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Review

Chemotherapy-induced mucositis: The role of mucin secretion and regulation, and the enteric nervous system

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ABSTRACT

Alimentary mucositis is a severe, dose-limiting, toxic side effect of cytotoxic chemotherapy and radiotherapy. Patients with mucositis often have reductions or breaks imposed on cytotoxic therapy, which may lead to reduced survival. Furthermore, there is an increased risk of infection and hospitalization, compounding the cost of treatment. There are currently limited therapeutic options for mucositis, and no effective prevention available. Mucin expression and secretion have been shown to be associated with mucositis. Furthermore, mucins exhibit protective effects on the alimentary tract through reducing mechanical and chemical stress, preventing bacterial overgrowth and penetration, and digestion of the mucosa. Additionally, a number of studies have implicated some key neurotransmitters in both mucositis and mucin secretion, suggesting that the enteric nervous system may also play a key role in the development of mucositis.

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1. Introduction

Alimentary mucositis (AM) is a severe, dose-limiting, toxic side effect of cytotoxic chemotherapy and radiotherapy, occurring in 40% of standard dose chemotherapy cases and 100% of high dose chemotherapy cases (Keefe et al., 2000, 1997; Pico et al., 1998). AM affects the entire alimentary tract, and there is no effective prevention available currently (Keefe et al., 2007; Rubenstein et al., 2004; Saltz et al., 1996; Saltz, 2003; Sonis et al., 2004a; Wadler et al., 1998). AM may reduce the chance of survival for cancer patients as a consequence of dose reductions (Elting et al., 2003; Savarese et al., 1997). Furthermore, AM may result in the requirement of nutritional adjuncts, including fluid replacement, liquid diets and total parenteral nutrition, compounding the cost of treatment. The currently accepted hypothesis for the development of AM suggests there are five intertwined phases, namely; (1) initiation; (2) up-regulation and generation of messenger signals; (3) signal amplification; (4) ulceration and (5) healing (Sonis, 2004a,b; Sonis et al., 2004a). This has been supported in both animal and human studies (Gibson et al., 2003; Gibson and Keefe, 2006; Logan et al., 2008b, 2007; Stringer et al., 2007; Yeoh et al., 2005). Of particular interest is the ulceration phase, where pathological evidence of mucositis is observed, both macroscopically (ulcers) and

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microscopically (cell changes). Some of these changes observed in animal models and in humans include histological damage (Gibson et al., 2003; Keefe, 2004; Keefe et al., 2000; Logan et al., 2008a) and mucin secretion during mucositis (Gibson et al., 2003; Stringer et al., 2009a,b,c).

2. Mucin protection of the epithelium

Mucins are high molecular weight acidic glycoproteins secreted by goblet cells, which hydrate in the lumen and form a gel to create a protective barrier over the epithelium (Jass and Walsh, 2001; Robbe et al., 2004; Specian and Oliver, 1991). Mucins can be divided into two types: secreted gel forming mucins and transmembrane mucins. There are currently 17 known mucin genes (MUC1-17), which are regulated by specific cytokines (interleukin (IL) 1, IL-6, tumour necrosis factor (TNF), IL-4 and IL-9) (Deplancke and Gaskins, 2001), and bacterial products, such as lipopolysaccharides (LPS), and other bacterial factors are thought to be involved in the regulation of mucins (Deplancke and Gaskins, 2001; Smirnov et al., 2005).

Mucins mediate between the luminal contents and the mucosa by protecting the mucosa from bacterial overgrowth and penetration, and digestion, also providing attachment sites for commensal bacteria (Robbe et al., 2004; Specian and Oliver, 1991). Mucins are believed to be involved in natural resistance as they maintain normal intestinal flora, by providing attachment sites for intestinal flora (Robbe et al., 2004), and by protecting the mucosa from bacterial overgrowth and penetration (Specian and Oliver, 1991). However mucins may also provide an environment for colonization of pathogenic bacteria in gastrointestinal disease (Robbe et al., 2004). The mucosa is protected from digestion from the microflora by mucins acting as substrates to microflora produced enzymes, such as α-galactosidase β-N-acetyl-galactosaminidase, sialidase, β-glucuronidase, blood group degrading enzymes and proteases (Specian and Oliver, 1991). Mucins may also contain sulphate or sialic acid groups, protecting against translocation of bacteria by increasing mucous viscosity and acidity (Allen et al., 1982; Fontaine et al., 1996), increasing the potential to resist bacterial enzyme-induced degradation (Fontaine et al., 1996; Rhodes, 1989). Mucins also protect the epithelium from mechanical and chemical stress (Robbe et al., 2004; Smirnov et al., 2005).

3. Mucositis and mucins

Mucin expression and secretion have been shown to be associated with mucositis (de Koning et al., 2007; Saegusa et al., 2008; Stringer et al., 2009b,c; Verburg et al., 2000). Dark Agouti (DA) rat models of mucositis have been used to determine a significant association with mucin secretion and expression (Stringer et al., 2009b,c). Cavitated goblet cells (goblet cells which have released mucins through exocytosis) have been shown to significantly increase ($p < 0.05$) from 96 h to 144 h after irinotecan treatment. Muc4 expression has also been shown to increase significantly ($p < 0.05$) in the jejunum compared to controls (Stringer et al., 2009c), which may suggest accelerated transmembrane mucus production. In another study, 5-fluorouracil (5-FU)-induced mucositis was shown to decrease goblet cell numbers in the jejunum significantly ($p < 0.05$) and increase the percentage of cavitated goblet cells significantly ($p < 0.05$) from 24 h to 72 h after treatment compared with controls (Stringer et al., 2009b). Furthermore, a Wistar rat model of five once daily injections of 50 mg/kg of 5-FU caused a significant ($p < 0.05$) decrease in mucin levels compared to controls in the jejunum and colon (Saegusa et al., 2008), suggesting mucin expression was decreased. Mucin expression has also been shown to be significantly ($p < 0.05$) decreased in a Wag/Rijrat model of methotrexate (MTX) induced mucositis (2 injections intravenously (iv) of 10 mg/kg 24 h apart), with Muc2 expression from 72 h to 144 h after MTX treatment significantly decreased (Verburg et al., 2000). However, these studies did not differentiate between cavitated and non-cavitated cells, providing expression data only.

In a MTX-induced mucositis model in muc2 knockout mice (2 injections (iv) of 25 mg/kg 24 h apart) a significant ($p < 0.05$) decrease in weight in MTX treated mice compared to controls was observed in wild type mice at 24 h, 48 h, 72 h and 120 h after administration, and in muc2 knockout mice at 24 h and 72 h, with significance almost reached at 48 h (de Koning et al., 2007). However, between 120 h and 144 h, 75% of the muc2 knockout mice died with significant weight loss, whereas all wild type mice survived. Furthermore, muc2 knockout mice were significantly ($p < 0.05$) lighter than wild type mice prior to administration of MTX, which was not taken into consideration when comparing wild type weight to muc2 knockout weight following treatment (de Koning et al., 2007).

Investigation of jejunum morphology between 24 h and 72 h has shown crypt length to increase significantly ($p < 0.05$) at 48 h in wild type mice compared to controls, with crypt length in wild type mice significantly ($p < 0.05$) larger than muc2 knockout mice at 48 h and 72 h (de Koning et al., 2007). Villous length was significantly ($p < 0.05$) decreased compared to controls in wild type mice at 72 h and 96 h and, in muc2 knockout mice at 24 h and 48 h (de Koning et al., 2007). Muc2 knockout mice had no recognizable crypts, and death by intestinal failure was ruled out by observing the colon, suggesting the importance of muc2 in the pathology of mucositis (de Koning et al., 2007). However, MTX-induced intestinal damage was comparable between muc2 knockout mice and controls; which may be due to IL-10, an anti-inflammatory cytokine, which was also shown to be upregulated in muc2 knockout mice prior to MTX administration compared to controls (de Koning et al., 2007). This suggests IL-10 may help prevent MTX induced mucositis through an anti-inflammatory mechanism and implicate mucus secretion in the development of mucositis.

4. Regulation of mucin secretion

The mechanisms of regulation of mucin secretion are largely unknown. A number of neurotransmitters and chemicals may be involved in the regulation of mucin secretion, including: vasoactive intestinal polypeptide (VIP) and nitric oxide (NO) (neurotransmitters), prostaglandin E2 (PGE2) and protease-activated receptor 2 (PAR2).

4.1. Vasoactive intestinal polypeptide

Vasoactive intestinal polypeptide (VIP) stimulates secretory activity (Dickson and Finlayson, 2009) by activation of two G-protein-coupled receptors, VPAC1 and VPAC2 (Martin et al., 2005; White et al., 2010), in the presence of extracellular Ca^{2+} (Bou-Hanna et al., 1994). Therefore, VIP may also be involved in stimulating secretary activity of goblet cells. Furthermore, VPAC2 may be involved in mucositis as it is desensitized and internalized by phosphorylated G protein-coupled receptor 2 (GRK2), which is augmented by protein kinase A (PKA) (Murthy et al., 2008), a protein involved in the mitogen activated protein kinase (MAPK) pathway, which is up-regulated in chemotherapy induced mucositis (Bowen et al., 2007b). VIP induces vascular endothelial growth factor (VEGF) production in human HaCaT keratinocytes via the MAPK pathway (Yu et al., 2010). MAPK signalling has been shown to be up-regulated in chemotherapy induced mucositis, therefore it is likely that changes in VIP expression may also be