Chemotherapy-induced mucositis: Mechanisms of damage, time course of events and possible preventative strategies.

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Abstract

Mucositis is a major oncological problem affecting large numbers of patients undergoing treatment for cancer. It can be produced by both chemotherapy and radiotherapy and can affect all areas of the gastrointestinal tract. This thesis has concentrated on several key gaps in current scientific knowledge that required investigation to enable a complete understanding of the cellular mechanisms associated with gastrointestinal mucositis. The different aspects of this thesis approach these fundamental knowledge gaps through a series of discrete research chapters which, when combined, provide evidence of the similarity in response to chemotherapy of the differing regions of the gastrointestinal tract. The research chapters investigated; (1, 2) the effects throughout the gastrointestinal tract of chemotherapeutic agents, Methotrexate (MTX) and Irinotecan, (3) the possible ameliorating potential of the cytokine Interleukin-11 in reducing the side effects of chemotherapy, (4) the expression of pro- and anti-apoptotic proteins and transcription factors along the gastrointestinal tract in normal human patients and (5) the time-course of development of oral mucositis in human patients.

Previous research has shown that cancer chemotherapy side effects within the gastrointestinal tract are predominantly due to apoptosis occurring in the crypts of the intestine. It is however unknown, whether there is a relationship between chemotherapy dose, apoptosis, p53/p21 expression and intestinal crypt cell proliferation. This thesis determined that low dose MTX (0.5 mg/kg) caused a high peak of apoptosis but minimal crypt cell hypoproliferation in the rat small intestine, and a lower peak of apoptosis with no crypt cell hypoproliferation in the colon. Higher doses of MTX (1.5; 2.5 and 5.0 mg/kg) caused lower peaks of apoptosis but severe crypt cell hypoproliferation in the small intestine. A change in p53 expression did not precede early high levels of apoptosis in the small intestine, whereas p53 upregulation was increased with late levels of apoptosis.

As mucositis is not specific to any one cytotoxic agent, a further aim of this thesis was to investigate if other cytotoxic drugs caused intestinal mucositis in a similar fashion to that described for MTX. Irinotecan is a commonly used chemotherapy agent, that causes severe mucositis in many patients. This thesis confirmed that Irinotecan acted in a similar fashion to MTX in the small intestine by causing severe small intestinal damage, with increased apoptosis and crypt cell hypoplasia. However, results also showed that

Irinotecan induced severe colonic damage with excessive mucus secretion, both of which are unusual for cytotoxic drugs.

A number of factors, such as interleukin-11 (IL-11), have been shown to have to be anti-mucotoxic potential – particularly for small and large intestinal symptoms. However, before they can be tested for their efficacy in humans, they must be shown to be safe, and not interfere with cytotoxic treatment or cause tumour growth. This part of my thesis aimed to determine if IL-11 ameliorated gastrointestinal mucositis. Results from this initial study also showed that administration of IL-11 caused no change in small intestine weight indicating IL-11 did not have any direct trophic effect on the small intestine. Following administration of MTX, IL-11 was able to ameliorate small intestinal mucositis by maintaining intestinal weight intestinal morphometry, despite being unable to prevent apoptosis. Although MTX induced hypoproliferation of crypt cells, IL-11 improved this reduction by helping to maintain villus area.

Our laboratory has previously shown that apoptosis occurs in the crypts of the small intestine shortly after the administration of chemotherapy and this may be directly related to the damage that is subsequently seen. Bcl-2 and other family members are either anti-apoptotic (Bcl-2, Bcl-xL, Bcl-w, Mcl-1) or pro-apoptotic (Bax, Bak, Bad, Bim) genes. Although there is still some uncertainty, the ratio of anti-apoptotic to pro-apoptotic proteins (eg Bcl-2:Bax) is believed to regulate apoptosis. The expression of both pro- and anti- apoptotic proteins, as well as the transcription factors, p53 and p21, are unknown in the normal human gastrointestinal tract. Therefore this thesis investigated the expression of 8 Bcl-2 family members and 2 transcription factors through seven regions of the gastrointestinal tract (oesophagus, antrum, duodenum, ileum, caecum, colon and rectum). Results clearly showed that for the majority of Bcl-2 family members, there was a lower level of expression within the oesophagus. The exception for this was the expression of Bcl-xL which showed no significant difference from other regions of the tract. Conversely, when the expression of the transcription factors p53 and p21 were examined it was found that p53 had a highly variable response. p21 also showed highly variable expression but as a general trend, decreased throughout the tract with highest levels observed in the oesophagus and lowest levels in the rectum.

Whilst there have been reports on treatment and prevention options of oral mucositis after chemotherapy, very little research has been conducted on the mechanisms behind oral mucositis. To date there are no conclusive data on the morphological changes in the

human oral mucosa following chemotherapy, furthermore, the time course of histological changes and the correlation with clinical symptoms and oral ulceration are not known. The final part of this thesis aimed to investigate the changes that occur in the oral mucosa of patients receiving chemotherapy and correlate these with chemotherapy "naïve" controls. The results from this study showed that the ultrastructural changes in the oral mucosa occur early after chemotherapy, and are not always correlated with histological changes as seen under a light microscope. Apoptosis within the basal cells of the oral mucosa occurred at day 1, 2, and 3 after chemotherapy and could be confirmed through transmission electron microscopy and TUNEL assay. In addition, expression of pro- and anti-apoptotic proteins and two transcription factors, p53 and p21, were examined. There was high variation in the expression of these, likely to be attributable to architectural changes. Therefore this study confirmed that, like the small intestine, changes occur early, with symptoms appearing later.

This thesis has provided tangible evidence to suggest that the entire gastrointestinal tract follows a similar pattern of development of mucositis. Results presented herein show that for the chemotherapeutic agents tested, a similar mode of action was reported in causing small intestinal mucositis. This is an important finding in developing and targeting appropriate preventative strategies. Apoptosis was also found to have a key role in mucositis and was detected in the oral cavity, small intestine and large intestine following chemotherapy. From this thesis it can be concluded that, although apoptosis was present following administration of chemotherapy, there was not always histological evidence of mucositis. Furthermore, results have also shown that cytokines such as IL-11, may have an important role to play in ameliorating mucositis. Further studies are now warranted in this area to optimise dose schedule and to clarify their mode of action.