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ORIGINAL ARTICLE

Characterisation of mucosal changes in the alimentary tract following administration of irinotecan: implications for the pathobiology of mucositis

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Abstract
Purpose The pathobiology of alimentary tract (AT) mucositis is complex and there is limited information about the events which lead to the mucosal damage that occurs during cancer treatment. Various transcription factors and proinflammatory cytokines are thought to play important roles in pathogenesis of mucositis. The aim of this study was to determine the expression of nuclear factor-κB (NF-κB), tumor necrosis factor (TNF) and interleukins-1β (IL-1β) and -6 (IL-6) in the AT following the administration of the chemotherapeutic agent irinotecan.
Methods Eighty-one female dark Agouti rats were assigned to either control or experimental groups according to a specific time point. Following administration of irinotecan, rats were monitored for the development of diarrhoea. The rats were killed at times ranging from 30 min to 72 h after administration of irinotecan. Oral mucosa, jejunum and colon were collected and standard immunohistochemical techniques were used to identify NF-κB, TNF, IL-1β and IL-6 within the tissues. Sections were also stained with haematoxylin and eosin for histological examination.
Results Irinotecan caused mild to moderate diarrhoea in a proportion of the rats that received the drug. Altered histological features of all tissues from rats administered irinotecan were observed which included epithelial atrophy in the oral mucosa, reduction of villus height and crypt length in the jejunum and a reduction in crypt length in the colon. Tissue staining for NF-κB, TNF and IL-1β and IL-6 peaked at between 2 and 12 h in the tissues examined.
Conclusions This is the first study to demonstrate histological and immunohistochemical evidence of changes occurring concurrently in different sites of the AT following chemotherapy. The results of the study provide further evidence for the role of NF-κB and associated pro-inflammatory cytokines in the pathobiology of AT mucositis. The presence of these factors in tissues from different sites of the AT also suggests that there may be a common pathway along the entire AT causing mucositis following irinotecan administration.

Keywords Mucositis · Nuclear factor-κB · Pro-inflammatory cytokines · Irinotecan · Chemotherapy

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Introduction

In recent years there has been increasing interest in the pathobiology of mucositis [2, 21, 28, 30]. It is now not

considered a purely epithelial phenomenon, but rather represents a complex interplay between all mucosal compartments [2]. Furthermore, mucositis is now recognized to involve the entire alimentary tract (AT) not just the oral mucosa [14]. There is, however, limited information about the specific events that lead to mucosal damage. The development of mucositis in patients during their cancer treatment places a significant clinical and financial burden on the provision of care. Importantly, mucositis can compromise treatment outcomes and, in itself, may increase mortality because of the increased risk of systemic infection [5]. Because of these adverse consequences there have been recent active endeavours to review the pre-clinical and clinical literature in order to develop management protocols for mucositis [15]. Furthermore, recognition of the fact that mucositis involves the entire AT has also further complicated the problem and created an increased challenge for clinical management.

The role of the transcription factor nuclear factor-κB (NF-κB) has been postulated as a key element in the development of mucositis [29]. This has been established using animal models as well as clinical studies [20, 34]. NF-κB is responsible for the upregulation of approximately 200 genes. In the context of mucositis however, it is the subsequent upregulation of tumor necrosis factor (TNF), interleukin-1β (IL-1β) and interleukin-6 (IL-6) and their amplification through positive feedback loops which are currently considered responsible for the tissue damage that occurs in a patient with mucositis [2]. The result of the upregulation of these pro-inflammatory cytokines is widespread apoptosis which occurs within the various epithelial and connective tissue compartments that make up the mucosal tissue.

Irinotecan hydrochloride is a chemotherapy agent that has been used to treat a variety of solid tumours. It has its cytotoxic effect by inhibiting DNA topoisomerase 1, which occurs following conversion of irinotecan to its active metabolite SN-38 by carboxylesterase [6]. The dose of irinotecan that can be safely administered is limited by severe side effects such as leukopaenia and severe diarrhoea [1, 12]. In the clinical setting, the occurrence of toxicities varies between patients [10]. Glucuronidation of SN-38 is protective against irinotecan toxicity. Hepatic and intestinal uridine diphosphate glucuronoslytransferase (UGT), particularly the UGT1A1 isoform, are involved in the glucuronidation of the active metabolite [10, 32]. It has been suggested that patients with low activity of UGT1A1 may have a genetic predisposition for increased irinotecan toxicity. Furthermore, the intestinal microflora may also have an important role to play in the toxicity of this drug. Many resident bacteria in the gut have β-glucuronidase activity and are able to convert the glucuronidated SN-38 back to the active metabolite within the intestine potentiating the toxicity of the drug resulting in an increase in side effects such as diarrhoea [31]. Furthermore, bacteria in damaged mucosa may directly stimulate NF-κB and pro-inflammatory cytokine expression [30] thereby potentiating tissue damage. The effect of irinotecan on the structure of the small intestine mucosa is well documented and includes increased apoptosis in the crypts of the jejunum and colon leading to loss of normal histology of the tissue as well as effacement of the normal structure of the crypts and villi [7]. These histological features of intestinal mucositis manifest clinically as diarrhoea, abdominal bloating and pain [13, 14, 16, 25].

Previous studies have postulated that, allowing for regional differences in the mucosa, the mechanism of mucositis development is similar regardless of the site of the AT [34]. The aims of the current study were:

1. To determine a time course for histological changes at different sites of the AT following administration of irinotecan.
2. To determine whether NF-κB and pro-inflammatory cytokines TNF, IL-1β and IL-6 are expressed in different regions of the AT following irinotecan administration.
3. To determine whether there is a difference in the timing of tissue levels of NF-κB and pro-inflammatory cytokines in different regions of the AT.

Materials and methods

This study was approved by the Animal Ethics Committees of The Institute of Medical and Veterinary Sciences and of The University of Adelaide and complied with the National Health and Medical Research Council (Australia) Code of Practice for Animal Care in Research and Training (2004). Due to the potentially severe nature of the diarrhoea that can be induced by irinotecan, animals were monitored four times daily and if any animal showed certain criteria (as defined by the Animal Ethics Committee) they were euthanised. These criteria included a dull ruffled coat with accompanying dull and sunken eyes, coolness to touch with no spontaneous movement, and a hunched appearance.

Eighty-one rats were randomly assigned to either a control or experimental group according to a specific time point. The control and experimental groups included three and six animals, respectively. Briefly, all rats in the experimental groups received 0.01 mg/kg subcutaneous atropine (to reduce any cholinergic reaction to irinotecan) immediately (within 2 min) prior to administration of a single intraperitoneal dose of 200 mg/kg of irinotecan (previously shown to cause reproducible gastrointestinal mucositis). Irinotecan (supplied by Pfizer) was administered in a sorbitol/lactic acid buffer (45 mg/ml sorbitol/0.9 mg/ml lactic acid

pH 3.4), required for activation of the drug. Rats in the control groups did not receive any treatment. Subsequent to administration of irinotecan the following endpoints were assessed four times per 24 h period: mortality, body weights, diarrhoea, and general clinical condition. Diarrhoea was classified according to the following criteria: mild diarrhoea (staining of anus); moderate diarrhoea (staining top of legs and lower abdomen); severe diarrhoea (staining over legs and higher abdomen as well as continual anal leakage).

Rats were killed at the following time points following administration of irinotecan: 30, 60 and 90 min, 2, 6, 12, 24, 48 and 72 h. Rats were killed by exsanguination and cervical dislocation. The GIT from the pyloric sphincter to the rectum was dissected out and flushed with chilled isotonic saline (0.9 w/v) to remove contents and then the wet weight of the small and large intestines was recorded. Samples (1 cm in length) of the small intestine (taken at 25% of the length of the small intestine from the pylorus) and the colon (taken at midcolon position) were dissected and removed for further analysis. In addition, samples of oral mucosa were also removed. All tissue samples were immediately fixed in 10% neutral buffered formalin before processing and embedding in paraffin wax.

Sample preparation for haematoxylin and eosin staining

Sections were cut at 4 μm and mounted on glass slides. The sections were dewaxed, rehydrated and stained in Lillie-Mayer's haematoxylin for 10 min. Sections were then differentiated in 1% acid alcohol and blued in Scott's tap water before being counterstained in eosin. The sections were dehydrated and mounted prior to histological examination. Photographs of the specimens were taken using an Olympus Altra 20 camera. Measurement of epithelial thickness in the oral mucosa, villus length (in the jejunum) crypt length (in the jejunum and colon) was undertaken using analySIS® FIVE software. Statistical analysis of the data was carried out using an unpaired *t* test (Graphpad Prism 5).

Expression of NF-κB, TNF, IL-1β and IL-6

The expression of NF-κB, TNF, IL-1β and IL-6 in the oral mucosa, jejunum and colon was detected using standard immunohistochemical techniques [34]. Briefly, serial 4 μm sections of the fixed paraffin tissues were cut and mounted on silane-coated microscope slides and dried on a hot-plate for approximately 2 h. The sections were deparaffinised in xylene before being rehydrated through a series of alcohols and distilled water, followed by two rinses in phosphate buffered saline (PBS, pH 7.5). Antigen retrieval was carried out by microwaving the slides whilst immersed in citrate buffer (pH 6.0) for 3 min on high power and 15 min on low power. The slides were allowed to cool to room temperature (20 min). They were dehydrated and endogenous peroxidase was blocked by immersion in 3% H_2O_2 in methanol for 1 min. The slides were covered with 50% horse serum in PBS for 30 min, rinsed with PBS, and incubated using the Avidin Biotin Blocking Kit (Vector Laboratories, Burlingame, CA, USA) to block endogenous avidin–biotin activity. NF-κB p65 (F-6) (Santa Cruz Biotechnology, Santa Cruz, CA, USA; sc-8008) is an affinity purified mouse monoclonal IgG1 antibody and was used at a dilution of 1:2,300; TNF (HP8001) (HyCult Biotechnology b.v.), a rabbit affinity purified polyclonal antibody, was used at a dilution of 1:1,000; IL-1β (H-153) (Santa Cruz Biotechnology, Santa Cruz, CA, USA; sc-7884), a rabbit affinity polyclonal antibody, was used at a dilution of 1:1,000; IL-6 (M-19) (Santa Cruz Biotechnology, Santa Cruz, CA, USA; sc-1265), a goat affinity polyclonal antibody, was used at a dilution of 1:1,000.

NF-κB, TNF, IL-1β or IL-6 antibody was then applied with 5% horse serum (Sigma) in PBS and the slides were left overnight (16 h) at 4°C in a humidified chamber. Following incubation with the primary antibody, sections were washed with two changes of PBS and incubated with a secondary antibody (Biotinylated anti-mouse immunoglobulin G purified antibody (Vector Laboratories)) at a dilution of 1:200 with 5% horse serum for slides treated with NF-κB, TNF, Il-1β and biotinylated anti-goat immunoglobulin G purified antibody (Vector laboratories) at 1:200 dilution with 5% horse serum for slides treated with IL-6 antibody, for 20 min at room temperature. Subsequent to this, the labeling reagent ultrastreptavidin peroxidase (Signet Pathology Systems Inc., Dedham, MA, USA) was applied for 20 min at room temperature. Antibody binding was visualised with 3,3′diaminobenzidine tetrachloride at room temperature. The slides were washed in two changes of distilled water and counterstained with Lillie-Mayer's haematoxylin for 5 min. The slides were dehydrated, cleared to xylene and mounted. Positive controls for NF-κB were rat breast adenocarcinoma tumour tissue and, for TNF, IL-1β and IL-6, normal rat lung tissue.

Qualitative immunohistochemistry was performed. Staining was observed using a light microscope. The intensity of staining for was scored as follows: 0 no staining, 1 weak staining, 2 moderate staining, 3 moderate to intense staining, 4 intense staining. This qualitative staining assessment has been previously validated by published grading systems [17–19] and is routinely used in our laboratory [3, 34]. All assessments were done in a blinded fashion by one investigator (RML). Due to the ordinal nature of the outcome data, the effect of irinotecan on the various outcome measures (i.e., tissue expression of NF-κB and pro-inflammatory cytokines) was assessed using ordinal logistic regression models. Statistical significance was set at

$P = 0.05$. All calculations were performed using SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Response to treatment

Treated rats began to demonstrate "clinical" signs and symptoms of gastrointestinal mucositis at 2 h following the administration of irinotecan. The main manifestation of this was diarrhoea. As reported previously [31], mild diarrhoea was observed in 23% of treated rats 2 h following the administration of irinotecan. Twelve hours following administration of the drug, 30% of rats had mild diarrhoea and 5% were classed as having moderate diarrhoea. Diarrhoea was most prevalent at 24 h where 39% of rats had mild diarrhoea and 5% had moderate diarrhoea. At 72 h, 33% of the rats in the treatment groups had mild diarrhoea. None of the rats in the control groups had diarrhoea. Chemotherapy-induced death did not occur in this study.

Histology

Oral mucosa

In the oral mucosa, changes were noted in the thickness of the epithelium over the 72 h period (Fig. 1). However, no obvious areas of ulceration were identified. The appearance of the oral mucosal epithelium at 48 h is shown in Fig. 1. Rats in the treatment groups demonstrated marked epithelial atrophy of the oral epithelium compared with controls at 60 min ($P < 0.0005$), 12 h ($P = 0.035$), 24 h ($P = 0.035$) and at 48 h ($P = 0.012$) (Fig. 2).

Jejunum

Marked histological evidence of mucositis was observed in the jejunum following treatment with irinotecan. This was evident from 6 h following administration of irinotecan (Fig. 1). These changes included the presence of degenerative enterocytes within the crypts followed by more gross architectural disturbances which were seen at the later time points where changes such as villus blunting, epithelial

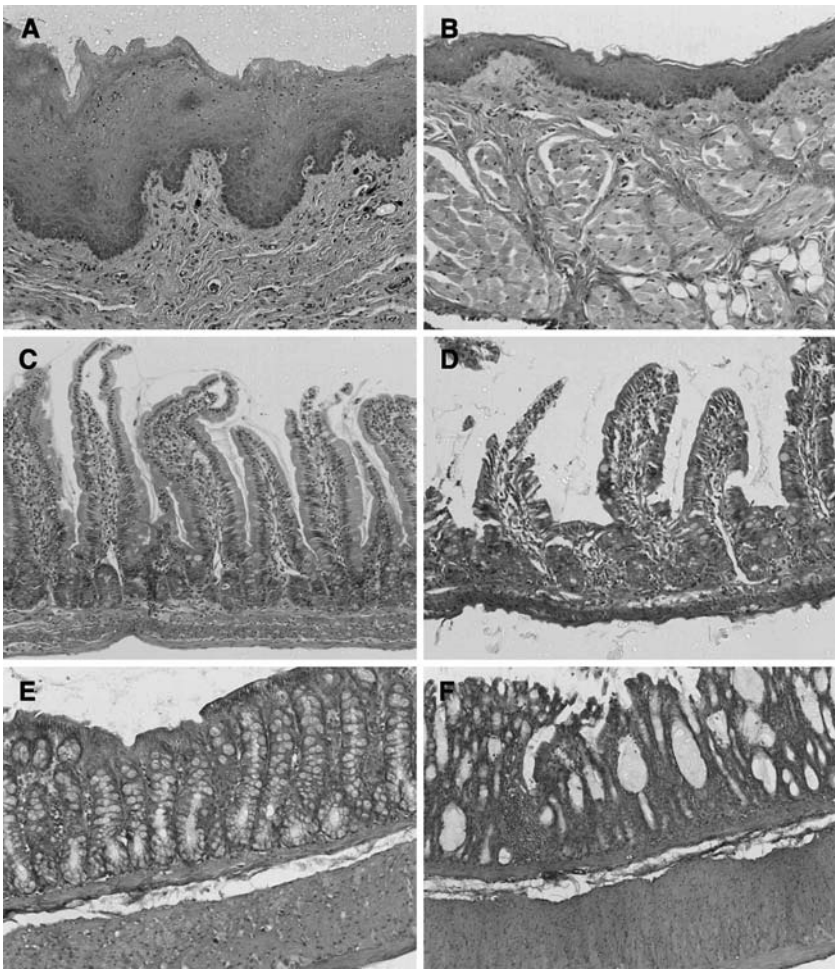

Fig. 1 Histological features of the oral mucosa (A, control and B, 48 h) jejunum (C, control and D, 72 h) and colon (E, control and F, 72 h) following irinotecan administration (original magnification ×20)

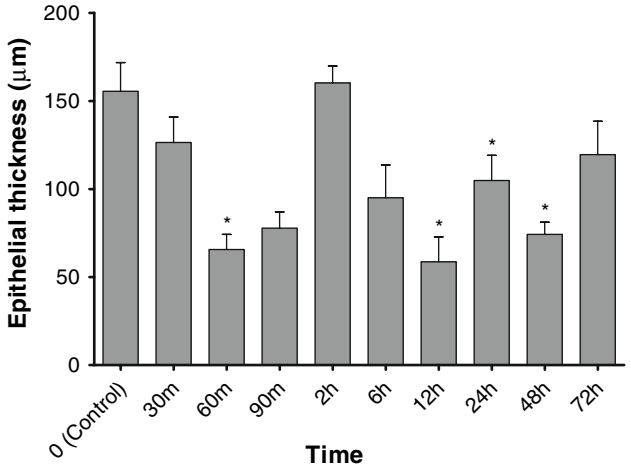

Fig. 2 Changes in epithelial thickness of the oral mucosa over 72 h following irinotecan administration

atrophy and increased intensity of inflammatory cell infiltration throughout the mucosal tissue were noted (Fig. 1). Changes in villus and crypt length (Fig. 3) were similar to that observed in the oral mucosa demonstrating a decrease in length at 60 min ($P = 0.0073$) and 30 min ($P = 0.017$), respectively, compared to the control group. This was followed by a resolution at 2 h followed by another significant reduction compared to controls at 12 h for villus length ($P = 0.0116$) and crypt length ($P = 0.0022$) before returning to lengths comparable to controls by 24 h.

Colon

Likewise, there was also marked histological evidence of mucositis in the colon following administration of irinotecan. Initial histological changes seen included the presence of individual degenerative enterocytes within the crypts. Whilst at later time points complete ablation of the crypts was observed (Fig. 1). Crypt length in the colon increased at 30 and 60 min ($P = 0.0006$ and 0.0036, respectively), returned to normal levels at 2 h and then increased again at 24 h ($P < 0.0001$) (Fig. 4).

Immunohistochemistry

NF-κB

Significantly elevated staining of NF-κB was observed in the oral mucosa, jejunum and colon. Levels peaked in the oral mucosa at 2 h following irinotecan administration (Fig. 5) and were consistently higher throughout the experimental period ($P = 0.0002$). In the jejunum, staining of NF-κB peaked at 12 h before subsiding and then slowly increasing over the later time points ($P = 0.0033$). In the colon, staining of NF-κB were also elevated at all time

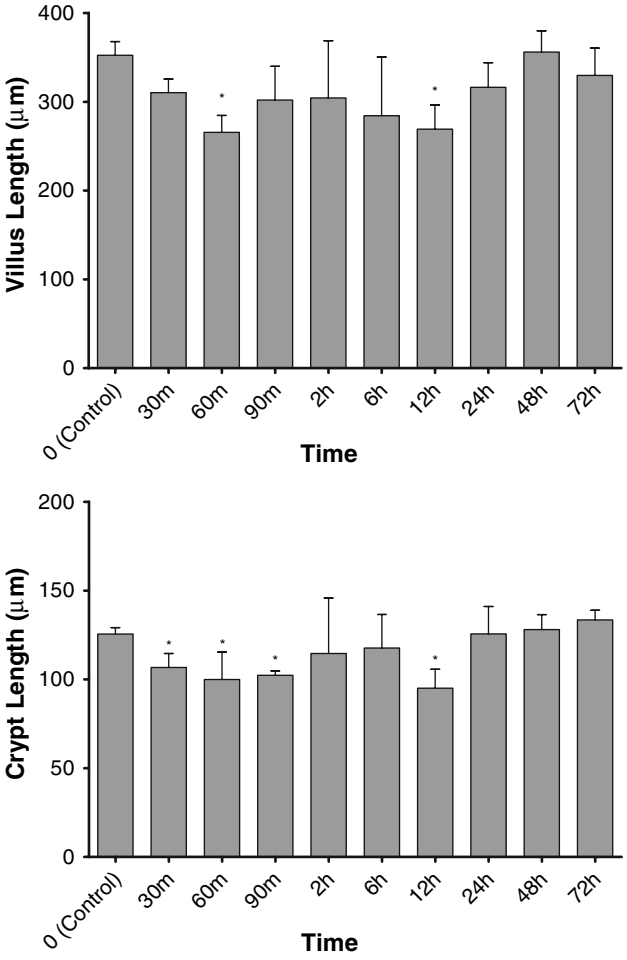

Fig. 3 Changes in villus and crypt length in the jejunum over 72 h following irinotecan administration

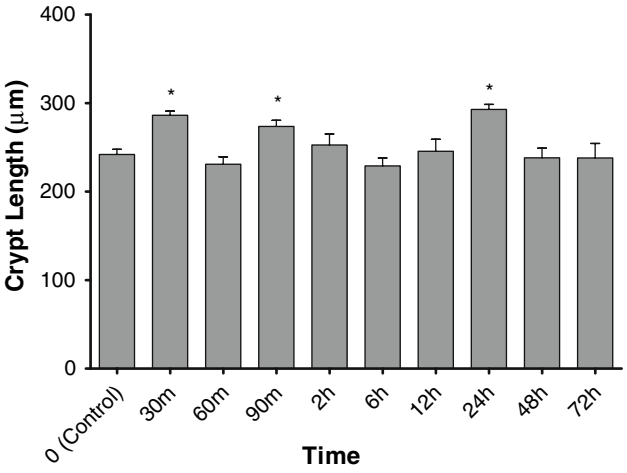

Fig. 4 Changes in crypt length in the colon over 72 h following irinotecan administration

points in the mucosa and the difference between the experimental and control groups was statistically significant ($P < 0.0001$).

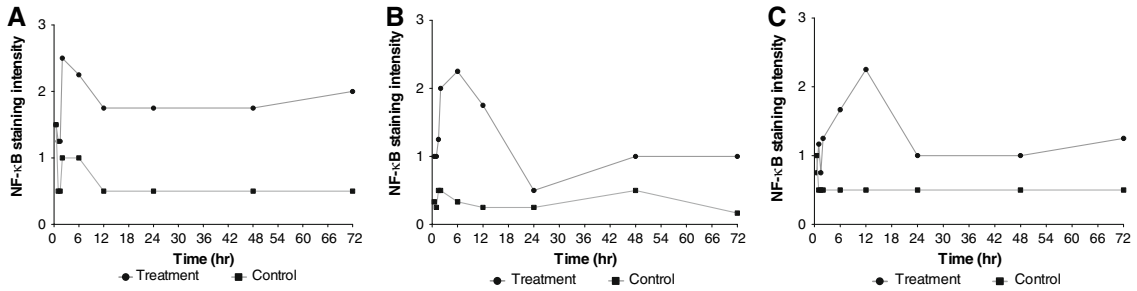

Fig. 5 NF-κB staining in the oral mucosa (**a**), jejunum (**b**) and the colon (**c**) following irinotecan administration

Positive staining for NF-κB was observed in the epithelium of all tissues as well as occasional fibroblasts and inflammatory cells in the submucosa.

TNF

The immunohistochemical staining for TNF was more intense in the experimental groups compared with the control groups in all of the sites of the rat AT that were examined (Fig. 6). When compared with the control group of rats significantly elevated tissue staining for TNF was observed in the oral mucosa peaking at 6 h ($P = 0.0101$). In the jejunum, the tissue levels of TNF were also elevated and peaked at 6 h although this was not significant ($P = 0.1235$). Significantly elevated tissue staining for TNF was observed in the colon compared to the control groups, peaking early at 2 h ($P = 0.0254$). The trend observed in TNF staining tended to parallel the expression of NF-κB in the tissues of the rat AT.

Positive staining for TNF was also evident in the fibroblast population as well as inflammatory cells in all the tissues examined.

IL-1β

Elevated staining for IL-1β was observed in the oral mucosa at the later time points investigated in the study (Fig. 4) however, this was not significant ($P = 0.0733$). Whilst in the jejunum, significantly elevated tissue staining in was observed compared to controls peaking at 6 h (Fig. 7) ($P = 0.017$). Following the peak, staining intensity then returned to the levels observed in the control rats. In the colon, the tissue levels of Il-1β peaked at 12 h (Fig. 7). As in the jejunum this result was statistically significant when compared with the control group of rats ($P = 0.0038$).

Occasional endothelial cell staining was noted in the oral mucosa and jejunum.

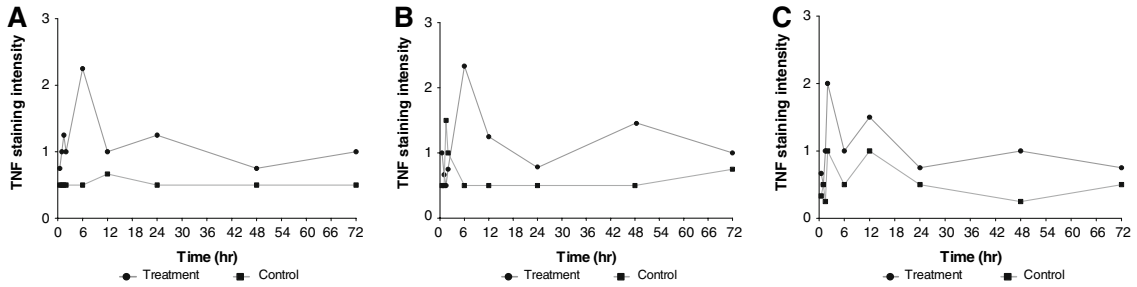

Fig. 6 TNF staining in the oral mucosa (**a**), jejunum (**b**) and the colon (**c**) following irinotecan administration

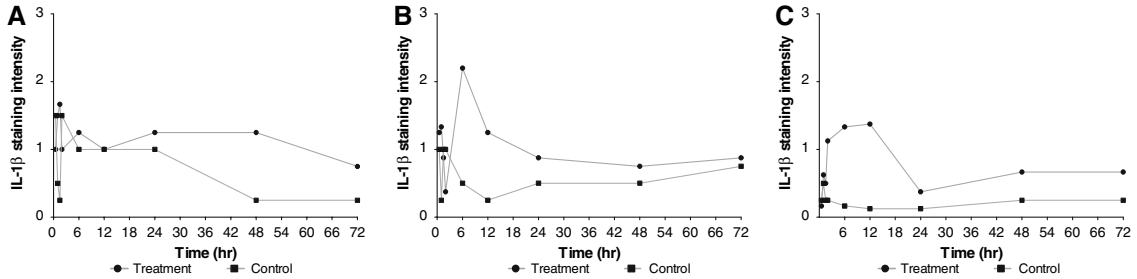

Fig. 7 IL-1β staining in the oral mucosa (**a**), jejunum (**b**) and the colon (**c**) following irinotecan administration

IL-6

As for Il-1β, tissue levels of IL-6 in the oral mucosa were significantly elevated at the later time points compared with control groups (Fig. 8) ($P = 0.0006$). There was a peak in tissue levels of IL-6 in the jejunum and colon at 6 h (Fig. 8), these results however, were not significant compared to controls ($P = 0.4230$ and 0.0976, respectively). The trend observed in the tissues examined with respect to changes in tissue levels of IL-6 over time was consistent with the other results.

Discussion

It has been postulated previously that the pathogenesis of mucositis is consistent throughout the AT with differences occurring as a result of anatomical and histological variation [14]. To the best of our knowledge, the results of this study are the first to indicate that this may indeed be the case. The current study demonstrated that NF-κB, thought to be a key driver of mucositis, and pro-inflammatory cytokines (TNF, IL-1β and IL-6) were expressed in various sites along the AT following the administration of irinotecan in the DA rat and that this coincided with histological evidence of tissue damage at early time points. Obviously there are limitations in this study in that it is not longitudinal; however, a true longitudinal study using serial biopsies from the same rat taken at different time points is not practical and would pose increased risk of mortality from procedures and compromise results. This study does have a large sample size and, as such, changes in cytokine expression over time can be implied.

The results from this study demonstrated that the staining for NF-κB, TNF, IL-1β and IL-6 in the oral mucosa, jejunum and colon was similar following the administration of irinotecan (Fig. 5, 6, 7, 8). Tissue staining was generally elevated between 2 and 12 h following chemotherapy. The peak in tissue staining of NF-κB and pro-inflammatory cytokines coincided with the initial signs of histological alteration of the tissues and initial occurrence of diarrhoea. However, the epithelial atrophy in the oral mucosa and reduction in villus and crypt lengths in the intestine resolved by 12 h. Gross architectural disturbances such as increased apoptotic bodies, inflammation, villus blunting and crypt ablation were not observed until the later time points. Likewise the early diarrhoea could be attributed to cholinergic effects of irinotecan in spite of the administration of atropine. More severe diarrhoea occurred at the later time points, coinciding with the more severe changes in mucosal histology seen in the intestine. This indicates that changes occur in the mucosa prior to the development of symptoms of mucositis. As well as demonstrating subclinical changes, the present study also demonstrated that these changes are similar regardless of anatomical site. This provides further evidence for a common pathway for mucositis development which is modified as a consequence of local structural differences in mucosae. There are obvious histological differences between the mucosae of the oral cavity and the intestinal mucosa. In the rat, the epithelial compartment of the oral mucosa is ortho-keratinised unlike in humans where the oral epithelium is generally non-keratinised or para-keratinised. The small and large intestinal mucosae in the rat have similar histology to that seen in humans in that it has structural features which are in keeping with its absorptive, digestive and protective functions [11]. These structural differences in the mucosa appear have implications for the resilience that different mucosae may exhibit in response to the effects of chemotherapeutic drugs.

The occurrence of subclinical changes in pro-inflammatory cytokines in the AT that were demonstrated in this study provides support for the hypothesis proposed by Sonis et al. [27, 28] whereby the onset on clinical symptoms of mucositis is preceded by a complex cascade of molecular events triggered by the production of reactive oxygen species within the mucosa including the activation of transcription factors such as NF-κB leading to upregulation of pro-inflammatory cytokines. These pro-inflammatory cytokines (such as TNF- IL-1β and IL-6) are responsible for the tissue damage that occurs as a result of promoting apoptosis within the mucosa. Recent studies in clinical settings have demonstrated the activation of NF-κB

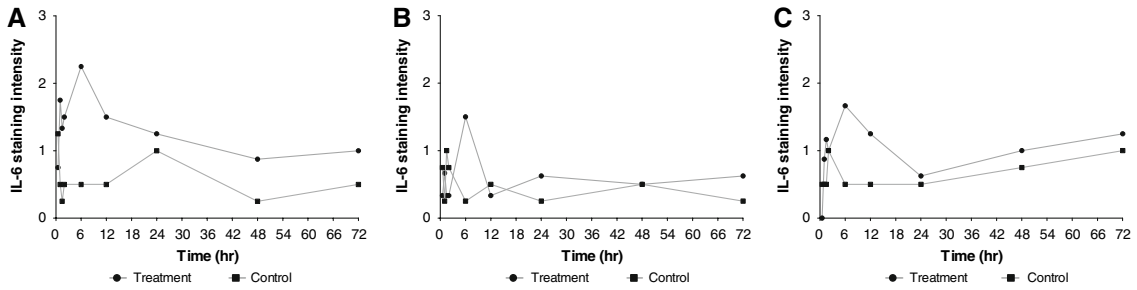

Fig. 8 IL-6 staining in the oral mucosa (**a**), jejunum (**b**) and the colon (**c**) following irinotecan administration

in the oral mucosa and small intestinal mucosa of patients that had undergone clinical chemotherapy and radiotherapy [20, 34]. In other clinical settings, for example inflammatory bowel disease (IBD), the role of cytokines is well established [24]. TNF has been demonstrated to have a host damaging role in this context [23]. It has also been suggested that NF-κB activation by IL-6 may also play a role in IBD [33]. The role of pro-inflammatory cytokines in IBD has led to the use of novel treatments that target and ultimately inhibit components of the inflammatory cascade, for example the anti-TNF monoclonal antibody, infliximab which has a beneficial effect in the management of Crohn's disease [24, 26]. As yet, to the best of our knowledge, there are no studies which have investigated similar targeted treatments in the context of mucositis. A recent publication by Melo et al. [22] indicated that irinotecan-induced diarrhoea in a mouse model may be modified by administering pentoxifylline and thalidomide. Both of these agents inhibit cytokine production. Pentoxifylline administration delayed the onset of diarrhoea as well as reducing the intestinal changes induced by irinotecan, including tissue levels of TNF and IL-1β [22]. Thalidomide, whilst not affecting diarrhoea onset also attenuated tissue changes in the intestine [22]. The authors of that study concluded that this provided evidence for the role of TNF and IL-1β in mucositis pathobiology and indicated that further investigations were necessary.

As well as the subclinical changes that occur in the intestinal mucosa before the development of diarrhoea, this study also suggests that ulceration in the oral mucosa is preceded by subclinical mucosal changes. Following irinotecan administration, alterations in NF-κB and pro-inflammatory cytokines occurred in the oral mucosa in the absence of ulceration. With respect to oral mucositis, many studies using animal models "induce" mucositis by traumatizing the mucosa by mechanical irritation following chemotherapy in order to create clinical evidence of ulceration [21]. This study is one of the first to demonstrate histological, as well as molecular changes occurring in the oral mucosa subsequent to chemotherapy in a rat model of mucositis *without* the need for trauma. This is important because, mechanical irritation of the mucosa may cause the induction of biological events which might complicate the histological and molecular changes induced by chemotherapy. The need to actually observe epithelial loss (i.e., ulceration) is based on a dated paradigm that mucositis is solely an epithelial phenomenon and purely relates to the development of ulceration, more recent ideas suggest that many subclinical events occur in the mucosa well before ulceration manifests clinically. The current study confirms this hypothesis. Other studies have also reported immunohistochemical, as well as ultrastructural, evidence of tissue damage irrespective of histological changes as well as an absence of clinical changes [8, 20]. We have previously demonstrated that elevated tissue levels of NF-κB occurred in the oral mucosa subsequent to chemotherapy. In that study, however, no histological evidence of structural alteration of the mucosa was apparent. Likewise, Gibson and colleagues demonstrated ultrastructural changes occurring in the oral mucosa in the absence of changes at the histological level. In the current study, the mucosa, particularly the epithelial barrier, although altered, remained intact providing additional support for the current pathobiology model of mucositis whereby many of the molecular events leading to ulceration occur before clinical evidence of mucositis is apparent [30]. In the rat, the distinct structural or phenotypic differences between the oral and intestinal mucosae, particularly in terms of keratinisation, may provide additional resilience of the oral mucosa to the effects of irinotecan.

In conclusion, the results of the study provide further evidence for the role of NF-κB and associated pro-inflammatory cytokines in the pathobiology of AT mucositis and addressed the three stated aims of the study. This is the first study to demonstrate changes in the tissue levels of these factors throughout the AT following administration of irinotecan. This study also indicates that there appears to be a common pathway for mucosal damage subsequent to irinotecan administration, irrespective of differences in structural variation between different regions of the AT. Other commonly used chemotherapy drugs cause mucositis (for example methotrexate and 5-fluorouracil) and have also been demonstrated to cause histological damage to tissues [4, 9]. Investigations into the tissue expression of cytokines subsequent to the administration of other drugs are warranted and are currently being undertaken by our group. Fully elucidating the pathobiology of mucositis is important as this should lead to better management of this debilitating side effect of cancer treatment ultimately leading to improved quality of life and treatment outcomes for patients.

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