate GI toxicity when given in combination with standard chemotherapy[19]. Therefore continued pharmaco-vigilance for GI toxicity is needed as the complexity of systemic chemotherapy of CRC rises with new treatment combinations.

MECHANISMS UNDERLYING CHEMOTHERAPY INDUCED MUCOSITIS

The manifestations of chemotherapy induced GI toxicity have been mainly attributed to the disruption of the mucosal barrier which lines the whole alimentary tract caused by the treatment; termed "mucositis". Previously thought as just an epithelial phenomenon when cells are exposed to chemotoxic agents or radiotherapy; it is in-

Table 1	gastrointestinal toxicity profile of fluoropyrimidine based chemotherapy used in colorectal cancer					
Regimen	Patient setting	CID overall	CID grade 3/4	Oral mucositis overall	Oral mucositis grade 3/4	Ref.
Flouropyrimidine monotherapy						
5-FU/LV bolus	Adjuvant	79%	21%-30%	28%	1%-8.1%	[93]
5-FU/LV infusion	Adjuvant	-	4%	-	2%	[9]
Capecitabine oral	Adjuvant	46%	11%	22%	2%	[10]
Combation therapy with Oxaliplatin/Irinotecan						
FLOX	Adjuvant	-	38%	-	-	[16]
FOLFOX	Adjuvant	56.3%	10.8%	41.6%	2.7%	[94]
XELOX	Adjuvant	60%	19%	21%	< 1%	[95]
FOLFOX	Advanced CRC	46%	5%	30%	1%	[96]
XELOX	Advanced CRC	50%	14%	28%	2%	[17]
FOLFIRI	Advanced CRC	63%	10%	35%	1%	[96]
XELIRI	Advanced CRC	-	47.5%	-	-	[15]

CID: Chemotherapy-induced diarrhea; CRC: Colorectal cancer; 5-FU/LV: Intravenous 5-fluorouracil and leucovorin; FLOX: Bolus 5-FU and oxaliplatin; FOLFOX: Infusional 5-FU and oxaliplatin; XELOX: Oral capecitabine and oxaliplatin; FOLFIRI: Infusional 5-FU and irinotecan; XELIRI: Oral capecitabine and irinotecan.

creasingly recognized that the pathobiology of mucositis is complex involving the mucosal immune system with an important role played by pro-inflammatory cytokine release. The clinical effects of mucositis vary according to anatomical site. Oral mucositis and mucositis affecting the upper GI tract causes painful ulcerations and dysphagia. Mucositis of the small and large bowel results in abdominal cramps, bloatedness and diarrhea[20].

The five stage model proposed by Sonis *et al*[21] is very useful in explaining the basic pathobiology of mucositis. In brief, the model comprises of 5 phases occurring sequentially; (1) initiation; (2) up-regulation and message generation; (3) signaling and amplification; (4) ulceration and inflammation; and (5) healing phase[22]. The initiation phase occurs when GI mucosa are exposed to cytotoxic agents resulting in cellular DNA damage and cell death mainly through the generation of oxidative stress and reactive oxygen species (ROS). ROS directly induce tissue injury and trigger a cascade of inflammatory pathways.

During the second phase, significant up-regulation of inflammatory mediators is observed and nuclear factor kappa-B (NF-κB) is thought to be pivotal in this process. Once activated by chemotherapy and ROS, NF-κB acts to induce gene expression and production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6, which in turn lead to tissue injury and apoptosis. NF-κB also causes up-regulation of gene expression of adhesion molecules and cyclooxygenase-2 (COX-2), with consequent angiogenesis.

During the third phase, a flood of pro-inflammatory mediators amplifies the whole inflammatory process *via* positive feedback loops, thus prolonging tissue injury. During this phase the process mainly occurs at the level of the submucosa and basal epithelium, therefore obvious damage to mucosal integrity is not observed clinically although the tissue biology is altered.

The fourth phase of mucositis is characterized by ulcerations and atrophic changes of the GI mucosa as a culmination event of tissue injury and stem cell death. GI epithelial integrity is destroyed and its function impaired. Patients are generally symptomatic during this phase. Bacterial colonization at the mucosa ulcers further induces inflammation by stimulating infiltration and activation of macrophages. Finally, the healing phase leads to renewal of epithelial proliferation and differentiation of the GI mucosa. This process occurs at approximately two weeks post chemotherapy and is also marked by angiogenesis implicating the importance of COX-2 in the process[22].

The histopathological changes associated with GI mucositis are well described. In humans, Keefe *et al*[23] studied patients undergoing chemotherapy with sequential duodenal biopsies pre and post treatment. They found that an increase in apoptosis was the first histological effect to be noted, with a seven-fold increase in apoptosis in intestinal crypts at day one post treatment. Reduction of intestinal villous area, crypt length and crypt proliferation then followed and the maximal effect was observed 3 d post treatment.

ANIMAL MODELS FOR STUDYING THE MECHANISM OF MUCOSITIS

Animal models have also been developed for the study of GI mucositis. Pertaining to chemotherapeutic agents used in CRC, Irinotecan and 5-FU based murine models are extensively researched and published.

IRINOTECAN HYDROCHLORIDE

Irinotecan hydrochloride (or CPT-11) exerts its antitumor effect by inhibiting DNA topoisomerase I[24]. The active metabolite, SN-38, induces irreversible DNA damage to tumor cells and its accumulation in the intestinal mucosa is thought to be responsible for enterotoxicity. SN-38 is glucuronidated in the liver to a non-toxic form (SN-38G) and excreted in the bile. Diarrhea is one of the major side effects of Irinotecan and patients encounter

two distinct types of diarrhea. Irinotecan induced early onset diarrhea occurs during or within several hours of administration and is cholinergically mediated and can therefore be prevented or ameliorated with atropine or anti-cholinergic agents. A second form of late onset diarrhea, which is not cholinergically mediated, ensues and mainly resulting from direct toxicity to GI mucosa in addition to other factors such as GI dysmotility[25].

Araki *et al*[24] reported that daily intraperitoneal injection of Irinotecan for 5 d causes severe diarrhea in athymic mice and haemorrhagic colitis by 7 d post treatment. Gibson *et al*[26] studied the histopathological changes associated with late onset irinotecan induced diarrhea on dark agouti (DA) rats by administrating daily intraperitoneal irinotecan for 2 d at varying doses and then examined the rats at fixed time points up to 96 h. They found irinotecan causes diarrhea by inducing apoptosis and hypoproliferation both in the small and large intestine. Additionally reduction in goblet cell numbers and mucin hypersecretion were noted in the colonic mucosa contributing to diarrhea. Similarly another study using a mouse model of irinotecan induced diarrhea found increased apoptosis together with structural changes in the GI mucosa and concluded that both malabsorption and mucin hypersecretion are likely to be at play[27].

Using the DA rat model, Bowen *et al*[28], 2007 looked at alterations in gene expression in Irinotecan induced diarrhea using microarray analysis and RT-PCR. They found multiple genes implicated in the mitogen-activated protein kinase (MAPK) signaling pathway were differentially regulated following Irinotecan treatment. These included IL-1 receptor, caspases, protein kinase C and dual-specificity phosphatase 6. Caspase-1 expression in jejunal tissue and was significantly increased 6 h after treatment and they conclude that GI damaged noted in chemotherapy utilizes the caspase cascade pathway, much like radiation induced damage and may be a potential target to prevent apoptosis following treatment. Logan *et al*[29] demonstrated with this model that in addition to histological changes noted in the GI mucosa, tissue staining for NF-κB, TNF-α, IL-1β and IL-6 were enhanced when compared to controls and peaked at between 2 and 12 h post administration. This provides further support for the role of pro-inflammatory cytokines in the pathogenesis of GI mucositis and the central role of NF-κB in the process. A mouse model of delayed diarrhea from Irinotecan also showed increase in pro-inflammatory cytokines and myeloperoxidase in intestinal tissue[30]. Additionally, they reported that thalidomide (known to have anti-TNF effects) and pentoxifylline (a methylxanthine derivative which reduces the expression of proinflammatory cytokines) decreased inflammatory infiltration and lesions induced by Irinotecan in treated mice. They conclude that cytokines regulate and amplify the immune response resulting in the injury and complications observed and that TNF-α, IL-1β and KC, (a mouse ortholog of human IL-8) are important mediators of this process. Using inducible nitric oxide synthase (iNOS) knock-out mice, the same group demonstrated that iNOS has an important role in the pathogenesis of mucositis. Furthermore, Infliximab, a monoclonal antibody against TNF-α, led to the reduction of intestinal expression of iNOS in irinotecan treated mice. Thus, suggesting that inflammatory cytokines and nitric oxide are among the main drivers of tissue damage in this model of mucositis[31].

5-FU

5-FU is an antimetabolite that acts on the enzyme thymidylate synthetase which in turn block DNA synthesis; thereby exerting its anti-tumor effects. Recognized common toxicities from 5-FU therapy include diarrhea and myelosuppression[32]. Several animal models exist for investigation of 5-FU associated toxicity and there is an increasing body of literature looking specifically at the mechanistic action of intestinal mucositis caused by 5-FU. Earlier studies conducted in mice models established the microscopic features of GI mucositis in 5-FU toxicity[33]. Pritchard *et al*[34] demonstrated in a murine model that 5-FU induced loss of crypt and villous cellularity through apoptosis and inhibition of cell cycle progression. Moreover these changes were significant reduced in p53 null mice; indicating that this process is p53 dependent.

Logan *et al*[12] examined GI mucositis in DA rats after a single administration of 5-FU (150 mg/kg intraperitoneally). They noticed shortening of crypt length, blunting and fusion of villi, enterocyte hyperplasia and increased apoptosis in the small intestine while decreased crypt length and increased apoptosis were noted in the colon. Interestingly immunochemistry on mucosal tissue of these rats showed elevation of TNF-α and IL-1β levels but no significant increased staining for NF-κB and IL-6. This indicates that apoptotic and inflammatory changes in 5-FU-induced mucositis may be secondary to pathways independent of NF-κB. In contrast, a study utilizing transcriptomic analysis was able to show that 1614 genes were upregulated in 5-FU-induced mucositis and that expression network revealed NF-κB as the central molecule in the process[35]. Furthermore bioluminescence imaging of transgenic mice showed increased NF-κB activity in the whole body 2 d post 5-FU administration which was most marked in the small intestine[36]. It has also been suggested the generation of reactive oxygen species (ROS) by NADPH oxidase 1 could also play a vital role at this stage[36]. Nevertheless, similar to Irinotecan, a pro-inflammatory process is initiated by 5-FU-induced intestinal damage and is likely that inflammatory cytokines mediate the subsequent apoptosis noted in intestinal crypts. Pro-inflammatory cytokines such as IL-1β are known to be capable of inducing apoptosis by altering the expression of apoptotic factors such as Bax and Bcl-2[37]. Work by Wu *et al*[38] showed that expression of IL-1 receptor antagonist (IL-1RA), a natural competitive antagonist of IL-1β, was increased in a mouse model of 5-FU-induced intestinal mucositis. Furthermore administration of exogenous IL-1RA resulted in significant reduction in

apoptosis and severity of diarrhea in this murine model; lending support for the role of IL-1β in the pathogenesis of mucositis[39].

A recent study also looked at intestinal mucositis induced by 5-FU in IL-4 knock-out mice. IL-4 is a critical mediator of intestinal inflammation and can function as either a pro- or anti-inflammatory molecule depending on the model of intestinal inflammation. In these mice they reported significantly reduced intestinal damage and inflammation induced by 5-FU after 72 h compared to wild type controls. Furthermore, pro-inflammatory cytokines were increased in wild type controls but not in mice lacking IL-4. The authors conclude that IL-4 has a role in 5-FU induced intestinal mucositis and that removing of IL-4 is effective in preventing pathological alterations secondary to such damage and may improve outcome; supporting the notion that strategy against IL-4 may be a novel logical therapeutic approach for this condition[40].

Keratinocyte growth factor (KGF) was shown to be effective in ameliorating 5-FU-induced intestinal mucositis and prolong crypt stem cell survival in a study by Farrell *et al*[41] but the exact mechanism by which KGF induces its protective effect is as yet not fully understood.

OXALIPLATIN

Oxaliplatin monotherapy seldom results in diarrhea but rather its main dose limiting toxicity results from drug associated neuropathy. As such, several animal models exist for oxaliplatin based toxicity but mainly looking at neurotoxicity, with little data on GI toxicity[42,43]. It is known that GI toxicity is potentiated in combination therapy of oxaliplatin with 5-FU in clinical studies but the exact mechanism behind this observed phenomenon is as yet not fully understood. Few studies have investigated GI mucositis resulting from combined 5-FU and oxaliplatin chemotherapy in the animal models and little data exists for the pathophysiology of mucositis with this combination[44]. Further research into the exact molecular pathways involved in mucositis induced by combination therapy is warranted.

TARGETED THERAPY

Monoclonal antibodies to EGFR such as cetuximab and panitumumab are known to cause diarrhea, though for cetuximab the severity is usually mild[45]. Bevacizumab, a monolconal antibody against VEGF seldom causes diarrhea but is associated with a risk of intestinal perforation, most likely secondary to tissue hypoxia due to inhibition of angiogenesis[46].

However, diarrhea is a well-recognized side effect of oral tyrosine kinase inhibitors. Small molecular targeted chemotherapeutic agents such as regorafenib have been shown to be efficacious in solid tumors and are being increasingly used in the treatment of metastatic colorectal cancer[47]. However it is likely that the mechanism behind their enterotoxicity is different from diarrhea generated by cytotoxic agents. In a rat model of diarrhea induced by lapatinib, an oral tyrosine kinase inhibitor used in the treatment of breast cancer, no significant histopathological changes was noted in the intestinal mucosa despite the development of diarrhea, suggesting an alternative pathway other than the inducement of GI mucositis. Further work to elucidate the exact pathogenesis of this GI specific side effect for this class of agent is warranted and is reportedly underway[48].

SURVIVAL BENEFIT

Chemotherapy is notable for significant toxicities that impact on patient quality of life during therapy and can lead to delay in treatment cycle, dose reduction or drug modification. However in some clinical studies it was noted that modifications to treatment secondary to side effects did not reduce the overall efficacy of the treatment regime[49]. Furthermore the occurrence of certain toxicities could serve as a predictive indicator for improved outcome post treatment. In the treatment of lung cancer with tyrosine kinase inhibitors the development of skin rash is associated with improved response rates[50]. Similarly, diarrhea consequent to sorafenib is a predictor of positive outcome in patients undergoing chemotherapy for advanced hepatocellular carcinoma[51]. With regards to treatment in the setting of CRC; an association between increased incidence of side effects and improved survival is observed. Twelves *et al*[52] demonstrated during post-hoc analysis of the X-ACT trial that the occurrence of hand-foot syndrome (HFS) was associated with better outcome in patients treated with capecitabine. Another study (AIO KRK-0104 trial) looked at the use of capecitabine in combination with other agents including oxaliplatin, irinotecan and cetuximab in the setting of metastatic CRC also found a correlation between skin toxicities triggered by capecitabine and progression-free and overall survival[53]. Hofheinz *et al*[54], 2012 performed a combined analysis of this trial and another rectal cancer trial using the same chemotherapy regimen and concluded that patients with HFS had improved survival compared to those with did not develop this skin toxicity. Interestingly GI toxicity and diarrhea were significantly more common in patients with HFS but not often coincident with haematological toxicities. The reason for this phenomenon is not yet fully understood but one may speculate that both the mucosal tissue and skin are more susceptible to chemotherapeutic agents that induce apoptosis compared with haematopoiesis. In contrast, the development of skin reaction during cetuximab therapy was shown to be associated with response and survival in metastatic CRC, although no increased GI toxicity was observed in a study by Cunningham *et al*[19] in 2004. This indicates that differential susceptibility of the mucosa to drug-induced toxicities and potential survival benefit may share a common underlying mechanism of action. There is as yet no study to suggest an association between CID and treatment response in chemotherapy for CRC but

this should be evaluated further in clinical studies.

CHEMOTHERAPY EFFECTS ON THE IMMUNE SYSTEM

As the chemotherapeutic agents used to treat cancer cells generate GI toxicity *via* the induction of apoptosis and subsequent inflammation; it is hypothesized that they may also have a beneficial effect on cancer survival by activating an anti-tumor immune response in cancer patients. This concept was supported by findings that cancer cell lines treated *ex-vivo* with certain cancer treatment modalities including chemotherapy can act as a cancer vaccine in animal studies[55,56]. It is now believed that a competent immune system plays a very important role in the efficacy of cancer therapy and that treatment will give the best chance of success when the tumor can be induced to undergo a process of programmed cell death that incites an adaptive immune response, the so called "immunogenic cell death" (ICD)[57]. This process, when activated, leads to the stimulation of T cells by antigen presenting cells such as dendritic cells (DC) through capture, processing and presentation of antigens to naive CD4^{+} and CD8^{+} T cells which in turn elicit an anti-tumor response[58].

While apoptosis is generally thought to be immunologically silent, ICD is characterized by the release or exposure of a range of substances called damage-associated molecular patterns (DAMPs), which can trigger an immune response. Of the DAMPs, it appears that the release of extracellular ATP, high mobility group protein B1 (HMGB1) and the exposure of calreticulin (CRT) on the outer membrane of the dying cell are vital for the initiation of ICD[59]. The emission of these DAMPs are triggered by anti cancer drugs and treatments with the ability to induce ICD; known as ICD inducers. These ICD inducers exert their influence in the release of DAMPs through the induction of endoplasmic reticulum (ER) stress in cancer cells and generation of reactive oxygen species (ROS). Both ER stress and ROS work to activate signaling pathways which help to traffic DAMPs to the extracellular space[60]. ICD inducers can be classified into two groups based on the selectivity for the ER in the generation of ER stress. Type 1 ICD inducers act on cytosolic proteins and targets not associated with ER to induce apoptotic cell death which in turn results in ER stress through secondary effects. Examples of type 1 ICD inducers include mitoxantrone, oxaliplatin, cyclophosphamide and γ-irradiation. In contrast, type 2 ICD inducers which include coxsackievirus B3 and hypericin-based photodynamic therapy (PDT) selectively target ER for the generation of ER stress by altering its homeostasis[59].

While the mode of action and the resultant ER stress could be qualitatively different between the ICD inducers, the components of DAMPs are shown to have an immunomodulatory function. Extracellular release of ATP is a strong "find me" signal for monocytes *via* P2Y2 receptors

and enhances their recruitment to apoptotic cancer cancers[61]. Exposure of CRT on cell surface of cancer cells undergoing ICD facilitates phagocytosis by DCs which present antigen and activate cytotoxic T-lymphocytes to give an anti-tumor response. Release of extracellular HMGB1 binds to various receptors such as TLR2, TLR4 and receptor for advanced glycosylation end products (RAGE) and in doing so stimulates an inflammatory reaction with the production of pro-inflammatory cytokines which has been found to be vital for the immunogenicity of ICD[61]. Indeed, the interaction between HMGB1 and the TLR-4 receptor on DCs is integral to this process, as a clinical study showed that a polymorphism of TLR-4 that affects the binding of HMGB1 is associated with early relapse of breast cancer[62]. This phenomenon was also observed in metastatic CRC, where Tesniere *et al*[63] showed that patients with normal TLR4 allele have an increased progression-free and overall survival compared with those bearing a loss-of-function TLR4 allele, in a trial involving the use of oxaliplatin-based chemotherapy regime. In addition, they found that this genetic polymorphism did not affect survival in patients with surgically resected CRC who did not undergo adjuvant chemotherapy; highlighting the major role of host immunity and inflammatory responses in determining outcome of chemotherapy in CRC.

CONCLUSION: SUPPORTIVE CARE FOR PATIENTS AND DEVELOPMENT OF NEW DRUGS

With chemotherapeutics in CRC have immunological benefits in addition to their cytotoxic effects, it is imperative that GI side effects are minimized to optimize dosing for treatment so that the best outcome can be achieved. Current management options for CID includes supportive care by symptomatic relief but there is increasing interest in regulating GI mucositis as a means to prevent and treat CID.

LOPERAMIDE

Loperamide is a non-analgesic opioid which helps with diarrhea by decreasing intestinal motility[25]. It is proven to be safe and commonly used in acute and chronic diarrhea in a variety of clinical settings[64]. It is also used as first line management of diarrhea in chemotherapy[65]. In regimens involving irinotecan, high dose loperamide was able to control symptoms to improve tolerability of the drug and to enhance effectiveness of therapy[66]. However its efficacy seems to be limited to mild to moderate diarrhea as a study showed that only 52% of patients who develop grade 3-4 CID responded to loperamide in a CRC cohort undergoing 5-FU-based chemotherapy[67]. Nevertheless its safety profile and affordability make it a worthwhile first line therapy to which other treatment options can be added.

OCTREOTIDE

Octreotide is a somatostatin analogue that has also shown to be effective in managing both secretory and malabsorptive diarrhea in several gastrointestinal disorders including short bowel syndrome and neuroendocrine tumors[68]. Its main mechanism of action is by binding to somatostatin receptors in the GI tract which affect a slow-down in transit time mainly in the small bowel. It also inhibits gut hormones reducing gastric, pancreatic and intestinal secretions, thereby helping to limit excess fluid that is needed to be resorbed by the colonic mucosa[69]. Several clinical studies have shown that the use of octreotide is effective in the treatment of CID[70-73]. There is also evidence that octreotide is more effective than loperamide in 5-FU based regimen[74]. Recent guidelines recommended the use of octreotide at a dose of ≥ 100 μg subcutaneously twice daily for the control of diarrhea in chemotherapy patients in whom loperamide fail to achieve an adequate response[75].

CELECOXIB

There has been an interest in the theoretical use of celecoxib in CID due to its anti-inflammatory properties, which were thought to ameliorate GI mucositis[76]. In addition, a supposedly anti-tumor effect with COX-2 inhibition makes it attractive as a potential adjunct in the treatment of solid malignancies. These anti-diarrheal and anti-tumor observations were demonstrated in rat models with irinotecan induced diarrhea[76]. However, a phas I study investigating the use of celecoxib in patients undergoing irinotecan based chemotherapy for advanced solid tumors did not show any benefit in CID[77]. Another study by Villalona-Calero *et al*[78], also found that the addition of celecoxib in combination with irinotecan did not improve tolerability of chemotherapy. Further work is needed to define the role of COX-2 inhibition in GI mucositis and its translation to clinical application in the treatment of CRC.

BUDESONIDE

Budesonide is a glucocorticoid with topical anti-inflammatory properties. It has been shown to be effective in the treatment of various inflammatory conditions, including inflammatory bowel diseases[79,80]. It has an extensive first pass metabolism effect in the liver and thus has limited systemic side-effect profile. Its efficacy in GI mucositis and CID was investigated in the clinical setting and an early short report noted improvement in the severity and duration of diarrhea in patients with irinotecan or 5-FU induced CID which was refractory to loperamide therapy[81]. A subsequent randomised placebo controlled trial also noted a reduction in the frequency of diarrhea when budesonide was used as a prophylactic measure but their study did not reach statistical significance. Based on their findings, it was concluded that further trials are warranted[82].

GLUCAGON-LIKE PEPTIDE-1 AND -2

Glucagon-like peptides (GLPs) are peptides which are synthesized and secreted by enteroendocrine L cells located in the GI tract. These molecules are involved in various homeostatic functions in our body, including the regulation of nutrient assimilation and satiety. When stimulated, L cells secrete GLP-1 and GLP-2 in equimolar quantities. Both peptides exert their effect by binding to their receptors, GLP-1 receptor (GLP-1R) and GLP-2 receptor (GLP-2R) respectively. GLP-1R is expressed widely in the body, including in pancreatic tissue, the GI tract, heart, kidney and nervous tissue. In contrast GLP-2R is expressed mainly in the GI tract and CNS. The differential distribution of their receptors partly explains the distinct physiological effects of GLP-1 and GLP-2; with GLP-1 exerting an influence in glucose homeostasis as an incretin hormone while GLP-2 has no significant incretin effects. Instead, GLP-2 has been noted to have potent intestinal trophic effect, promoting crypt cell proliferation and villous growth of the jejunum and ileum[83]. In addition, GLP-2 enhances intestinal barrier function and has a cytoprotective effect on intestinal mucosa[84]. Exogenous GLP-2 has been shown to be protective against various intestinal insults, including ischemia-reperfusion-induced and irradiation induced injury[85,86]. In animal models of inflammatory bowel disease, the administration of GLP-2 was shown to have significant anti-inflammatory effects, and ameliorated weight loss associated with ileal and colonic inflammation[87]. There is therefore an intense interest in the ability of GLP-2 to reduce inflammation in GI mucositis and CID. In a murine model of CID, Boushey *et al*[88] demonstrated that a GLP-2 analogue was able to enhance survival and reduce weight loss while having little effect in chemotherapy effectiveness on the tumor. Furthermore they observed that this effect was driven in part by anti-apoptotic effects on intestinal cells expressing GLP-2R. Yamazaki *et al*[89], 2004 showed that increasing GLP-2 levels by pharmacological means significantly attenuated intestinal damage measured by reduction of small intestinal wet weight in 5-FU treated mice. Other studies also noted similar changes and a reduction in inflammatory cells suggesting an immunomodulatory effect of GLP-2 in CID[90,91]. Intriguingly GLP-1 has also been found to have an intestinal trophic effect and treatment with GLP-1 ameliorated GI mucositis induced by 5-FU in mice[92]. Clinical studies are therefore warranted to translate such encouraging pre-clinical data to the treatment of CID *via* the GLP pathway.

CONCLUSION

GI toxicity from systemic chemotherapy in CRC remains a significant burden to patients limiting quality of life and impacting on optimal dosing for effective treatment. Recent advances highlight the importance of inflammation in the pathophysiology of GI mucositis and also bring to the attention its potential role for enhanced cancer survival post chemotherapy by triggering immunogenic cell

death. Strategies to nullify the undesirable yet common side effects of GI toxicity by addressing inflammatory changes triggered during mucositis are currently in development; with agents targeting the GLP pathway showing great promise in pre clinical studies. However, it is important to note that any such agents developed should not interfere with the efficacy of chemotherapy treatment and the complex interplay between side effects of inflammation and inflammation driven immunogenicity will need to be considered.

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