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ORIGINAL ARTICLE

Is the pathobiology of chemotherapy-induced alimentary tract mucositis influenced by the type of mucotoxic drug administered?

Richard M. Logan · Andrea M. Stringer · Joanne M. Bowen · Rachel J. Gibson · Stephen T. Sonis · Dorothy M. K. Keefe

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Abstract
Purpose Alimentary tract (AT) mucositis is a serious problem complicating cancer treatment, however, its pathobiology remains incompletely understood. Nuclear factor-κB (NF-κB) and pro-inflammatory cytokines are considered to have important roles in its development. This has been previously demonstrated in different sites of the AT following administration of irinotecan in an animal model using the Dark Agouti rat. The aim of the present study was to determine whether the changes that occur in the AT are affected by the type of mucotoxic drug.
Methods Female DA rats were given a single dose of either methotrexate (1.5 mg/kg intramuscularly) or 5-fluorouracil (150 mg/kg intraperitoneally). Rats were killed at 30, 60, 90 min, 2, 6, 12, 24, 48 and 72 h. Control rats received no treatment. Samples of oral mucosa, jejunum and colon were collected. Haematoxylin and eosin stained sections were examined with respect to histological evidence of damage and standard immunohistochemical techniques were used to demonstrate tissue expression of NF-κB, TNF, IL-1β and IL-6.
Results Both MTX and 5-FU administration caused histological evidence of tissue damage in the AT as well as changes in tissue expression of NF-κB and specific pro-inflammatory cytokines. This study, however, demonstrated that there were differences in the timing of histological changes as well as the timing and intensity of pro-inflammatory cytokine tissue expression caused by the different drugs.
Conclusions The results from this study suggest that there are differences in the mucositis pathobiology caused by different drugs. This may have important ramifications for the management of mucositis particularly with respect to the development of treatment regimens for mucositis. Further investigations are required to determine the exact pathways that lead to damage caused by the different drugs.

Keywords Mucositis · Pro-inflammatory cytokines · Chemotherapy · Methotrexate · 5-Fluorouracil

R. M. Logan (✉)
Discipline of Oral Pathology, School of Dentistry,
Faculty of Health Sciences, The University of Adelaide,
North Terrace, Adelaide, SA 5005, Australia
e-mail: richard.logan@adelaide.edu.au

R. M. Logan
Division of Tissue Pathology,
Institute of Medical and Veterinary Sciences,
Adelaide, SA 5000, Australia

A. M. Stringer · J. M. Bowen · R. J. Gibson · D. M. K. Keefe
Department of Medical Oncology,
Royal Adelaide Hospital, Adelaide, SA 5000, Australia

A. M. Stringer · J. M. Bowen · R. J. Gibson · D. M. K. Keefe
Division of Medicine, Faculty of Health Sciences,
The University of Adelaide, Adelaide, SA 5005, Australia

S. T. Sonis
Brigham and Women's Hospital, Boston, MA, USA

Introduction

Alimentary tract (AT) mucositis remains an important clinical problem as it is a potentially serious complication for patients undergoing cancer treatment [9, 12, 13, 29]. The development of mucositis is thought to be "driven" through the activation of nuclear factor-κB (NF-κB) which promotes the upregulation of key cytokines such as tumour necrosis factor (TNF), interleukin-1β (IL-1β) and interleukin-6 (IL-6). These pro-inflammatory cytokines are considered

to play important roles in the overlapping phases of mucositis development, in particular upregulation and message generation, signal amplification and ulcerative phases [21, 25, 26, 29]. In the clinical setting, a wide variety of drugs may cause mucositis. We have previously published histological and immunohistochemical data illustrating changes that occur concurrently at different sites of the AT subsequent to the administration of irinotecan in the Dark Agouti (DA) rat [20]. Irinotecan, a topoisomerase-1 inhibitor is well known to cause mucositis, particularly small intestinal mucositis and causes significant clinical problems for patients undergoing treatment protocols that include this drug. Following administration of irinotecan in the DA rat, histological evidence of tissue damage was observed in the oral mucosa, jejunum and colon. This coincided with changes in the tissue expression of NF-κB as well as TNF, IL-1β and IL-6 [20].

Different drugs obviously act through different pathways and, in themselves, may promote or inhibit different pro-inflammatory cytokines [18, 22, 31]. The idea therefore, that a single pathway leads to mucosal damage, particularly in the context of chemotherapy, is simplistic. Is, therefore, the type of chemotherapy drug important in terms of this damage? In order to determine whether different drugs caused similar changes in the AT to that observed with irinotecan, our original study was replicated, substituting irinotecan with either methotrexate (MTX) or 5-fluorouracil (5-FU) both of which are commonly used drugs in clinical practise.

Although both of these drugs ultimately kill tumour cells through inhibiting DNA synthesis, they do this through different mechanisms. MTX is a folate antagonist and inhibits the enzyme dihydrofolate reductase which is required for the production of thymidine; as a result, DNA synthesis is blocked. 5-FU is a pyrimidine analogue and interferes with DNA synthesis by blocking thymidylate synthetase; in addition it is also incorporated into RNA thereby affecting RNA function and protein synthesis. MTX also has anti-inflammatory actions which are exploited in the management of diseases such as rheumatoid arthritis. It is well established that each of these drugs cause mucositis both in the oral mucosa and in the small intestine [5, 11, 23, 24]. MTX causes damage to the small intestine which is characterised by increased crypt apoptosis and villus atrophy [11]. 5-FU administration also results in increased apoptosis and decreased cellularity in the small intestine [24]. The specific mechanisms by which MTX and 5-FU cause mucosal toxicity, particularly in the oral mucosa, are not well understood. In the context of the current hypothesis regarding mucositis pathobiology, these drugs demonstrate some paradoxical behaviour. For example, with regard to its anti-inflammatory action, MTX causes a reduction in pro-inflammatory cytokine gene expression, particularly IL-1, 2 and 6 as well as interferon-γ [18]. Furthermore, MTX has been demonstrated to suppress NF-κB in Jurkatt cells [22]. Likewise, 5-FU has also been shown to decrease NF-κB activation [2, 3]. As NF-κB activation and subsequent pro-inflammatory cytokine production have been implicated to play an important role in mucositis pathobiology, given these drug effects, is it possible that MTX and 5-FU might cause mucosal damage in the AT through alternative pathways?

Accordingly, the aims of this study were to:

1. Determine the histological changes that occur in the oral mucosa, jejunum and colon in rats following administration of either MTX or 5-FU.
2. Determine whether there is immunohistochemical evidence of changes in tissue expression of NF-κB and pro-inflammatory cytokines (TNF, IL-1β and IL-6) at different sites of the AT following the administration of either MTX or 5-FU.
3. Determine whether there was a difference in histological and immunohistochemical features according to the type of drug administered.

Based on the fact that MTX and 5-FU have different mechanisms of action, it was hypothesised that the changes observed in the AT of the DA rat following chemotherapy would be influenced by the type of drug administered.

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). 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.

To investigate the effect of MTX and 5-FU on the different mucosae, two separate experiments were designed. In each experiment, 81 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 a single intramuscular dose of 1.5 mg/kg of MTX or a single intraperitoneal dose of 150 mg/kg of 5-FU (both doses of drugs previously shown to cause gastrointestinal mucositis in rats [7, 10]. Rats in the control groups did not receive any treatment. Subsequent to administration of the drug the following endpoints

were assessed four times per 24 h period: mortality, diarrhoea, and general clinical condition.

Rats were killed by exsanguination and cervical dislocation at the following time points following administration of the drug: 30, 60 and 90 min, 2, 6, 12, 24, 48 and 72 h. A section of the AT extending from the pyloric sphincter to the rectum was dissected out and flushed with chilled isotonic saline (0.9 w/v) to remove contents. 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

Tissue samples were embedded in paraffin wax, cut at 4 μm sections 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 then 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. In order to compare epithelial thickness in the oral mucosa and crypt length in the jejunum and colon over time and between the different drugs, a general linear model was fitted to the data. In the model a generalised estimating equation was used to account for the dependence in observations from the same rat. The analysis was done using SAS version 9.1 (SAS Institute Inc. Cary, NC, USA).

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

NF-κB, TNF, IL-1β and IL-6 expression in the oral mucosa, jejunum and colon were detected using standard immunohistochemical techniques [32]. 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) to block endogenous avidin–biotin activity. NF-κB p65 (F-6) (Santa Cruz Biotechnology, Santa Cruz, CA; 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; 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; 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 labelling reagent ultrastreptavidin peroxidase (Signet Pathology Systems, Inc., Dedham, MA) 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 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 [15–17] and is routinely used in our laboratory [4, 32]. All analyses were done in a blinded fashion by one investigator (RML).

Due to the ordinal nature of the outcome data, the effect of treatment on the various outcome measures 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

The rats tolerated both MTX and 5-FU well. Clinical indications of deterioration were not observed in the experimental groups of rats with respect to either drug. Furthermore, significant diarrhoea was not observed in either group of rats.

Histology

Oral mucosa

In the oral mucosa both drugs caused a decrease in epithelial thickness (Figs. 1a, b, 2a, b), however, neither drug caused ulceration. For rats given MTX, this occurred by 90 min ($p = 0.0059$) following drug administration followed by brief return to baseline thickness then a gradual decline over the rest of the time period (12 h, $p = 0.004$; 24 h, $p = 0.001$; 48 h, $p = 0.0004$; 72 h, $p = 0.0019$) (Fig. 3a). 5-FU administration resulted in a decrease in epithelial thickness at 2 h ($p = 0.0002$) followed by a brief return to baseline levels between 6 and 24 h followed by a gradual decline over the rest of the 72-h period (48 h, $p < 0.0001$; 72 h, $p < 0.0001$) (Fig. 3a).

Post hoc comparison of this data demonstrated that MTX caused earlier changes in epithelial thickness compared to 5-FU at 90 min ($p = 0.0005$), however, no difference was observed at later time points.

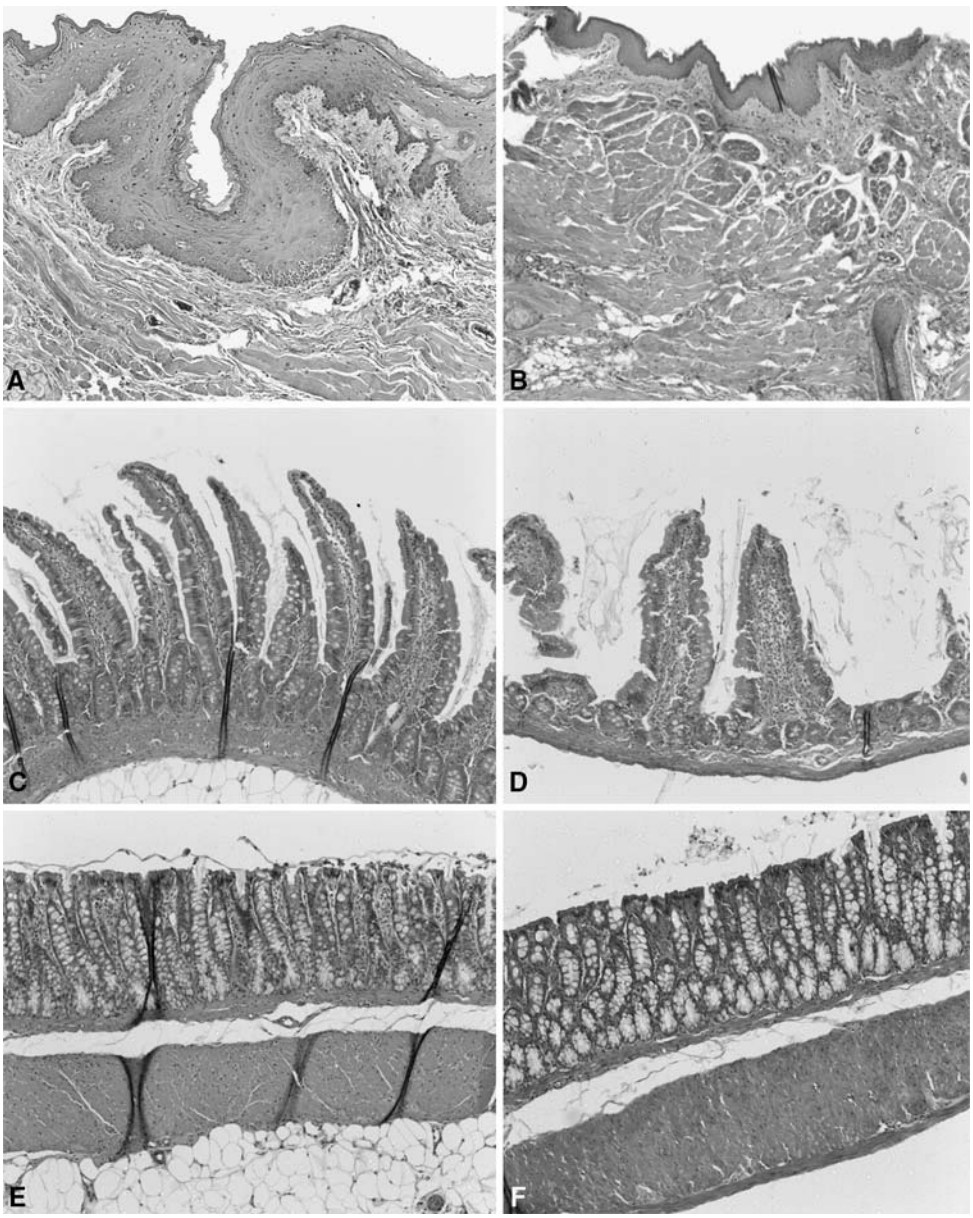

Fig. 1 Histological features of the alimentary tract following methotrexate administration: oral mucosa **a** control and **b** 12 h; jejunum **c** control and **d** 12 h; colon **e** control and **f** 24 h

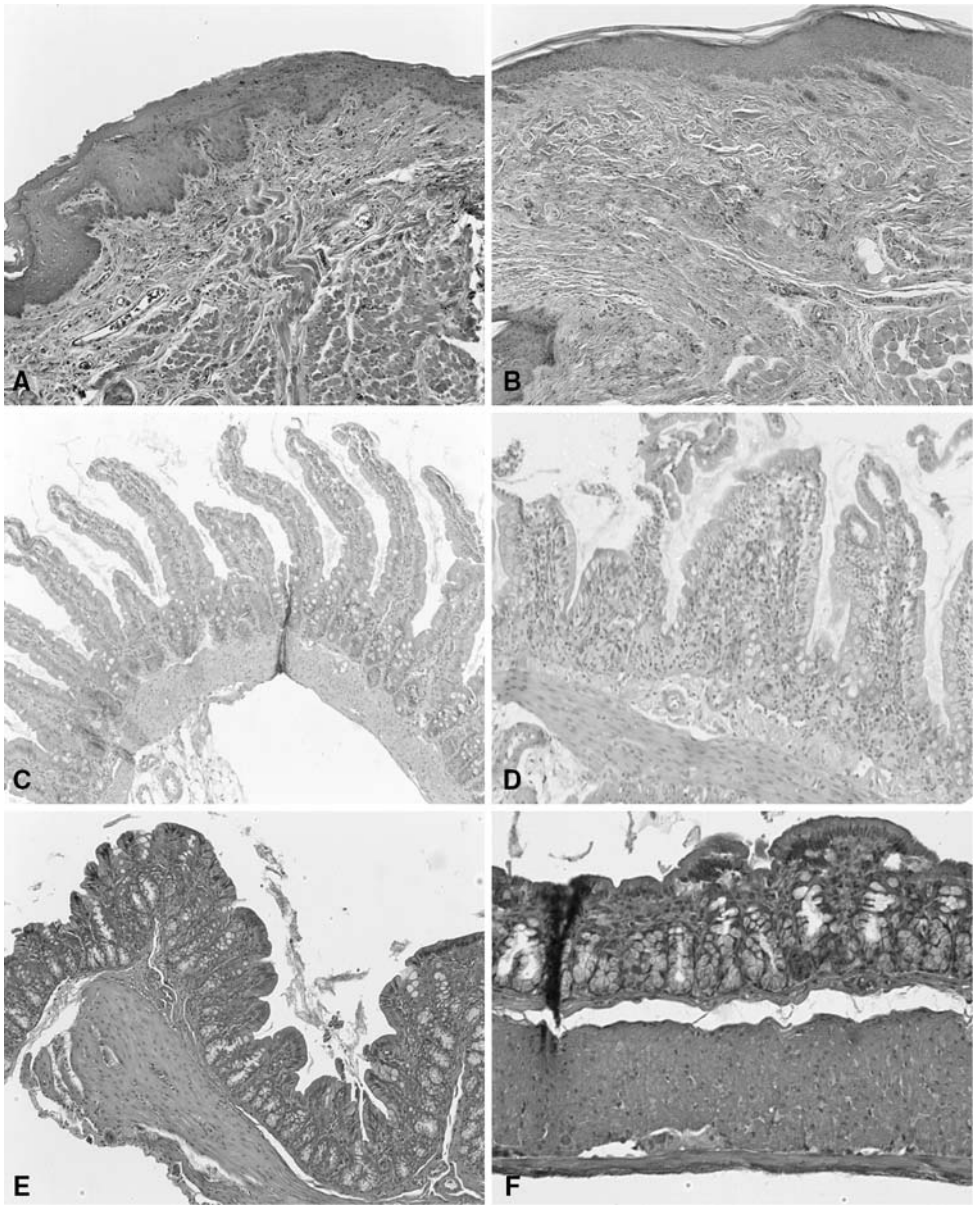

Fig. 2 Histological features of the alimentary tract following 5-fluorouracil administration: oral mucosa **a** control and **b** 12 h; jejunum **c** control and **d** 12 h; colon **e** control and **f** 24 h

Jejunum

In the jejunum crypt a reduction in crypt length was observed (Fig. 1c, d) following MTX administration from 6 h ($p < 0.0001$) and throughout the remaining time period (12 h, $p < 0.0001$; 24 h, $p < 0.0001$; 48 h, $p < 0.0001$; 72 h, $p = 0.0061$; Fig. 3b). Other signs of mucosal damage included blunting and fusion of the villi and obliteration of the crypts. 5-FU caused blunting and fusion of the villi as well as enterocyte hyperplasia (Fig. 2c, d). Increased apoptotic bodies were observed at 12 h. 5-FU administration also caused changes in jejunal crypt length. After remaining stable during the early time points, a reduction in crypt length occurred from 12 h ($p = 0.0038$) extending over the remainder of the 72-h time period (24 h, $p < 0.0001$; 48 h $p < 0.0001$; 72 h, $p = 0.0109$) (Fig. 3b).

Post hoc comparisons between MTX and 5-FU demonstrated that 5-FU caused a greater reduction in crypt length compared with MTX which was significant at 24 and 48 h ($p < 0.0001$ and $p = 0.0598$, respectively).

Colon

Histological examination revealed minimal changes in the colon in rats given either MTX or 5-FU (Figs. 1e, f, 2e, f). However, for MTX treated rats, an increase in crypt length was observed between 2 and 6 h ($p = 0.0207$ and $p < 0.0001$, respectively) followed by a return to baseline levels by 12 h

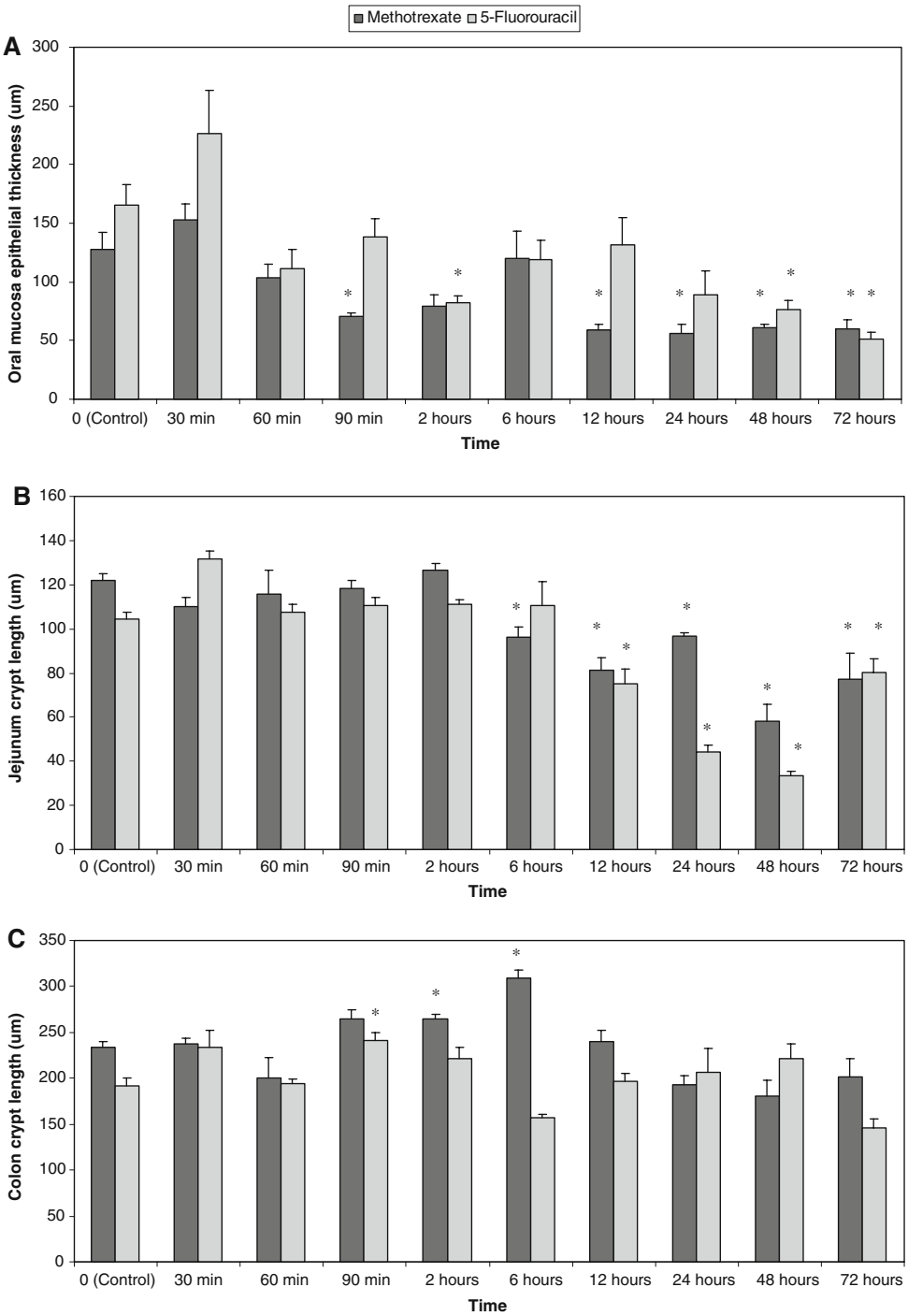

Fig. 3 Changes in oral epithelial thickness (**a**) and jejunum (**b**) and colon (**c**) crypt lengths following administration of methotrexate and 5-fluorouracil

(Fig. 3c). 5-FU administration resulted in increased numbers of apoptotic bodies within the deep aspects of the crypts. With respect to crypt length, increased length was noted 90 min ($p = 0.0007$) followed by a brief reduction at 6 h ($p = 0.0036$) a further reduction occurred by 72 h ($p = 0.00145$) (Fig. 3c, f).

Post hoc comparison demonstrated that there was a difference in crypt length changes between MTX and 5-FU administration at 6 h. MTX caused a marked increased in crypt length whereas there was a decrease in crypt length in the rats given 5-FU ($p < 0.0001$).

Immunohistochemistry

NF-κB

The level of staining for NF-κB within the oral mucosa was elevated at the majority of time points subsequent to MTX administration (Fig. 4a). Although approaching significance, the results for the tissue levels of NF-κB expression were not significant ($p = 0.078$). In the jejunum and colon statistically significant levels of tissue staining for NF-κB

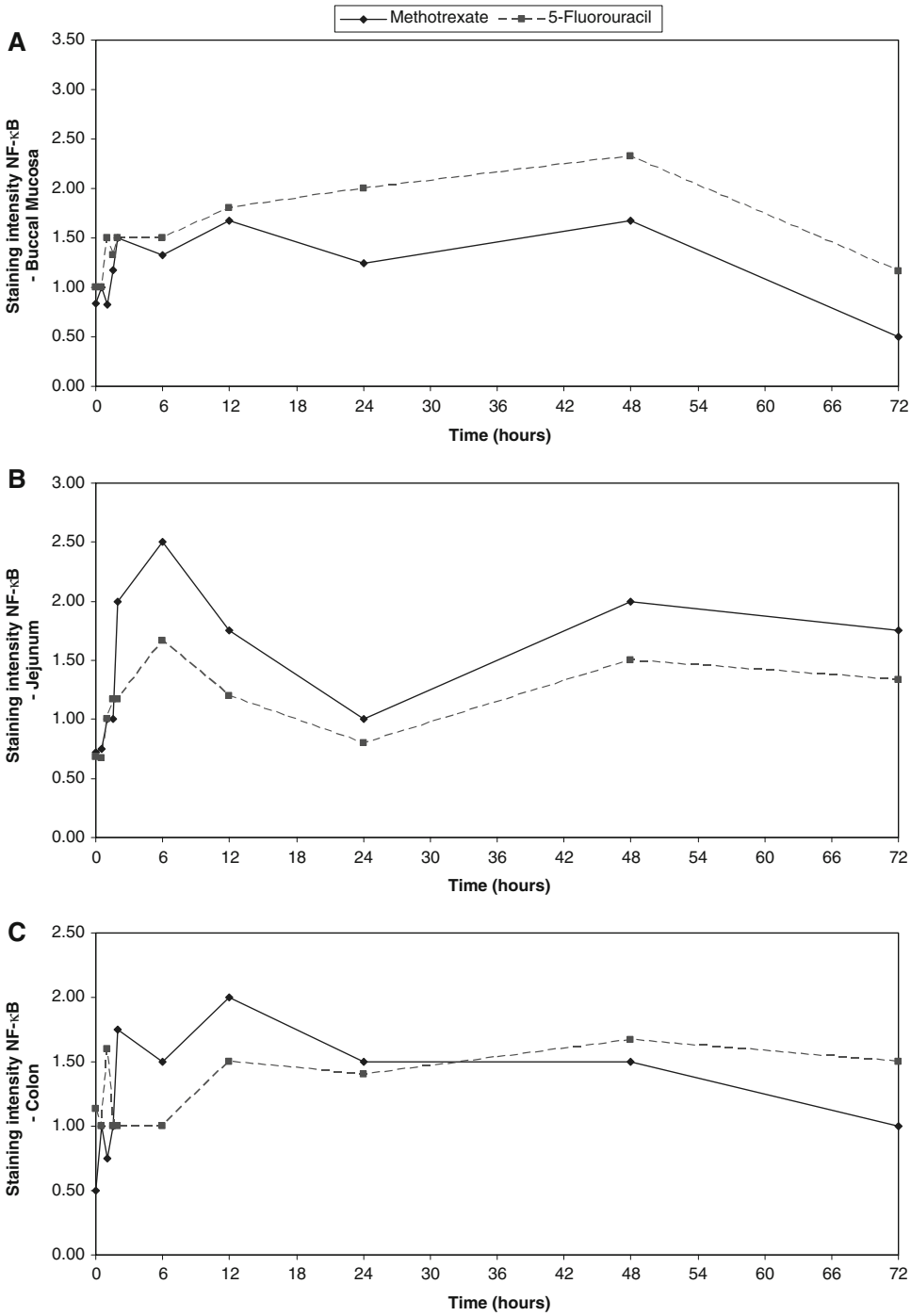

Fig. 4 NF-κB staining of the oral mucosa (**a**), jejunum (**b**) and colon (**c**) following administration of methotrexate and 5-fluorouracil

were observed peaking at 6 h in the jejunum ($p = 0.008$) and at 12 h in the colon ($p = 0.005$) (Fig. 4b, c, respectively).

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

5-FU administration did not cause significantly elevated tissue staining for NF-κB within the oral mucosa, jejunum or the colon (Fig. 4a–c, respectively). Again, the epithelial cells stained positively for NF-κB as well as fibroblasts and inflammatory cells within the underlying submucosal tissue.

TNF

Significantly elevated levels of TNF were observed in the oral mucosa, jejunum and colon subsequent to MTX administration ($p = 0.0005$, $p = 0.0007$ and $p = 0.002$, respectively) (Fig. 5a–c, respectively). In the oral mucosa the tissue staining for TNF peaked at between 2 and 6 h, in

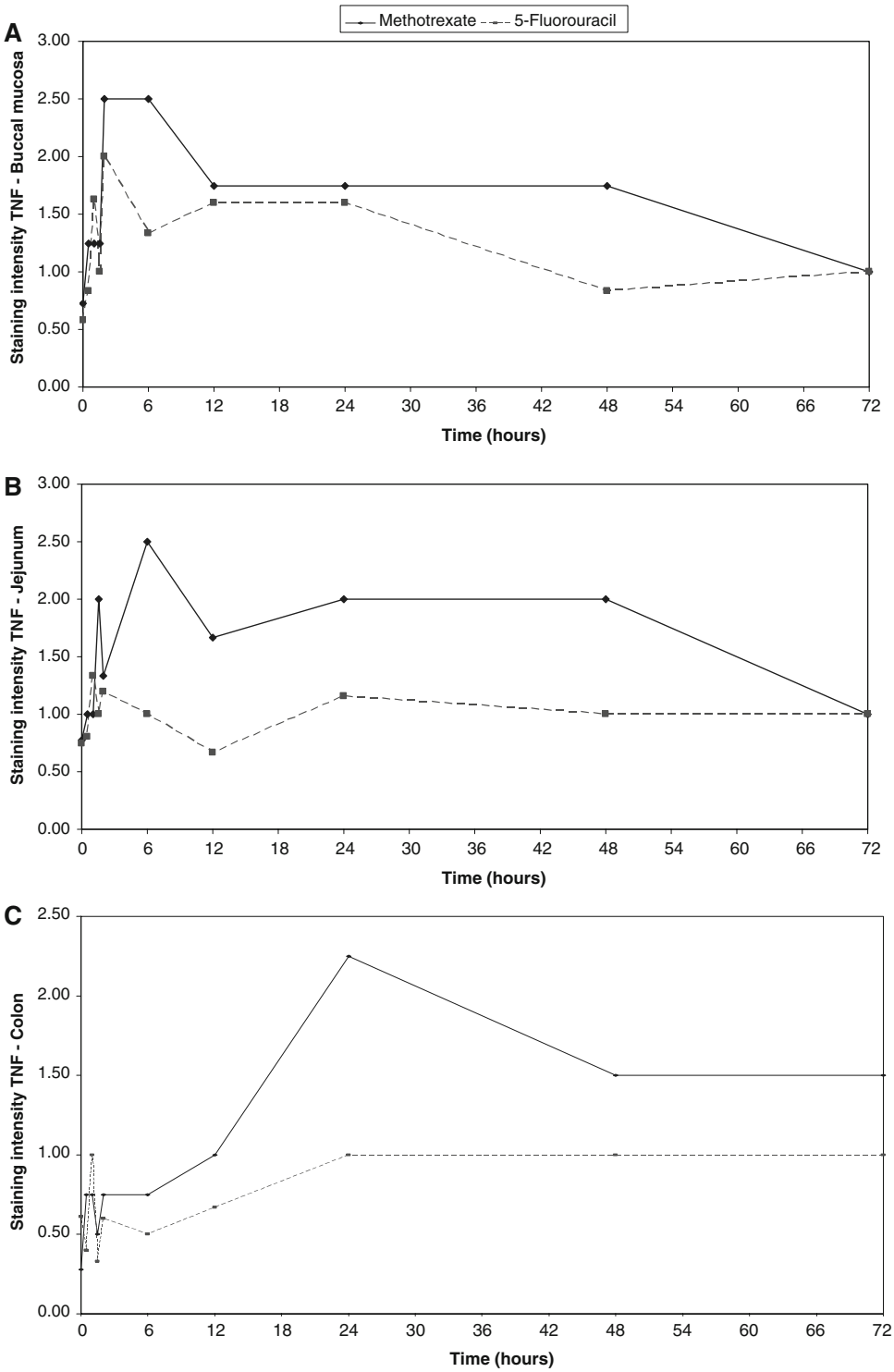

Fig. 5 TNF staining of the oral mucosa (**a**), jejunum (**b**) and colon (**c**) following administration of methotrexate and 5-fluorouracil

the jejunum at 6 h, whilst in the colon the elevation of TNF was more prolonged and peaked at 24 h.

TNF staining was significantly elevated in the oral mucosa and colon following administration of 5-FU ($p = 0.008$, oral mucosa; $p = 0.001$, jejunum; $p = 0.0002$, colon). As for MTX, TNF levels peaked earlier in the oral mucosa and jejunum, whilst in the colon the peak levels occurred later and were more prolonged (Fig. 5a–c, respectively).

*IL-1*β

Following MTX administration, significantly elevated levels of IL-1β were observed in the oral mucosa, jejunum and

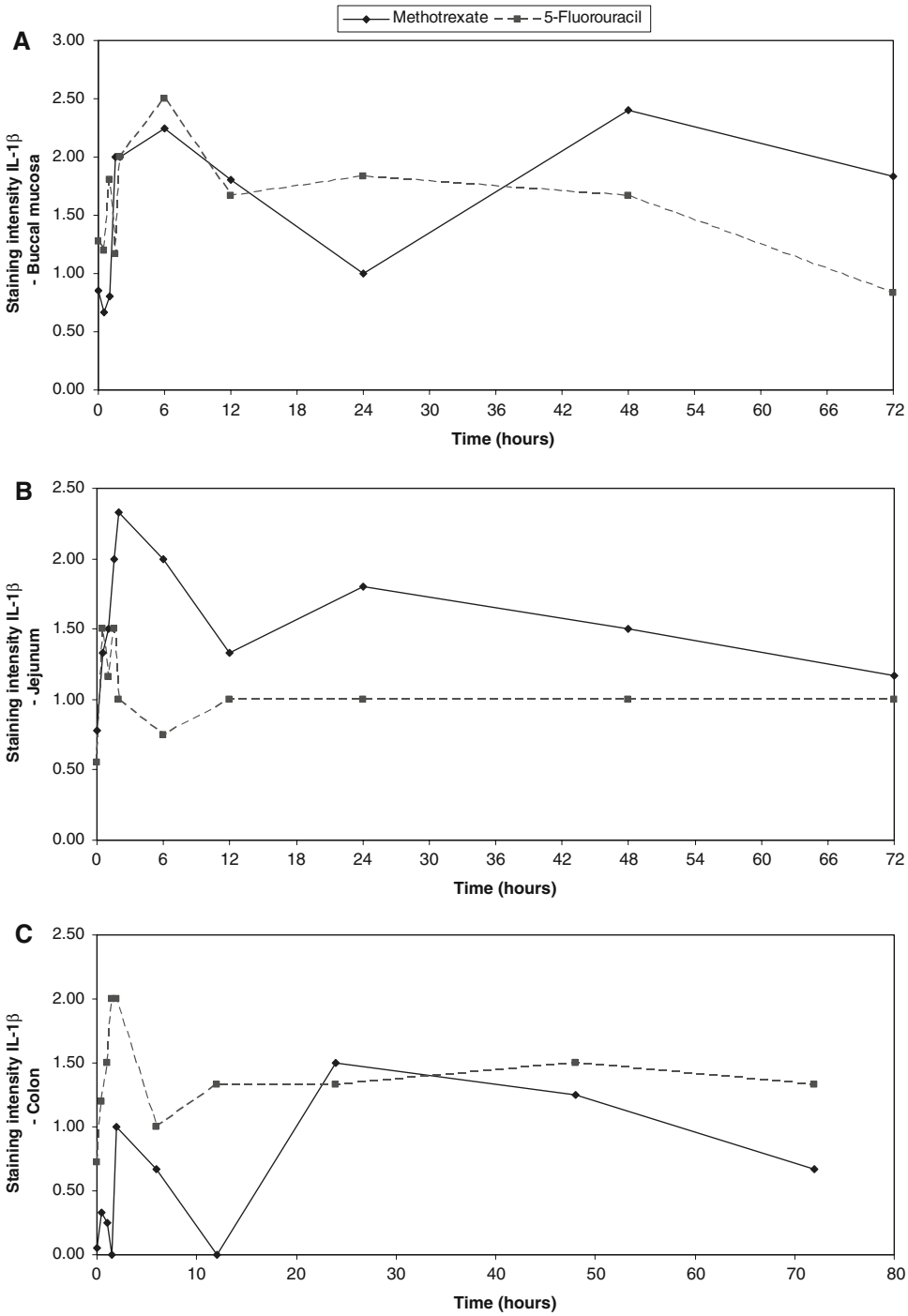

Fig. 6 IL-1β staining of the oral mucosa (**a**), jejunum (**b**) and colon (**c**) following administration of methotrexate and 5-fluorouracil

colon ($p = 0.0016$, $p < 0.0001$ and $p = 0.0064$, respectively). In each of the three AT sites examined there appeared to be two peaks of IL-1β, at 6 and 48 h in the oral mucosa, at 2 and 24 h in the jejunum and colon (Fig. 6a–c, respectively).

With respect to IL-1β levels following 5-FU administration, only oral mucosa demonstrated significant results ($p = 0.0013$) with a peak in staining intensity observed at 6 h (Fig. 6a). Although not significant, peaks of IL-1β were observed in the jejunum and colon at 90 min (Fig. 6b, c, respectively).

IL-6

The staining intensity of IL-6 demonstrated in the tissues following MTX administration was significantly elevated

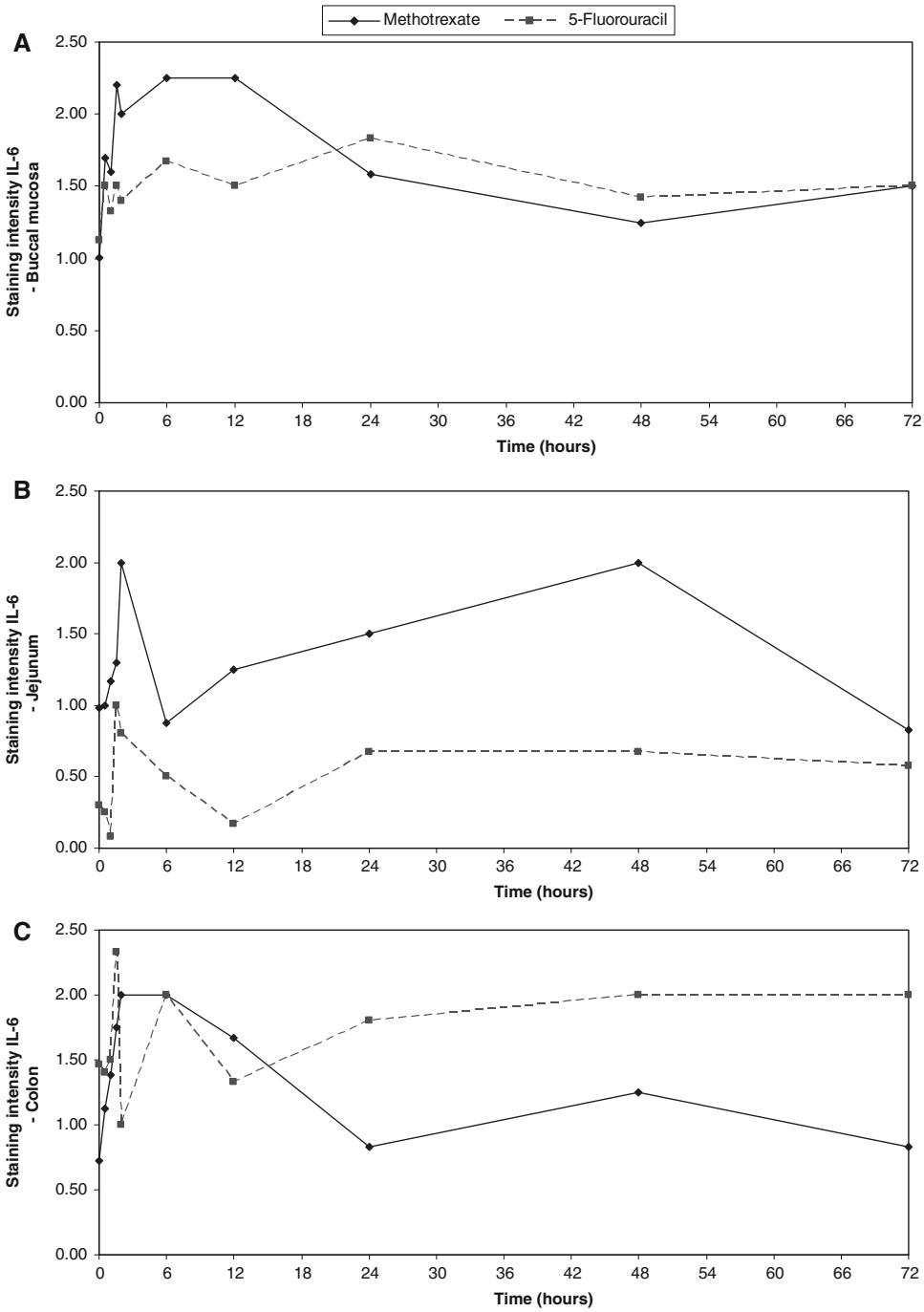

Fig. 7 IL-6 staining of the oral mucosa (**a**), jejunum (**b**) and colon (**c**) following administration of methotrexate and 5-fluorouracil

in the oral mucosa, jejunum and colon ($p = 0.0004$, $p = 0.0083$ and $p = 0.0013$, respectively). Early peaks in IL-6 staining were observed in the oral mucosa and colon at 6 h (Fig. 7a, c, respectively), whilst in the jejunum, IL-6 staining peaked at 2 h and again at 48 h (Fig. 7b).

Following the administration of 5-FU, no significant staining for IL-6 was observed in any of the tissues examined (Fig. 7a–c, respectively).

Table 1 summarises the changes in tissue levels that were observed following administration of irinotecan [20], MTX and 5-FU.

Discussion

We have previously demonstrated that in the DA rat, administration of irinotecan causes histological changes consistent with AT mucositis in different anatomical sites of the AT as well as alterations in tissue expression of proinflammatory cytokines [20]. This observation supported the hypothesised role of NF-κB activation and subsequent elevation of tissue levels of TNF, IL-1β and IL-6. The main objective of the current study was to determine whether different drugs with different modes of action caused similar changes in the AT of the DA rat.

Table 1 Summary of timing of peak tissue levels of NF-κB and pro-inflammatory cytokines following administration of irinotecan, methotrexate or 5-fluorouracil

	Irinotecan [20]		Methotrexate		5-Fluorouracil	
NF-κB	O	2 h	O	NS	O	NS
	J	12 h	J	6 h	J	NS
	C	12 h	C	12 h	C	NS
TNF	O	6 h	O	2–6 h	O	2 h
	J	NS	J	6 h	J	NS
	C	2 h	C	24 h	C	24 h
IL-1β	O	NS	O	6 and 48 h	O	6 h
	J	6 h	J	2 and 24 h	J	NS
	C	12 h	C	2 and 24 h	C	NS
IL-6	O	6 h	O	6 h	O	NS
	J	NS	J	2 and 48 h	J	NS
	C	NS	C	6 h	C	NS

O oral mucosa, *J* jejunum, *C* colon, *NS* no significant change

The type of histological changes observed in the tissues was similar to that described in other studies [7, 10], however these focussed predominantly on small intestinal mucositis. In spite of this, the rats in the current study were administered similar doses of MTX and 5-FU to these other studies based on the fact that mucositis is considered to occur throughout the AT. Gibson et al. [10] demonstrated a decrease in crypt length at 6 h following MTX administration which corresponds to that observed in the current study. Following 5-FU administration, Cool et al. [7] demonstrated decreased crypt length at 48 h which again is comparable to the results in this study. We also demonstrated these changes at earlier time points following 5-FU administration. Although MTX or 5-FU administration caused similar of histological changes by 72 h, differences were observed with respect to the timing of changes between the two groups. MTX administration resulted in an initial decrease in epithelial thickness of the oral mucosa followed by a brief increase to normal levels and then a subsequent decrease in epithelial thickness over the 72 h time period. Similar changes were observed in the jejunum and colon with respect to crypt length; however, the initial decrease was not significant. 5-FU administration, on the other hand, although not significant in the oral mucosa and colon caused an initial increase in oral epithelial thickness and intestinal crypt length followed by a gradual reduction over the remainder of the 72-h period. An interesting feature was the apparent increased resilience of the colon compared to other sites of the AT. In this site, an increase in crypt length occurred following administration of MTX and 5-FU. A decrease in crypt length was either brief (in the case of MTX) or apparent only at the 72 h time point. This "hyperplastic" response in the colon was also observed following irinotecan administration [20]. Does this difference between the colon and the other mucosae reflect a true increased resilience of the colon? If so, what protective mechanisms are present in the colon that promote this hyperplastic response? It has previously been suggested that the location of stem cells within the colon renders them more resistant to damage, so that although apoptosis may be increased this does not necessarily lead to tissue damage occurring [10].

We have previously demonstrated that irinotecan causes similar histological changes in the AT compared to those that resulted from MTX and 5-FU administration. It is considered that this indicates that the mucosa may have a relatively limited repertoire in its response to the insult caused by drug administration [14]. Irinotecan administration, however, appeared to cause histological changes that were more severe than those resulting from MTX and 5-FU [20]. The timing of changes observed following irinotecan were also different and indicated a shorter, more intense insult with evidence of resolution by 72 h [20] unlike in the current study where oral epithelial thickness and jejunum crypt length remained reduced compared to baseline levels at 72 h. It is unclear why irinotecan caused this difference in timing and intensity, however, it is postulated that the cholinergic side effects of irinotecan may potentiate the damage that occurs following its administration. As mentioned the same type of damage appears to be caused by MTX and 5-FU, however, this is protracted compared to irinotecan; it would be interesting to observe ongoing changes at time points beyond 72 h to determine whether the pattern of damage is similar. As this damage appears to occur over a more extended time period following MTX and 5-FU administration it is likely that this results in reduced severity of the histological changes observed.

With respect to tissue levels of NF-κB and pro-inflammatory cytokines, further differences were observed between the two experimental groups. Similar staining to that seen previously with irinotecan was observed following the administration of MTX whereby an early peak in tissue staining for NF-κB, TNF, IL-1β and IL-6 occurred [20]. 5-FU, however, showed no significant increase in tissue staining for NF-κB or IL-6 in the experimental groups compared with controls.

Why was there a difference in the changes caused by different drugs? It is well documented that MTX, 5-FU and irinotecan all cause mucositis [6, 11, 19, 20] and the histological examination of the tissues in this study demonstrated damage particularly in the jejunum and colon. As mentioned previously, MTX has been demonstrated to suppress NF-κB expression [22] as well as IL-6 [18]. This, however, does not appear to have occurred in the AT of the DA rats subsequent to administration of MTX. Apart from direct effects of MTX it is possible that the increased

expression of NF-κB and pro-inflammatory cytokines may be a secondary phenomenon that occurs subsequent to MTX-induced tissue damage via other unidentified pathways as there are many other factors that can activate NF-κB apart from chemotherapy [27]. Furthermore, protective factors in the gut (e.g., trefoil factors) have also been shown to be affected by MTX, possibly rendering the AT more susceptible to subsequent tissue damage [8, 30]. This damage would then expose the tissues to other aggravating factors that may promote NF-κB activation such as cell wall products from resident bacteria within the gut [28].

Like MTX, 5-FU administration has been shown to inhibit NF-κB activation [2, 3] in this instance the results of this study are supportive in that there was no observed difference over the 72 h time period. A murine model describing 5-FU-induced intestinal damage demonstrated that intestinal toxicity arose due to the combined effects of apoptosis and inhibition of cell proliferation. These events lead to reduced cellularity in the crypts and villi of the intestine [24]. The same authors suggest that the mechanisms that lead to such damage were dependant on p53. It has been suggested that nitric oxide (NO) may a play a role in 5-FU induced mucositis through NO-dependent activation of p53 as well as members of the Bcl-2 family [19]. Further investigation into the mechanisms through which 5-FU causes mucosal toxicity is required.

The results from this study confirmed our hypothesis that changes observed in the AT of the DA rat following chemotherapy would be influenced by the type of drug administered. We demonstrated that the pattern of histological changes that occurred at different sites of the AT appeared to be affected by the type of drug. Furthermore, the tissue expression of NF-κB and pro-inflammatory cytokines, as indicated by intensity of immunohistochemical staining, was also determined by the type of drug administered. Of course, in the clinical setting, the mechanism of drug may not be the only variable that determines whether a patient will develop mucositis. Other factors may also impact on a patient's risk for developing mucositis including hormonal factors, underlying systemic illness and genetic factors, not to mention factors in the local tissue environment [1]. In addition, many treatment protocols involve the combination of different drugs which adds further complexity to the mechanisms through which AT toxicity occurs. Further investigation is required so that more effective, or even targeted, strategies can be developed for the treatment of mucositis.

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