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ORIGINAL ARTICLE

Tight junction defects are seen in the buccal mucosa of patients receiving standard dose chemotherapy for cancer

Hannah R. Wardill[1] · **Richard M. Logan**[2] · **Joanne M. Bowen**[3] · **Ysabella Z. A. Van Sebille**[3] · **Rachel J. Gibson**[1]

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Abstract
Purpose Oral mucositis is one of the most common and debilitating side effects of chemotherapy treatment. Patients are often unable to eat and drink, which can lead to poor clinical outcomes and extensive resource utilisation. The primary aim of this study was to determine the molecular integrity of oral epithelial tight junctions in patients undergoing chemotherapy. The secondary aim was to correlate these changes with proinflammatory cytokines and matrix metalloproteinase profiles.
Methods Patients ($n = 23$) were recruited from the Royal Adelaide Hospital between 2000 and 2003. Reach patient underwent two oral buccal mucosa biopsies (4 mm): one prior to chemotherapy treatment and a second one after chemotherapy treatment. Oral buccal mucosa biopsies were also taken from seven healthy volunteers with no history of cancer, chemo- or radiotherapy treatment or inflammatory disorders. Routine haematoxylin and eosin staining was performed to determine epithelial thickness. Immunohistochemical staining was performed for claudin-1, zonular occludens-1, occludin, interleukin-1β, tumour necrosis factor, interleukin-6, matrix metalloproteinase-2 and metalloproteinase-9.
Results Patients receiving standard dose chemotherapy had significant epithelial atrophy. Elevations in all cytokines and matrix metalloproteinases were seen, with significant lamina propria staining for interleukin-6 and tumour necrosis factor. Matrix metalloproteinase-2 appeared most upregulated within the oral epithelium. These changes coincided with altered tight junction staining properties. Changes in the staining intensity and localisation were both noted, with clear cytoplasmic staining for zonular occludens-1 and claudin-1 in patients treated with chemotherapy.
Conclusions Chemotherapy causes defects in oral tight junctions, coupled with altered cytokine and matrix metalloproteinase profiles. Tight junction disruption in the epithelium may contribute to ulcer development or lead to poor tissue integrity, and the timing of these events may be a target for preventative treatment.

Keywords Mucositis · Chemotherapy · Oral toxicity · Tight junctions · Clinical · Oral mucosa

✉ Hannah R. Wardill
hannah.wardill@adelaide.edu.au

Richard M. Logan
richard.logan@adelaide.edu.au

Joanne M. Bowen
joanne.bowen@adelaide.edu.au

Ysabella Z. A. Van Sebille
ysabella.vansebille@adelaide.edu.au

Rachel J. Gibson
rachel.gibson@adelaide.edu.au

[1] Discipline of Anatomy and Pathology, School of Medicine, University of Adelaide, North Terrace, Adelaide, South Australia 5005, Australia

[2] School of Dentistry, University of Adelaide, Adelaide, South Australia, Australia

[3] Discipline of Physiology, School of Medicine, University of Adelaide, Adelaide, South Australia, Australia

Introduction

Chemotherapy treatment is associated with a host of debilitating side effects with varying effects on patient quality of life, resource utilisation and treatment efficacy. Over the past decade, there has been an appreciation gained for the impact of chemotherapy-induced alimentary mucositis on patient quality of life, leading to vast improvements in our understanding of its pathobiology [1, 2]. Mucositis is characterised by severe

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ulceration along the entire alimentary tract [3]; however, oral lesions are most easily accessed and therefore diagnosed. In fact, oral mucositis is frequently described as the most common dose-limiting factor for patients undergoing chemotherapy treatment, affecting 80–100 % of those receiving high-dose treatment [4, 5]. The development of oral mucositis in patients during cancer treatment places a significant clinical and economic burden on the provision of care. Additionally, oral mucositis can compromise treatment outcomes and, in itself, increases mortality through heightened infection risk. Despite its prevalence and clinical impact, there is limited data on the molecular mechanisms that underpin or initiate this toxicity.

It is currently accepted that the pathobiology of alimentary toxicity, in which oral mucositis is included, can be described using a continuous and overlapping 5-phase model proposed by Sonis in 2004 [6, 7]. This model was the first to recognise that alimentary toxicity is not purely an epithelial phenomenon, highlighting the dynamic interactions that occur between the epithelium, extra cellular matrix (ECM), submucosa and the chemotherapeutic agent itself. Consequently, the pathobiology is defined as the collective consequences of direct cytotoxicity, induced by the chemotherapeutic agent, as well as inflammatory-driven indirect cytotoxicity primarily controlled through nuclear factor kappa B (NFκB). Although this model of alimentary mucositis remains universally accepted, recent advances in our understanding have identified complimentary molecular mediators of toxicity. One such example is the emerging role of tight junctions [8] in regulating barrier dysfunction commonly observed following cytotoxic treatment.

Tight junctions are highly dynamic signalling complexes vital to epithelial homeostasis. Located at the apico-lateral boundary of adjacent epithelial cells, tight junctions are integral in maintaining epithelial adhesion as well as regulating paracellular permeability [9]. Tight junctions are primarily formed of four protein groups; claudins, zonular occludens (ZO), junctional adhesion molecules (JAMs) and occludin. Importantly, the molecular interactions of these proteins cause tight junctions to be highly malleable and plastic structure that assemble, grow, recognise and disassemble in response to various physiological and pathological cues. Based on their highly plastic nature, particularly in response to inflammatory mediators, tight junctions have gained significant attention in a number of inflammatory-based gastrointestinal pathologies, including mucositis [10, 11]. Tight junctions were first identified to be involved in the pathobiology of gastrointestinal (GI) mucositis in 1997, with Keefe and colleagues [12] showing increased and uncontrolled intestinal permeability in patients receiving high-dose chemotherapy. In 2000, ultrastructural changes in small intestinal tight junctions were identified in patients receiving various chemotherapeutic treatment regimens [5]. Since the early 2000s, several studies have identified molecular defects in intestinal tight junctions following chemotherapy treatment, with downregulation, redistributing and phosphorylation of occludin, ZO-1 and claudin-1 consistently reported [13–17]. Tight junction disruption is therefore emerging as a key player in the pathobiology of mucositis.

Modification of tight junction proteins, particularly post-translationally, is a well-documented phenomenon and forms the basis of many inflammatory pathologies [18–20]. In the setting of both oral and GI mucositis, the interaction between proinflammatory cytokines, matrix metalloproteinases (MMP) and tight junctions is compelling given the strong inflammatory component of mucositis [21] and documented changes in MMP profiles [22]. The ability of proinflammatory cytokines and MMPs to degrade tight junctions is well established [23, 24], highlighting a potential interaction between mediators of mucositis and tight junction disruption. Importantly, these mediators are not only found at elevated levels in the gut but also the oral cavity [3] and circulating serum [21] therefore suggesting that tight junction disruption may also play a role in the pathobiology of oral mucositis. This study therefore aims to determine the phenotype of oral epithelial tight junctions in patients receiving chemotherapy and correlate with established changes in proinflammatory cytokines (IL-1β IL-6, TNF) and MMP profiles (MMP-2, MMP-9). Results from this study will determine if tight junction disruption is a common mechanism of oral and GI mucositis and may shed light on the underlying mechanisms responsible for barrier dysfunction.

Materials and methods

Patients

Tissue samples were sourced from a previously conducted study [25] published by Gibson et al. 2006. This previous study was approved by Royal Adelaide Hospital Human Ethics Committee. Briefly, patients were recruited from the Department of Medical Oncology at the Royal Adelaide Hospital between 2000 and 2003 ($n = 23$). The study included 7 male and 16 female patients with a median age of 52.4 years (32–86 years) [25]. Patients were excluded if they were undergoing concurrent radiotherapy to the head and neck or if they had pre-existing mucosal damage. Tumour type was heterogeneous amongst patients and included breast, non-Hodgkin's lymphoma, Hodgkin's lymphoma, colorectal, lung and neuroendocrine pancreatic. Standard dose chemotherapy was used in all patients, administered over 1–4 h [25]. Treatments included ABVD, AC, CMF, DOX, Docetaxel, CHOP, 5-FU/Folinic Acid, CAV and Streptozocin. For tabular breakdown of patient demographics and treatment regimens, please refer to Gibson et al. 2006.

Patients had a single oral buccal mucosa biopsy prior to the commencement of their first chemotherapy cycle and a second after cessation of their treatment (mean 4.8 days; range 3–11 days). Seven healthy volunteers (3 M:4F), with no history of cancer, chemotherapy treatment and pre-existing mucosal

damage, were also recruited for the study. All biopsies were performed by a single operator. Pre-chemotherapy biopsies were taken on one side of the mouth, and post-chemotherapy biopsies were taken on the opposite side. The surrounding buccal mucosa was injected with local anaesthetic, and a small (4 mm) punch biopsy was taken. A single stitch was placed at the site of the biopsy if necessary. The number of previous chemotherapy cycles undergone by each patient was recorded at recruitment to determine if these contributed to histological or molecular changes in the oral cavity.

Clinical assessment of oral mucositis

Case note reviews were used to identify the presence/absence of mucositis in this patient cohort at the time of sample collection. Institutional reporting guidelines did not require mandatory reporting of oral mucositis symptoms in patient case notes, and therefore, oral toxicities were not as comprehensively reported in this archival patient group as would be required today. Gibson et al. (2006) reported that 50 % of patients had mucositis symptoms of WHO grades 1–2 (relatively mild) ranging from mouth ulcers, loss of taste, mouth dryness, 'thick' feeling over the tongue and cheek area and fissured tongues [25]. For full tabular breakdown of mucositis severity and symptoms, please refer to Gibson et al. (2006) [25].

Histological analysis

Oral buccal mucosa biopsies were cut at 5 μm using the Leica Microtome and mounted onto glass microscope slides. Routine haematoxylin and eosin staining was conducted on all buccal mucosa biopsy samples. Briefly, sections were dewaxed and rehydrated through graded ethanols. Sections were placed in Harris Haematoxylin for 2 min before being placed in 0.5 % ammonia for 1 min. Sections were washed and placed in eosin for 2 min before being dehydrated, cleared and coverslipped. Slides were scanned using a NanoZoomer (Hamamatsu Photonics, Japan) and analysed using NanoZoomer Digital Pathology software (Histalim, Montpelier, France). Epithelial thickness was measured ten times across the width of the tissue section and an average determined [26]. All analyses were conducted in a blinded fashion.

Immunohistochemistry

Immunohistochemistry (IHC) was carried out on 4 μm sections of oral buccal mucosal cut on a rotary microtome and mounted onto FLEX IHC microscope slides (Flex Plus Detection System, Dako, Denmark; #K8020). Immunohistochemical analysis was performed for three tight junction proteins (claudin-1, ZO-1 and occludin), proinflammatory cytokines (IL-1β, IL-6, TNF) as well as MMP-2 and MMP-9 (Table 1). Immunohistochemical analysis was performed using Dako reagents on an automated machine (AutostainerPlus, Dako, Denmark) following standard protocols supplied by the manufacturer. Briefly, sections were deparaffinised in histolene and rehydrated through graded ethanols before undergoing heat mediated antigen retrieval using an EDTA/Tris buffer (0.37 g/L EDTA, 1.21 g/L Tris; pH 9.0). Retrieval buffer was preheated to 65 °C using the Dako PT LINK (pre-treatment module). Slides were immersed in the buffer and the temperature raised to 97 °C for 20 min. After returning to

Table 1 Antibody specification and application

Antibody	Distributor Catalogue #	Dilution	Polymer type Incubation period
Occludin	Invitrogen	5 μg/ml	EnVision™ FLEX+ Rabbit LINKER
Mouse monoclonal	33–1500		60 min
Claudin-1	Abcam	2 μg/ml	EnVision™ FLEX+ Rabbit LINKER
Rabbit polyclonal	ab15908		60 min
ZO-1	Invitrogen	2.5 μg/ml	EnVision™ FLEX+ Rabbit LINKER
Rabbit polyclonal	61–7300		60 min
TNF	Abcam	10 μg/ml	EnVision™ FLEX+ Rabbit LINKER
Rabbit polyclonal	ab6671		30 min
IL-1β	Abcam	2 μg/ml	EnVision™ FLEX+ Rabbit LINKER
Rabbit polyclonal	ab9787		30 min
IL-6	Abcam	1.67 μg/ml	EnVision™ FLEX+ Rabbit LINKER
Rabbit polyclonal	ab6672		30 min
MMP-2	Abcam	1.25 μg/ml	EnVision™ FLEX+ Rabbit LINKER
Rabbit polyclonal	ab58803		30 min
MMP-9	Abcam	1.25 μg/ml	EnVision™ FLEX+ Mouse LINKER
Mouse monoclonal	ab37150		30 min

65 °C, slides were removed and placed in the Dako AutostainerPlus and stained following manufacturer's guidelines. Briefly, endogenous peroxidase was blocked using the FLEX peroxidase block followed by a serum-free protein block (Dako, Denmark; #X0909). Primary antibodies were suspended in the EnVision™ FLEX Antibody Diluent (Dako, Denmark; #K8006) and applied for 60. Negative controls had the primary antibody omitted. The EnVision™ FLEX+ Rabbit/Mouse LINKER (Dako, Denmark; #K8019) was then applied for 30–60 min before DAB was used to visualise the target protein. Slides were removed from the automated stainer, counterstained in Harris Haematoxylin, dehydrated and coverslipped. Slides were scanned using the NanoZoomer (Hamamatsu Photonics, Japan) and assessed with NanoZoomer Digital Pathology software (Histalim, Montpellier, France). Healthy control samples were used as an internal positive control for tight junction proteins. Human tonsil was used as a positive control for IL-1β, IL-6, TNF, MMP-2 and MMP-9.

Slides were scanned using a NanoZoomer (Hamamatsu, Japan) and analysed using NanoZoomer Digital Pathology software (Histalim, Montpellier, France). Tight junction staining was analysed in the superficial/intermediate, prickle cell and basal epithelium as well as the endothelium of the lamina propria (Fig. 1), whilst IL-1β, IL-6, TNF, MMP-2 and MMP-9 staining was analysed in the whole oral epithelium and lamina propria. Staining intensity was analysed using a validated semi-quantitative grading system [26] from 0 to 3; where 0 = no staining, 1 = mild staining, 2 = moderate staining and 3 = intense staining (Fig. 2) and was conducted in a blinded fashion [26]. In addition, the characteristics of tight junction staining, including membrane specificity and location, were assessed qualitatively.

Statistical analysis

Epithelial thickness and immunohistochemical staining were compared between healthy control samples, pre-chemotherapy samples and post-chemotherapy samples using GraphPad Prism 7.0. Data was assessed for normality using the D'Agostino-Pearson omnibus test. When normality was confirmed, a two-way analysis of variance (ANOVA) was performed with a Tukey's post hoc. If normality was not achieved, a Kruskal-Wallis with a Dunn's multiple comparison was performed. To determine the relationship between previous chemotherapy cycles and epithelial thickness, a linear regression model was applied and the coefficient of determination (r^2) was determined. A p value < 0.05 was considered significant.

Results

Chemotherapy causes significant epithelial atrophy

Epithelial atrophy was observed both before ($p = 0.0008$) and following chemotherapy cycles ($p < 0.0001$; Fig. 3a, c). Given that patients were not naïve to chemotherapy treatment, it is likely that the atrophy observed prior to treatment was due to the previous cycles patients underwent. This was confirmed by a strong correlation between epithelial thickness and the

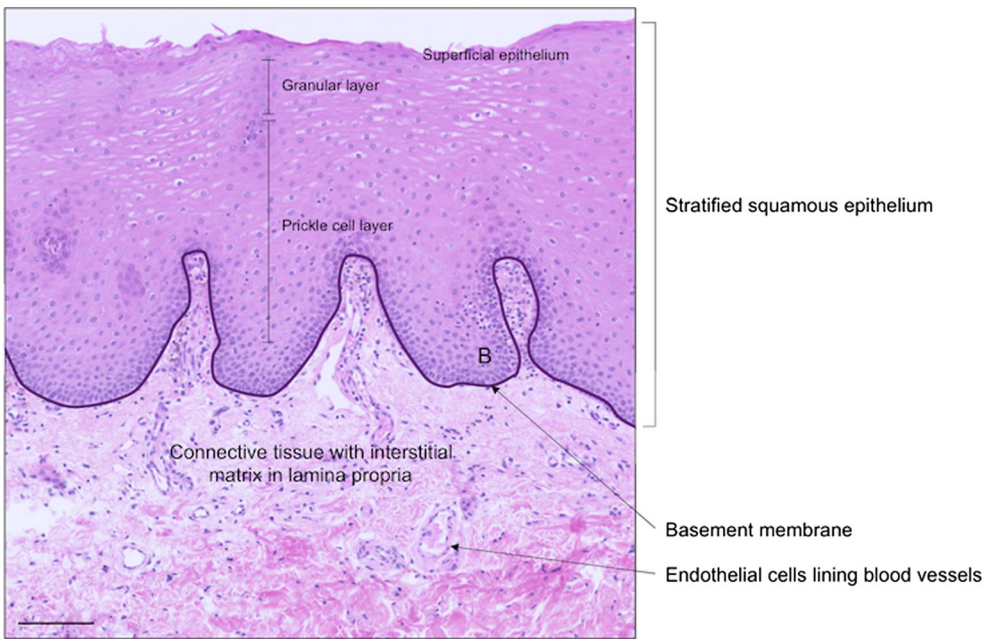

Fig. 1 Histology of the human oral mucosa. A photomicrograph of the oral cavity stained with haematoxylin and eosin (original magnification 40×; *scale bar* shows 100 μm). The stratified squamous epithelium of the human oral cavity is non-keratinised, displaying four distinct layers: basal cells (*B*), prickle cell layer, the intermediated layer and the superficial epithelium. Mitotically active basal cells reside on the basement membrane, separating the epithelium from the underlying lamina propria. The lamina propria consists of loose connective tissue with rich vasculature and immune capabilities

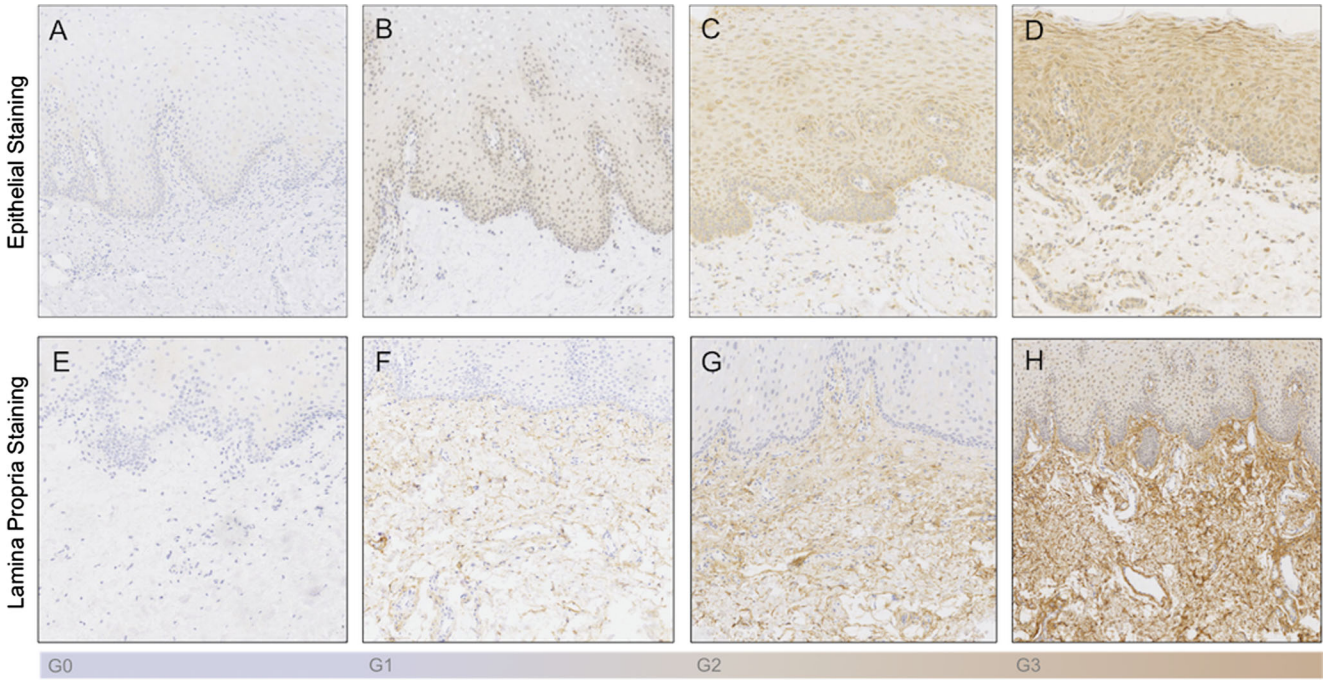

Fig. 2 Semi-quantitative grading system representative images. Representative photomicrographs of the oral epithelium showing immunohistochemical staining of varying intensities. **a**–**d** indicate epithelial staining intensities, whilst lamina propria staining is shown in images **e**–**h**. Staining is graded on a scale of 0–3, where 0 = no staining, *1* = mild staining, *2* = moderate staining and *3* = intense staining

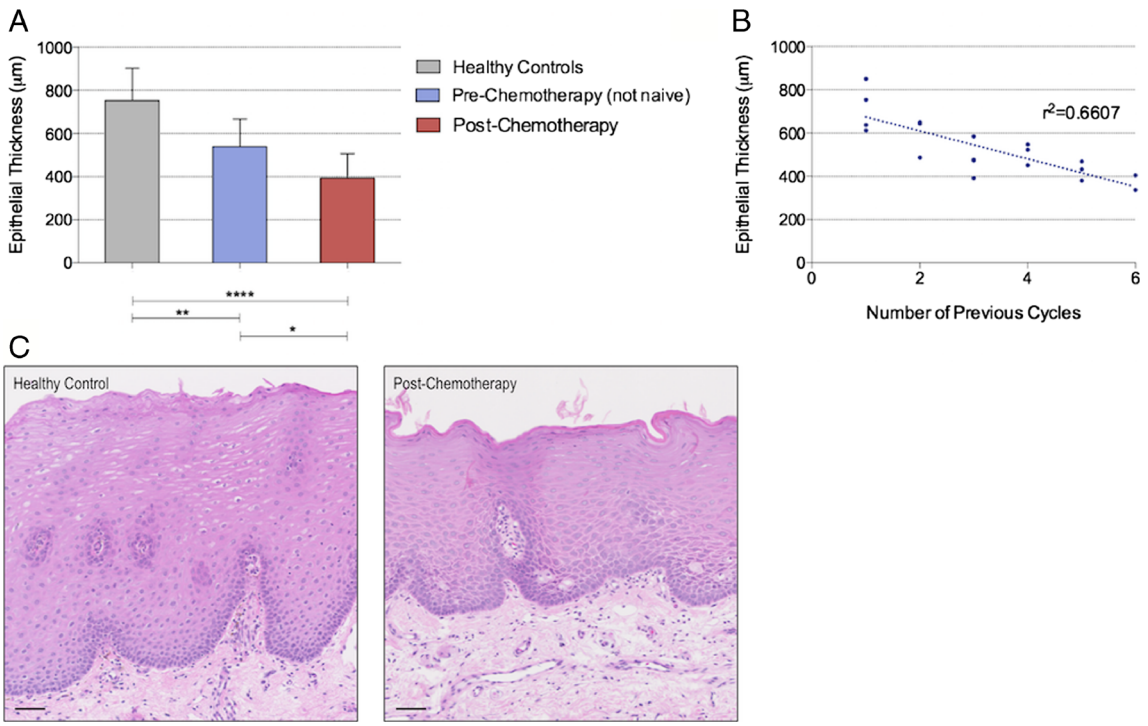

Fig. 3 **a** Epithelial atrophy was observed in cancer patients prior to chemotherapy (**$p = 0.0008$) and following chemotherapy (****$p < 0.0001$). Epithelial atrophy was significantly more severe in patients following chemotherapy compared with those prior to the onset of treatment (*$p = 0.0042$). **b** Correlation between epithelial thickness and previous cycles of chemotherapy. Data presented as mean ± SEM (**b**) or individual points (**b**) with a linear regression model. **c** Representative histological images showing epithelial thickness for healthy controls and patients following chemotherapy treatment. Original magnification 40×; *scale bars* show 50 µm.

number of previous chemotherapy cycles patients had undergone ($r^2 = 0.66$; Fig. 3b).

Chemotherapy increases proinflammatory cytokines and alters MMP profiles

Increases were seen in all proinflammatory cytokines and MMPs subtypes following chemotherapy (Fig. 4). IL-1β and IL-6 showed increased expression in the epithelium of patients treated with chemotherapy ($p = 0.0017$, $p = 0.0167$, respectively). Although no significant change was seen in the epithelial expression of TNF across all groups ($p > 0.05$), there was a significant increase in the lamina propria following chemotherapy treatment ($p < 0.0001$). This was consistent with the changes seen in IL-6, with significant increases in patients treated with chemotherapy ($p < 0.0001$). Both IL-6 and TNF appeared most prominent in the fibrous material and amorphous ground substance of the lamina propria (Fig. 4b). MMP-9 staining remained showed mild increases in staining expression in both the epithelium ($p = 0.0039$) and lamina propria ($p = 0.0409$) of patients treated with chemotherapy. MMP-2 staining was most significant in the epithelium of patients treated with chemotherapy ($p = 0.001$), with clear cytoplasmic staining in the prickle layer indicating active secretion. The vasculature and fibroblasts in the lamina propria also showed positive MMP-2 staining in patients treated with chemotherapy.

Residual inflammatory signalling was evident in the oral cavity of patients exposed to previous chemotherapy treatment, with pre-chemotherapy biopsies displaying increased TNF in the lamina propria ($p < 0.0001$).

Tight junction defects are seen following chemotherapy

Claudin-1 and ZO-1 protein expression decreased most notably in the basal (claudin-1: $p = 0.0130$, ZO-1: $p < 0.0001$) and prickle cell layers (claudin-1: $p = 0.0078$, ZO-1: $p < 0.0001$). Despite only modest changes in the overall staining intensity of tight junction proteins, clear changes in their localisation were evident (Fig. 5). In healthy controls, ZO-1 and claudin-1 displayed strong specificity for the membrane, with epithelial staining showing the typical 'cobblestone' appearance. In patients treated with chemotherapy, claudin-1 expression appears disrupted, particularly in the basal epithelium, and less specific for the membrane. Membrane specificity is not evident until more superficial epithelial layers. This redistribution is also clear in ZO-1 staining characteristics, with clear cytoplasmic staining evident.

Discussion

Recent clinical practice guidelines [8] and preclinical research outcomes [10] have highlighted the growing evidence

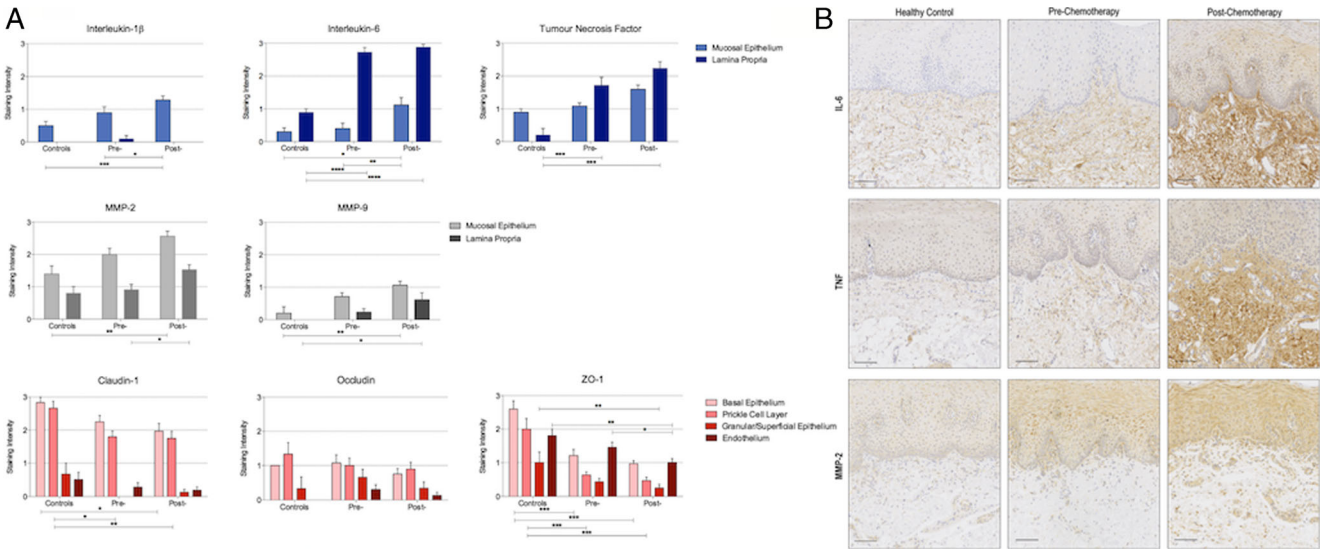

Fig. 4 **a** Immunohistochemical analysis of proinflammatory cytokines, MMPs and tight junction protein expression. All staining was analysed using a validated, semi-quantitative grading system (*0–3*). Data expressed as mean ± SEM. *$p < 0.05$; **$p < 0.002$; ***$p = 0.0001$; ****$p < 0.0001$. **b** Representative staining for IL-6, TNF and MMP-2 in the oral mucosa of healthy controls and patient samples prior to and following chemotherapy. Staining intensity for IL-6 and TNF was most apparent in the lamina propria, staining fibrous material and amorphous ground substance. Low-grade epithelial staining was evident in patients treated with chemotherapy. MMP-2 staining was most significant in the epithelium, with clear cytoplasmic staining in the prickle layer indicating active secretion. The vasculature as fibroblasts in the lamina propria showed positive MMP-2 staining in patients treated with chemotherapy. Original magnification 40×; *scale bars* on images **a**–**c** show 100 μm.

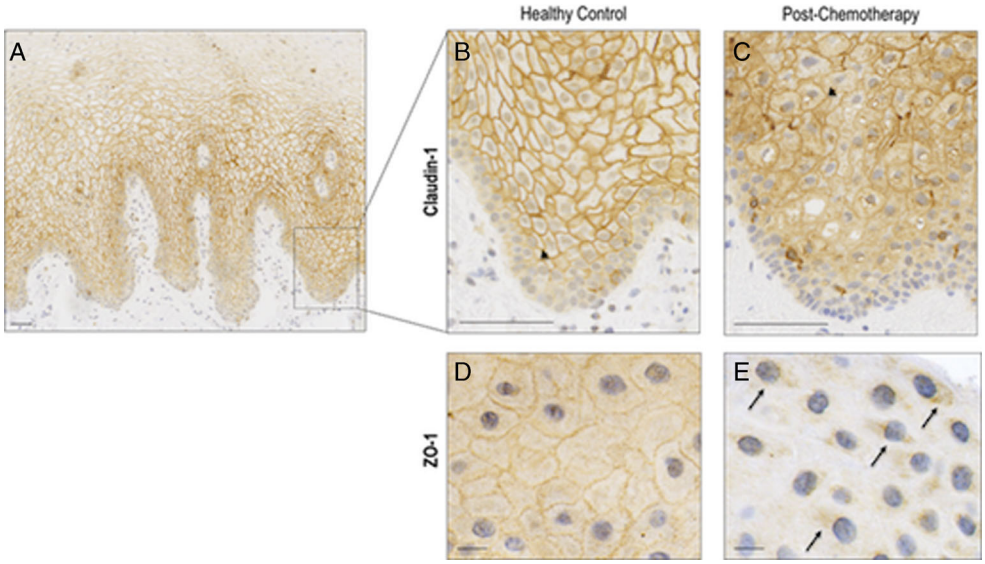

Fig. 5 Representative staining for claudin-1 (**a**–**c**) and ZO-1 (**d**–**e**) in the oral epithelium of healthy controls and patients treated with chemotherapy. The membrane specificity of claudin-1 staining is evident in the deep layers of the basal epithelium in health control patients (**a**, **b**: *arrow head*). In treated patients, staining is disrupted and less specific for the membrane, showing redistribution to the cytoplasm. Membrane specificity is not evident until more superficial epithelial layers (**c**: *arrow head*). ZO-1 staining displays typical cobblestone appearance in health controls (**d**). Following chemotherapy, internalisation of ZO-1 is evident showing clear cytoplasmic staining (**e**: *arrows*). Original magnification 40×; *scale bars* on images **a**–**c** show 100 μm; *scale bars* on images **d**–**e** show 10 μm

indicating the impact of tight junction disruption in the development of chemotherapy-induced mucositis. In light of this new research avenue, the current study utilised archival tissue samples obtained from patients undergoing standard chemotherapy, with the aim of determining oral epithelial tight junction integrity and correlating with established changes in pro-inflammatory cytokine and MMP profiles.

An unexpected finding from the current study was significant epithelial atrophy seen in the buccal mucosa biopsies taken prior to chemotherapy treatment. Importantly, all patients recruited for the original study had received previous cycles of cytotoxic treatment indicating that treatment causes persistent, long-term changes in the oral cavity. Epithelial thickness strongly correlated with the number of previous treatments patients underwent. These results support the idea that affected tissue exhibits long-term ultrastructural changes. These changes in epithelial thickness were also accompanied by residual inflammation and extra cellular matrix signalling, with elevated staining intensity compared to healthy controls. Unfortunately, we were unable to access information regarding the timing of previous cytotoxic treatment and correlations could not be drawn.

This study is the first to identify chemotherapy-induced oral epithelial tight junction disruption in patients receiving chemotherapy. In fact, it is one of only a few clinical studies that have documented changes in tight junctions from clinical patient samples. Keefe and colleagues (2000) showed altered tight junction integrity in the duodenum of patients undergoing chemotherapy [5]. These changes, detected by transmission electron microscopy, were the first to suggest that tight junction disruption may contribute to ulceration, loss of tissue integrity and diarrhoea development in patients undergoing chemotherapy. Consequently, chemotherapy-induced tight junction disruption may indeed be a critical aspect of oral ulceration—a major clinical aspect of mucositis. More importantly, however, tight junctions provide an important paracellular barrier to potential pathogens and thus disruption may promote bacterial translocation and increase the risk of local, or systemic, infection in already immunocompromised patients. This is a well-documented risk associated with tight junction disruption in the gastrointestinal tract, with chemotherapy-treated rats showing increased bacterial translocation to the mesenteric lymph nodes and spleen [27] coupled with severe tight junction impairment. Implications for oral epithelial tight junction disruption may therefore not only promote mucosal breaches but have detrimental effects on patients' clinical health outcomes.

Tight junctions are highly plastic complexes, with the ability to change in response to a wide variety of physiological and pathological cues. Although reduced expression of key tight junction proteins is most widely documented, cytoplasmic redistribution of these proteins has also been shown to drastically affect their function. For example, Nassour et al. (2014) showed that application of STb, a low molecular weight heat-resistance toxin produced by enterotoxigenic Escherichia coli, caused significant translocation of claudin-1 to the cytoplasm of T84 cells [28]. This was accompanied by increased permeability of T84 monolayers and poor transepithelial resistance. In similar studies, redistribution of claudin-1 from the membrane to a more soluble form was associated with marked alterations in F-actin

stress fibres [29]. F-actin filament dissolution and condensation were also accompanied by redistribution and fragmentation of ZO-1 and occludin. This relationship has also been demonstrated in response to IL-1β treatment, with altered subcellular localisation of claudin-1 and ZO-1 shown in both thyroid cells [19] and cultured human corneal epithelial (HCE) cells [30]. In the setting of chemotherapy-induced tight junction disruption, it has also been shown that downregulation and redistribution of ZO-1 drastically affects the function of intestinal tight junctions. For example, Hamada and colleagues showed that methotrexate-induced diarrhoea resulted in significantly increased permeability to fluorescein isothiocyanate-dextran coupled with internalisation of ZO-1 in colonic epithelial cells [14, 15]. Although shown in a variety of cell types and in response to varying cues, these studies emphasise the significance of cytoplasmic redistribution of tight junction proteins and may offer mechanistic avenues to explore.

The current study has shown clear increases in several proinflammatory cytokines and MMP subtypes. This change comes as no surprise given the vast amount of research showing a strong inflammatory component to alimentary toxicity [3, 21, 26, 31]. However, few studies have assessed cytokine and MMP expression in the oral epithelium of patients receiving chemotherapy, with most research coming from preclinical animal models. For example, our laboratory has previously shown elevations in IL-1β, TNF and IL-6 in the oral mucosa of tumour-bearing rats receiving chemotherapy [3], paralleling the clinical changes observed in the current study. These results compliment earlier clinical findings showing increased NFκB and cyclooxygenase-2 expression in the oral cavity of patients following cytotoxic chemotherapy [32]. Recent research has also shown elevated MMP-9 expression in the ventral surface of the tongue of tumour-bearing rats treated with chemotherapy [26]. This parallels earlier research showing a time-dependent increase in both MMP-2 and MMP-9 in the jejunum following irinotecan administration [31]. Although more substantial changes were seen preclinically, particularly for MMP-9, results again reflected the changes observed clinically. Importantly, the changes in proinflammatory cytokine and MMP profiles observed in our present study were clearly coupled with changes in tight junction integrity.

The idea that both proinflammatory cytokines and MMPs regulate tight junctions is not a new phenomenon, with strong supportive in vitro and in vivo evidence. The earliest evidence for proinflammatory cytokine-dependent tight junction disruption was seen in the setting of inflammatory bowel disorders, with clear changes in claudin-1, ZO-1 and occludin coinciding with peak relapse and remission phases [33]. Recent in vitro research has solidified the modulatory roles of proinflammatory cytokines on tight junction integrity, showing that IL-1β and TNF are able to disrupt tight junction integrity [34–36]. Comparable effects have also been documented following exposure to MMPs [37], although much of the research to date has only focused on their effects on endothelial tight junctions. Importantly, however, interactions between proinflammatory cytokine signalling, MMP activity and epithelial tight junction integrity have been documented. In fact, treatment with TNF has been reported to activate both MMP-2 and MMP-9 resulting in tight junction disruption and epithelial hyper-permeability [38].

More recently, MMP-tight junction interactions have been demonstrated using human airway epithelial models [39] and human embryonic kidney cell lines [39]. In both cases, MMP-9 activation caused altered expression and localisation of occludin, claudin-1 and ZO-1, tight junction strand breaks and epithelial apoptosis, thus highlighting a clear role of MMPs in the regulation of tight junctions and barrier function. Given the wealth of supportive literature showing cytokine- and MMP-mediated tight junction disruption, the idea that these interactions underpin chemotherapy-induced oral toxicity is compelling. Given that these interactions have also been reported to contribute to chemotherapy-induced gut toxicity and associated diarrhoea, this study therefore indicates that tight junction defects occur throughout the entirety of the alimentary tract, regardless of anatomic site. This provides further evidence for a common pathway for mucositis development, which is modified as a consequence of local structural differences in the mucosae. These differences are overwhelming when comparing the oral mucosa to the gastrointestinal tract; however, these structural differences may have implications for the resilience that different mucosae may exhibit in response to the effects of chemotherapeutic drugs.

Conclusions

Chemotherapy causes defects in key tight junction proteins of the oral cavity, characterised by decreased expression and cytoplasmic redistribution. This is the first study to identify changes in oral epithelial tight junctions of patients undergoing chemotherapy. This provides further evidence for a common pathway for alimentary mucositis, with regional differences the result of structural variations in the alimentary mucosae. Changes in oral epithelial tight junctions were coupled with altered cytokine and MMP profiles, and the timing of these events may be a target for preventative treatment. It is therefore critical that these results be assessed in a more controlled manner to assess if tight junction disruption is in fact the cause of oral mucositis or purely an effect. It must also be acknowledged that not all patients undergoing chemotherapy treatment developed clinical mucositis. Despite this, subclinical evidence of mucositis was apparent in the form of apoptosis [25], inflammation, atrophy and perhaps tight junction defects. For a stronger understanding of the temporal relationship between mediators of inflammation, tight junctions and mucositis development to be establish, these investigations should now be extended into controlled animal studies as well

as into larger patient cohorts with heterogeneous diagnoses and more detailed reporting of mucositis onset, severity and duration.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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