Pre-treatment with insulin-like growth factor-I partially ameliorates 5-fluorouracil-induced intestinal mucositis in rats

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Abstract

Insulin-like growth factor-I (IGF-I) has been demonstrated to enhance mucosal repair following intestinal damage induced by chemotherapeutic agents (intestinal mucositis). However, the potential for prophylactic IGF-I to protect the intestine remains undefined. We investigated the effects of IGF-I pre-treatment on chemotherapy-induced mucositis in rats. Male Sprague Dawley rats were treated for 7 days with 0 or 4.3 mg/kg/day IGF-I delivered systemically via osmotic mini-pump. Rats received an intraperitoneal injection of 0 or 150 mg/kg 5-fluorouracil (5-FU) on day 7 and were killed 48 h later for assessment of intestinal damage and repair. Compared to normal controls, 5-FU decreased epithelial proliferation by 86%, concurrently increasing the incidence of apoptosis 87-fold, whilst decreasing small intestinal (SI) length by 14%, SI weight by 30% and total gut weight by 24%. 5-FU decreased villus height in the duodenum (23%), jejunum (20%) and ileum (30%) with crypt depths decreased by 31%, 27% and 33% in these gut regions. These effects were less profound in IGF-I pre-treated rats in which apoptosis was increased 48-fold, with SI length decreased by 7%, SI weight by 18% and total gut weight by 24%. 5-FU decreased villus height in the duodenum (23%), jejunum (20%) and ileum (30%) with crypt depths decreased by 31%, 27% and 33% in these gut regions. These effects were less profound in IGF-I pre-treated rats in which apoptosis was increased 48-fold, with SI length decreased by 7%, SI weight by 18% and total gut weight by 24%. 5-FU decreased villus height in the duodenum (23%), jejunum (20%) and ileum (30%) with crypt depths decreased by 31%, 27% and 33% in these gut regions. These effects were less profound in IGF-I pre-treated rats in which apoptosis was increased 48-fold, with SI length decreased by 7%, SI weight by 18% and total gut weight by 24%. 5-FU decreased villus height in the duodenum (23%), jejunum (20%) and ileum (30%) with crypt depths decreased by 31%, 27% and 33% in these gut regions. These effects were less profound in IGF-I pre-treated rats in which apoptosis was increased 48-fold, with SI length decreased by 7%, SI weight by 18% and total gut weight by 24%. 5-FU decreased villus height in the duodenum (23%), jejunum (20%) and ileum (30%) with crypt depths decreased by 31%, 27% and 33% in these gut regions. These effects were less profound in IGF-I pre-treated rats in which apoptosis was increased 48-fold, with SI length decreased by 7%, SI weight by 18% and total gut weight by 24%.

Keywords: Insulin-like growth factor-I; Mucosa; Chemotherapy; Intestinal injury; Rats

1. Introduction

Chemotherapeutic agents, such as 5-fluorouracil (5-FU), are commonly used in the treatment of many breast and intestinal cancers, acting by inhibition of cell division processes resulting in reduced cellular proliferation [1,2]. 5-FU targets rapidly dividing cells indiscriminately, such that cells lining the intestine and malignant cells are equally susceptible [3]. The resultant damage to the small intestinal epithelium manifests as a condition known as intestinal mucositis. Intestinal mucositis is a major cause of morbidity and mortality during
treatment or cancer [4] with symptoms that include nausea, dehydration, vomiting, diarrhoea, and in some cases serious bacterial infection that may be fatal [1]. Approximately 80% of patients receiving 5-FU therapy experience mucositis [5] with intestinal damage characterized by apoptosis of regenerative stem cells [6], reduced mucosal DNA, RNA, and protein content, blunted villi and crypts [7] and perturbed brush-border hydrolase activity [8].

Although a variety of strategies have been investigated which either prevent or treat mucositis, none of these approaches have been shown to be sufficiently effective [9]. However, there have been several recent reports of efficacy following treatment with growth factors. (reviewed in [10]). Pre-treatment with 5 mg/kg/day keratinocyte growth factor (KGF), a heparin-binding member of the fibroblast growth factor family, for 3 days has been demonstrated to increase mucosal thickness, indicated by increased villus height and crypt depth, that persisted during 5-FU chemotherapy suggesting that KGF could be therapeutically useful to lessen side effects of current cancer therapy regimens [11]. Other growth factors such as granulocyte-colony stimulating factor [12], interleukin-11 [13] and transforming growth factor-β1 [14] have been shown to reduce the severity of mucositis when applied as pre-treatments and/or in the repair phase. Insulin-like growth factor-I (IGF-I) is another candidate for potential therapeutic application in the context of intestinal mucositis.

The gastrointestinal tract has been identified as one of the most sensitive target tissues for IGF-I [15,16]. When administered systemically, IGF-I has been demonstrated to promote gut growth and maturation in normal rats [18,17] and to stimulate proliferation [18] and decrease apoptosis [19] in intestinal epithelia. Under these conditions such as the partially resected small bowel [20], experimental radiation enteritis [21] and inflammatory bowel disease [22], IGF-I has been shown to enhance repair of the small bowel. These responses suggest that IGF-I peptides have potential therapeutic applications to stimulate gut growth and repair. IGF-I increases growth of the small intestine in rats by increasing proliferation and decreasing apoptosis in the epithelium [23], though the precise mechanisms involved remain poorly understood. The regulation of IGF-I actions in the gastrointestinal tract remains unclear, however, in vivo evidence indicates that locally expressed IGF binding proteins (IGFBPs) may play a role [24].

Few studies have investigated the role of IGF-I and its application following damage to the bowel induced by chemotherapy agents. In one such study, Howarth et al. [25] studied the effects of IGF-I administration on the intestinal mucosa following damage by methotrexate, an antimetabolite used widely in cancer chemotherapy. Administration of IGF-I was found to improve regrowth of the damaged small intestine, with significant effects on villus height and crypt depth in the ileum [25]. However, although IGF-I is known to induce growth and mucosal repair of the bowel, it is unknown whether pre-treatment with IGF-I conveys any protection from bowel damage.

Since orally administered IGF-I is known to degrade rapidly in the gastric environment [26], the aim of the current study was to investigate whether pre-treatment with systemically delivered IGF-I could protect the small intestine from chemotherapy-induced mucositis. More specifically, we hypothesized that IGF-I administered before chemotherapy would increase tissue mass, thereby increasing functional surface area, hence maintaining integrity of the mucosal brush border and rendering the small bowel less susceptible to the damaging consequences of chemotherapy.

2. Materials and methods

2.1. Animals and experimental design

Male Sprague Dawley rats were maintained in Tewniplast metabolism cages for acclimatization and collection of baseline data. The environment of the animals was maintained at 25°C with a 12 h light/dark cycle. Rats had continual access, unless otherwise indicated, to water and a standard casein-based diet [27]. Thirty-two rats were randomly allocated into four treatment groups (Group 1: vehicle for 7 days followed by injection with saline; Group 2: IGF-I for 7 days followed by injection with saline; Group 3: vehicle for 7 days followed by injection with 5-FU; Group 4: IGF-I for 7 days followed by injection with 5-FU) (n = 8 rats per group). When average bodyweight had reached 135 g, each animal was anesthetized with isoflurane and an osmotic minipump (Alzet model 2002, Alza, Palo Alto, CA, USA) containing 0.1 M acetic acid vehicle or 4.3 mg/kg/day IGF-I (recombinant human IGF-I, GroPep Pty Ltd, Adelaide, South Australia), as used previously [25], was subcutaneously implanted in the suprascapular region. Following pump removal on day 7, animals received a single intraperitoneal injection of saline or 150 mg/kg 5-FU (Sigma, St. Louis, MO, USA) and killed 48 h later. Rats were injected with 50 mg/kg 5-bromo-2′-deoxyuridine (BrdU) (BrdU, DAKO, Carpinteria, CA, USA) 1 h prior to kill to label S-phase nuclei for studies of proliferation. BrdU is an analog of thymidine that is incorporated into DNA during the S phase of the cell cycle.

2.2. Collection of gut tissues

Rats were killed on day 9 by CO2 asphyxiation and cervical dislocation. Trunk blood was collected and placed into heparinized tubes and plasma separated by centrifugation and frozen at −80°C for later assay of
IGF-I levels and detection of IGF binding proteins (IGFBPs). The abdomen was opened surgically by a midline incision, and the duodenum separated from the jejunum by cutting at the Ligament of Treitz. The gut was removed intact and placed onto an ice-cold slab. The stomach, duodenum, jejunum, ileum, caecum, and colon were weighed empty of contents, and the lengths of the small intestinal components measured unstretched. Samples of duodenum, jejunum, and ileum were frozen at −80 °C for analysis of sucrase activity and fixed for 24 h in 10% v/v formalin fixative for histological processing. Other organs including the spleen, liver, thymus, heart, kidneys, epididymal fat pads and adrenals were weighed. This protocol followed the Australian Code of Practice for the Care and Use of Animals and was approved by the Animal Ethics Committee of the Adelaide Women’s and Children’s Hospital, North Adelaide, South Australia.

2.3. Total small intestinal sucrase activity determination

In vivo determination of sucrase activity by breath testing was utilized as an indication of brush border integrity. Breath tests were performed on days 0, 7, and 9 of the trial period. After collection of a baseline sample, rats were gavaged with 1 g/ml sucrose (naturally containing 13C) and breath samples collected at 30, 60, and 90 min after gavage. 13C-sucrose is converted to glucose and fructose by sucrase and absorbed across the gastrointestinal wall where it enters the bloodstream and is converted to 13CO2. Breath 13CO2 levels following the ingestion of 13C-sucrose represent the activity of sucrase and the digestive and absorptive capacity of the small intestine. Breath samples were analyzed for 13CO2 by an isotope ratio mass spectrometer (Europa Scientific, Crewe, UK) to determine the ratio of 13C/12C in each sample relative to the calcium carbonate primary standard (Pee-Dee Belemnite Limestone, SC, USA). The change in breath 13CO2 levels from baseline at each time point was calculated and the data expressed as area under the curve of breath 13CO2 levels over time.

Sucrase activity was measured in homogenates of jejunal and ileal tissues by methods described previously [28]. Briefly, jejunal homogenates were diluted 1/80 and 1/50 with 50 mM phosphate buffer containing Triton-X, and ileal homogenates diluted 1/50 and 1/30. Fifty microliters of 0.2 M sucrose was added to 50 μl of each homogenate and incubated at 37 °C for 30 min. The sucrose substrate is cleaved to its constituents, glucose and fructose by sucrase in the homogenates. Glucose production was then detected colorimetrically by measuring the optical density of samples at 490 nm (Dynatech MR7000 microplate reader, Dynatech, Denkendorf, Germany). Assays were performed in quadruplicate, corrected for background absorbency, and included relevant controls to allow for inter-assay variability.

2.4. Histological assessment

Segments of duodenum, jejunum, and ileum were placed in formalin fixative for 24 h and then transferred to 70% ethanol. For histological examination, transverse sections of 4 μm were stained with haematoxylin and eosin, and examined with a light microscope (Olympus BH-2, Tokyo, Japan). A semi-quantitative histological assessment of intestinal damage was utilized to obtain an overall score of damage severity [29]. Untreated rat intestinal tissue was used as a baseline reference to grade the histological criteria. Quantitative histological analysis was conducted on images acquired with a colour video camera (Sony Hyper HAD model SSC-DC50P, Japan) and digitised by Image-Pro Plus software (Media Cybernetics, Silver Springs, MD, USA). Average villus height and crypt depth measurements for each region were derived from 40 villi and cryps per section, respectively.

2.5. 5’ Bromo-2’-deoxyuridine immuno-staining

BrdU labeling was used as an index of cell proliferation in intestinal tissues. Immuno-staining of 4 μm sections of distal ileum was performed according to previously published methods [30]. For each animal, 10 crypt fields were examined under a light microscope and the total number of BrdU-labeled crypt cells and total crypt areas in that field were used to provide the BrdU-labeling density (cells/mm²) for each treatment group.

2.6. Apoptotic cell density measurement

Haematoxylin and eosin stained sections of ileal specimens were used to identify apoptotic cells. Morphological features of apoptosis include chromatin and cytoplasmic condensation, shrinkage from adjacent cells and apoptotic bodies forming through nuclear and cytoplasmic fragmentation [30]. In each section, 10 crypt fields were measured for the total crypt area and the total number of apoptotic cells present to calculate the apoptotic cell density (cells/mm²) as described in [30].

2.7. Plasma IGF-I levels

Plasma samples were assayed by a radioimmunoassay (RIA) as described in [25] to determine the total amount of IGF-I in the plasma of rats at kill. Plasma samples were diluted 1:4 in RIA buffer and assayed in triplicate.

2.8. Statistical analysis

Statistical comparisons were made using the Instat program V3.05 (Graph Pad, San Diego, CA, USA). For the semi-quantitative scoring of intestinal damage,
data are presented as medians and ranges and each region was compared statistically using the Kruskal–Wallis non-parametric analysis of variance (ANOVA), and where significance was identified ($p < 0.05$), the Tukey–Kramer multiple comparison test was used. For all other measurements, data are presented as mean ± standard error of the mean (SEM) and were analyzed by a one-way ANOVA and when the significance level was $p < 0.05$ a post hoc analysis of groups was performed using a Tukey’s test.

3. Results

3.1. Effects of 7 days IGF-I pretreatment

Mean initial bodyweights were 142.3 ± 5.6 g for the vehicle treated animals (Group 1) and 142.5 ± 5.6 g for the IGF-I treated rats (Group 2). At completion of the 7-day experimental period, bodyweight gain was 9% greater following IGF-I treatment (85.2 ± 1.2 g) compared to the vehicle-treated control group (78.2 ± 2.2 g, $p < 0.05$, Table 1). There were no significant differences in food and water intake or urine output between the IGF-I and vehicle treatments. Liver weight increased by 22% after IGF-I treatment (12.8 ± 0.35 g) compared to vehicle (0.5 ± 0.5 g, $p < 0.001$, Table 2), while the spleen (vehicle: 0.61 ± 0.05 g vs. IGF-I: 0.75 ± 0.03 g, $p < 0.001$) and thymus (vehicle: 0.77 ± 0.04 g vs. IGF-I: 0.94 ± 0.06 g, $p < 0.05$) weights were greater by 24% and 21%, respectively. There were no differences between treatment groups in the weights of the other organs (heart (vehicle: 0.97 ± 0.03 g vs. IGF-I: 1.03 ± 0.03 g); kidneys (vehicle: 2.02 ± 0.05 g vs. IGF-I: 2.08 ± 0.06 g); lungs (vehicle: 1.34 ± 0.09 g vs. IGF-I: 1.33 ± 0.05 g); adrenals (vehicle: 0.04 ± 0.01 g vs. IGF-I: 0.04 ± 0.01 g); epididymal fat pads (vehicle: 1.86 ± 0.14 g vs. IGF-I: 1.58 ± 0.14 g)).

Infusion of IGF-I for 7 days significantly increased small intestinal length by 11% (114.6 ± 1.2 cm (IGF-I) vs. 103.6 ± 3.1 cm (vehicle), $p < 0.05$), small intestinal weight by 21% (7.49 ± 0.45 g (IGF-I) vs. 6.77 ± 0.20 g (vehicle), $p < 0.001$) and total gut weight by 19% (9.72 ± 0.45 g (IGF-I) vs. 8.74 ± 0.23 g (vehicle), $p < 0.001$) compared with normal control animals (Fig. 1). There were no significant differences in sucrase activity as measured in vivo by breath testing on day 0 or day 7 of the experimental period (Fig. 2) or measured biochemically in homogenates of proximal jejunum (20% small intestine (SI)) or distal ileum (90% SI) between IGF-I and vehicle-treated animals (Fig. 2). Semi-quantitative histology scores and duodenal, jejunal and ileal villus heights and crypt depths, did not differ significantly between IGF-I treated rats and normal controls.

### Table 1

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Vehicle + saline</th>
<th>Vehicle + 5-FU</th>
<th>IGF-I + saline</th>
<th>IGF-I + 5-FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bodyweight change (g)</td>
<td>78.2 ± 2.2</td>
<td>62.5 ± 2.1b</td>
<td>85.2 ± 1.2c,d</td>
<td>67.1 ± 2.0b</td>
</tr>
<tr>
<td>Total food intake (g)</td>
<td>179.1 ± 4.6</td>
<td>173.6 ± 3.3</td>
<td>179.2 ± 2.7</td>
<td>165.9 ± 2.2a</td>
</tr>
<tr>
<td>Total water intake (ml)</td>
<td>234.5 ± 10.2</td>
<td>239.8 ± 7.7</td>
<td>247.0 ± 9.8</td>
<td>250.5 ± 11.8</td>
</tr>
<tr>
<td>Total urine output (ml)</td>
<td>120.6 ± 6.2</td>
<td>137.9 ± 9.1</td>
<td>122.0 ± 7.9</td>
<td>126.5 ± 9.7</td>
</tr>
</tbody>
</table>

Values are means ± SE ($n = 8$ per group). Comparisons were analysed using a one-way ANOVA and a Tukey’s test. Statistical significance is vs. vehicle + saline treatment group. ($p < 0.05$, b $p < 0.001$), vs. vehicle + 5-FU treatment (c $p < 0.001$) or vs. IGF-I + 5-FU treated group (d $p < 0.001$).

### Table 2

<table>
<thead>
<tr>
<th>Organ</th>
<th>Treatment group</th>
<th>Vehicle + saline</th>
<th>Vehicle + 5-FU</th>
<th>IGF-I + saline</th>
<th>IGF-I + 5-FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>Vehicle + saline</td>
<td>0.97 ± 0.03</td>
<td>0.97 ± 0.02</td>
<td>1.03 ± 0.03</td>
<td>0.96 ± 0.02</td>
</tr>
<tr>
<td>Liver</td>
<td>10.5 ± 0.51</td>
<td>9.97 ± 0.35</td>
<td>12.8 ± 0.35c,d,e</td>
<td>10.7 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.61 ± 0.05</td>
<td>0.33 ± 0.02c</td>
<td>0.75 ± 0.03e,d,f</td>
<td>0.37 ± 0.01c</td>
<td></td>
</tr>
<tr>
<td>Kidneys</td>
<td>2.02 ± 0.05</td>
<td>1.95 ± 0.05</td>
<td>2.08 ± 0.06</td>
<td>2.03 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>1.34 ± 0.09</td>
<td>1.40 ± 0.10</td>
<td>1.33 ± 0.05</td>
<td>1.38 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Thymus</td>
<td>0.77 ± 0.04</td>
<td>0.45 ± 0.02c</td>
<td>0.94 ± 0.06d,f</td>
<td>0.51 ± 0.03c</td>
<td></td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>EFP</td>
<td>1.86 ± 0.14</td>
<td>1.86 ± 0.24</td>
<td>1.38 ± 0.14</td>
<td>1.46 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

Values are means (g) ± SE ($n = 8$ per group). Comparisons were analysed by a one-way ANOVA, with a Tukey’s test. Statistical significance is vs. vehicle + saline treatment group (c $p < 0.05$, b $p < 0.01$, c $p < 0.001$), vs. vehicle + 5-FU treatment (d $p < 0.001$) or vs. IGF-I + 5-FU treated group (e $p < 0.01$, f $p < 0.001$). EFP: epididymal fat pads.
There were no statistically significant differences in muscularis thickness in the three regions of the small intestine analyzed after IGF-I treatment (data not shown). Similarly, IGF-I administration did not significantly affect either the numbers of apoptotic cells (Fig. 4) or the BrdU labeling index (Fig. 5) compared to normal controls. There was no significant difference in total plasma IGF-I levels in IGF-I treated animals compared to vehicle (49.1 ± 5.6 ng/ml (vehicle) vs. 51.8 ± 8.2 ng/ml (IGF-I)).

3.2. Effects of 5-FU administration

Although there were no significant differences in food and water intake and urine output between 5-FU treated and normal control groups, 5-FU treatment significantly reduced bodyweight gain by 20% over the 9 day experimental period, (78.2 ± 2.2 g compared to 62.5 ± 2.1 g) \((p < 0.001)\) (Table 1). Spleen weight decreased significantly by 46% after 5-FU treatment (vehicle: 0.61 ± 0.05 g vs. 5-FU: 0.33 ± 0.02 g, \(p < 0.001\)) compared to normal animals (Table 2), while thymus weight was reduced by 42% (vehicle: 0.77 ± 0.04 g vs. 5-FU: 0.45 ± 0.02 g, \(p < 0.001\)). There were no significant effects of 5-FU on the weights of any other organs. (heart (vehicle: 0.97 ± 0.03 g vs. 5-FU: 0.97 ± 0.02 g); kidneys (vehicle: 2.02 ± 0.05 g vs. 5-FU: 1.95 ± 0.05 g); lungs (vehicle: 1.34 ± 0.09 g vs. 5-FU: 1.40 ± 0.10 g); adrenals (vehicle: 0.04 ± 0.01 g vs. 5-FU: 0.04 ± 0.01 g); epididymal fat pads (vehicle: 1.86 ± 0.14 g vs. 5-FU: 1.86 ± 0.24 g) liver (vehicle: 10.5 ± 0.51 g vs. 5-FU: 9.97 ± 0.35 g)).

The single 5-FU injection significantly reduced small intestinal length and weight by 14% (88.9 ± 2.7 cm (5-FU) vs. 103.6 ± 3.1 cm (Saline), \(p < 0.001\)) and 30% (4.76 ± 0.08 g (5-FU) vs. 6.77 ± 0.20 g (saline), \(p < 0.01\)), respectively, compared with the corresponding saline-injected control (Fig. 1) whereas total gut weight decreased by 24% (6.67 ± 0.09 g (5-FU) vs. 8.74 ± 0.22 g (saline), \(p < 0.01\)). There was no significant difference in sucrase activity detected by breath analysis between vehicle and 5-FU treated animals, on day 0, day 7 or day 9 of the experimental period (Fig. 2). However, sucrase activity in the proximal jejunum (20% SI), assessed biochemically, was significantly affected by 5-FU injection (vehicle: 509 ± 18 nmol gluc/min/cm vs. 5-FU: 388 ± 22 nmol gluc/min/cm, \(p < 0.05\) (Fig. 2) although there were no significant effects evident in the distal ileum (90% SI) (vehicle: 42 ± 5 nmol gluc/min/cm vs. 5-FU: 34 ± 3 nmol gluc/min/cm) 5-FU treatment resulted in significant damage to all small intestinal regions when assessed by the semi-quantitative severity scoring system (all regions \(p < 0.001\)) (Table 3). Duodenal, jejunal and ileal villus heights and crypt depths were also significantly reduced compared to controls (Fig. 3). Duodenal villus height and crypt depth decreased by 23% (vehicle: 793 ± 8 \(\mu\)m vs. 5-FU: 611 ± 15 \(\mu\)m, \(p < 0.01\)) and 31% (vehicle: 202 ± 11 \(\mu\)m vs. 5-FU: 139 ± 4 \(\mu\)m, \(p < 0.001\)), respectively. Jejunal villus height and crypt depth decreased by 20% (vehicle: 685 ± 24 \(\mu\)m vs. 5-FU: 548 ± 11 \(\mu\)m, \(p < 0.001\)) and 27% (vehicle: 128 ± 4 \(\mu\)m vs. 5-FU: 93 ± 3 \(\mu\)m, \(p < 0.01\)), while ileal

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**Fig. 1.** Small intestine length (cm) (A), small intestine weight (g) (B), and total gut weight (g) (C) 2 days after intraperitoneal 5-fluorouracil (150 mg/kg) or saline injection in rats pre-treated with vehicle (0 mg/kg/day IGF-I) or 4.3 mg/kg/day IGF-I for 7 days. Bars are means ± SE (\(n = 8\) rats per group). Data were compared statistically using a one-way ANOVA and a Tukey's test. Statistical significance is vs. vehicle + saline treatment group (a: \(p < 0.01\), b: \(p < 0.001\)), vs. vehicle + 5-FU treatment (c: \(p < 0.05\), d: \(p < 0.001\)) or vs. IGF-I + 5-FU treated group (e: \(p < 0.001\)). SI: small intestine.
villus height and crypt depth decreased by 30% (vehicle: 473 ± 9 μm vs. 5-FU: 332 ± 10 μm, p < 0.001) and 27% (vehicle: 272 ± 19 μm vs. 5-FU: 199 ± 4 μm, p < 0.05). Two days after administration of 5-FU there was an 87-fold increase in crypt cell apoptosis (9 ± 2 apoptotic cells/mm² mucosa (saline) vs. 915 ± 249 apoptotic cells/mm² mucosa (5-FU), Fig. 4), and an 85% reduction in crypt cell proliferation as assessed by the BrdU labeling index (7532 ± 1290 BrdU labeled cells/mm² mucosa (saline) vs. 1151 ± 354 BrdU labeled cells/mm² mucosa (5-FU), Fig. 5). Plasma IGF-I levels were not significantly affected by 5-FU administration compared to saline-injected controls (49.1 ± 5.6 ng/ml (vehicle) vs. 49.5 ± 8.5 ng/ml (5-FU)).

Table 3

Effects of administration of 5-fluorouracil (150 mg/kg) or saline in rats pre-treated with 4.3 mg/kg/day IGF-I or vehicle for 7 days on the quantitative histological assessment of small intestinal damage

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Vehicle + saline</th>
<th>Vehicle + 5-FU</th>
<th>IGF-I + saline</th>
<th>IGF-I + 5-FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>0 (0–0)</td>
<td>12 (11–14)b</td>
<td>0 (0–0)c,d</td>
<td>8 (6–10)a</td>
</tr>
<tr>
<td>Jejunum 1 (10% SI)</td>
<td>0 (0–0)</td>
<td>16 (14–17)b</td>
<td>0 (0–0)c,d</td>
<td>12 (11–15)a</td>
</tr>
<tr>
<td>Jejunum 2 (25% SI)</td>
<td>0 (0–0)</td>
<td>15 (14–17)b</td>
<td>0 (0–0)c,d</td>
<td>12 (10–13)a</td>
</tr>
<tr>
<td>Jejunum/Ileum (50% SI)</td>
<td>0 (0–0)</td>
<td>12 (10–14)b</td>
<td>0 (0–0)c,d</td>
<td>10 (9–13)a</td>
</tr>
<tr>
<td>Ileum (75% SI)</td>
<td>0 (0–0)</td>
<td>12 (10–13)b</td>
<td>0 (0–0)c,d</td>
<td>10 (9–12)a</td>
</tr>
<tr>
<td>Ileum (90% SI)</td>
<td>0 (0–0)</td>
<td>11 (10–12)b</td>
<td>0 (0–0)c,d</td>
<td>10 (9–11)a</td>
</tr>
</tbody>
</table>

Values are medians. The range of data is indicated in parentheses (n = 8 per group). Comparisons were analysed using the Kruskal–Wallis non-parametric ANOVA and the Dunn’s test. Significance is vs. vehicle + saline treatment group (a: p < 0.05, b: p < 0.01), vs. vehicle + 5-FU treatment (c: p < 0.001) or vs. IGF-I + 5-FU treated group (d: p < 0.05). SI: small intestine.
3.3. Effects of IGF-I pre-treatment on 5-FU induced damage

IGF-I pre-treatment could not prevent the reductions in bodyweight gain induced by 5-FU as the combined treatment resulted in a bodyweight gain which was reduced by 14% (67.1 ± 2.0 g) over the 9-day experimental period compared to vehicle-pre-treated and saline-injected normal controls (78.2 ± 2.2 g), and was not significantly different from the 5-FU treated group (62.5 ± 2.1 g) (Table 1). There were no significant effects on the weights of the visceral organs between IGF-I and vehicle pre-treated rats receiving 5-FU (Table 2).

Total gut weight was significantly decreased by 15% by the combination of IGF-I pre-treatment and 5-FU injection (7.49 ± 0.19 g, p < 0.001) compared to normal controls receiving vehicle pre-treatment followed by saline injection (8.74 ± 0.22 g). Although the combination of IGF-I pre-treatment and 5-FU injection decreased small intestinal weight similarly by 18% (5.51 ± 0.16 g, p < 0.001), compared to normal controls (6.77 ± 0.20 g), small intestinal length was not significantly affected (Fig. 1). Nevertheless, the combination of IGF-I pre-treatment and 5-FU injection increased total gut weight by 16% (7.74 ± 0.12 g) compared to rats receiving vehicle pre-treatment and 5-FU injections (6.67 ± 0.09 g, p < 0.05). Small intestinal weight was increased by 12% in rats pre-treated with IGF-I and injected with 5-FU (5.33 ± 0.11 g) compared to vehicle pre-treatment and 5-FU injection (4.76 ± 0.08 g, p < 0.05) although small intestinal length was not significantly affected.

There was no significant difference in total sucrase activity as measured by breath testing between the combination of IGF-I and 5-FU treatment and either normal controls or vehicle pre-treated rats receiving 5-FU,
on day 0, day 7 or day 9 of the experimental period (Fig. 2). Similarly, IGF-I pre-treatment could not prevent 5-FU induced reductions of sucrase activity as measured biochemically in both proximal jejunum and distal ileum (Fig. 2).

IGF-I pre-treatment could not reduce the adverse effects of 5-FU on the small intestinal regions as assessed by the semi-quantitative severity score system (Table 3).

However, quantitative villus height and crypt depth measurements showed that IGF-I pre-treatment partially protected the small intestine. Compared to 5-FU treated controls (villus height: $611 \pm 15\mu m$), pre-treatment with IGF-I increased duodenal villus height by 19% ($727 \pm 28\mu m$, $p < 0.05$), accompanied by a trend towards increases in villus height and crypt depth in the jejunum and ileum (Fig. 3). While IGF-I pre-treatment
did not affect crypt cell proliferation as assessed by BrdU labeling (Fig. 5) it considerably diminished apoptosis from an 87-fold increase (915 ± 249 apoptotic cells/mm² mucosa (5-FU)) to a 48-fold increase (432 ± 104 apoptotic cells/mm² mucosa (IGF-I)), although statistical significance was not attained (Fig. 4). Moreover, in 5-FU treated animals, there was also no significant difference in plasma IGF-I levels between vehicle pre-treated and IGF-I pre-treated rats. (49.5 ± 8.5 ng/ml (5-FU with vehicle pre-treatment) vs. 40.5 ± 6.1 ng/ml (5-FU with IGF-I pre-treatment)).

4. Discussion

There have been several investigations describing the potential therapeutic benefits of recombinant growth factors in the setting of chemotherapy-induced mucositis (reviewed in [10]). However, these studies have been largely confined to EGF [8] and KGF [11]. Indeed, KGF is currently undergoing clinical trial as a therapeutic modality for mucositis. Nevertheless, few of these experimental studies have focused on the potential for growth factors to act as a preventative, or prophylactic therapy for mucositis. Efficacy, however, has not been restricted to individual growth factors, and has been reported following oral administration of an extract of growth factors derived from bovine cheese whey [29]. Since receptors for IGF-I are strongly expressed in the intestinal crypts, [31] and exogenously-administered IGF-I has resulted in potent intestino-trophic responses [15,20,21], and mucosal repair [22,25] we investigated the capacity for IGF-I to protect the intestine from damage utilizing a model of chemotherapy-induced intestinal mucositis.

In the current study, a single dose of IGF-I, only, was investigated; selected on the basis of previous studies in which potent intestine-trophic effects had been described [15,17]. IGF-I infusion did not alter food or water intake, consistent with experimental studies associated with administration of other intestine-trophic growth factors including EGF [8], KGF [11] and more recently, glucagon-like peptide-2 (GLP-2) [32]. The increased bodyweight gain over the experimental period was consistent with previous reports of exogenous IGF-I administration to rats [15,17]. 5-FU chemotherapy resulted in a decrease in body weight. However, pre-treatment with IGF-I did not abrogate this effect. This is in contrast to findings reported with KGF [11]. Farrell et al. [11], utilizing a more severe model of mucositis in the mouse, described a significant amelioration of weight loss after intestinal injury. However, the two studies are not directly comparable, since the KGF study employed a more complex chemotherapy regimen associated with multiple dosing of 5-FU and methotrexate in combination with radiotherapy. Future studies could benefit from exploring the dose-responsive effects of IGF-I, and the possible use of IGF-I analogs that bind poorly to IGF binding proteins, such as des (1–3) IGF-I and long-R²-IGF-I that have previously demonstrated greater potency than native IGF-I with respect to intestinotrophism [15].

In the current study, small intestinal length was maintained in chemotherapy-treated rats pre-treated with IGF-I, consistent with previous studies identifying IGF-I as one of the few biological agents capable of stimulating linear growth of the intestine [17], and hence, presumably optimizing digestive function during chemotherapy. Total gut weight was increased by prior treatment with IGF-I, and sustained 48 h after chemotherapy. However, this effect was less pronounced in the small intestine, the site of maximal injury. Indeed, this result was consistent with a study of prophylactic KGF administration [11] that also failed to maintain small bowel weight after chemotherapy.

5-FU administration significantly reduced sucrase activity in the jejunum and decreased overall sucrase activity by 22% when assessed non-invasively in the breath. However, prior infusion of IGF-I for 7 days had no demonstrable effect on sucrase activity by either method when assessed 48 h after cessation of IGF-I administration. Brush border enzyme activity would appear to be a sensitive indicator of chemotherapy-induced mucositis and response to therapeutic intervention. Both EGF [33] and IGF-I [25] have been reported to restore brush border hydrolase activity after chemotherapy. However, administration of EGF prior to 5-FU-induced mucositis [34] has actually been reported to increase brush border damage and hence, the incidence of mucositis, a finding attributed to an increased susceptibility to the stomatotoxic effects of 5-FU by an increase in the rate of basal cell proliferation induced by EGF. Together these findings identify the time-interval between cessation of growth factor administration and commencement of chemotherapy as an important determinant of epithelial integrity and the severity of mucositis.

In the current study, IGF-I pre-treatment partially attenuated 5-FU induced reductions in villus height and crypt depth in all regions of the small intestine studied, with maximal growth localized to the jejunum, the region known to be most responsive to exogenous IGF-I [15,17]. To this end, the capacity for IGF-I to ameliorate the atrophic properties of certain chemotherapy drugs is similar to KGF that has been reported to prevent villus atrophy and improve crypt survival in the intestine of chemotherapy-treated mice (reviewed in [35]). Although a sustained increase in mucosal thickness has been reported following chemotherapy in mice pre-treated with KGF [11], more comprehensive, time-course studies are required to provide this information for IGF-I. Should further studies identify IGF-I as a potential prophylactic therapy for intestinal mucositis, confirming its safety and efficacy in a neoplastic setting.
will be vital. Indeed, Gibson et al. [36] have reported only a modest intestinal growth, and no protection from mucositis in studies of prophylactically-administered KGF to tumour-bearing rats, despite a positive effect on reducing tumour size. Further studies of IGF-I pre-treatment in tumour-bearing rats could therefore, potentially, prove rewarding.

IGF-I has previously been demonstrated to increase proliferation of crypt cells in the small intestine [37,38]. However, proliferation was not altered by IGF-I in the present study. Since 47 h had elapsed between the cessation of IGF-I infusion and administration of the BrdU proliferation marker, it was possible that the proliferative properties of IGF-I had not been sustained for this period of time, once again defining the value of conducting a comprehensive time-course following cessation of IGF-I administration in a chemotherapy setting. Nevertheless, proliferation was decreased by 85% after 5-FU administration similar in magnitude to previous reports with the related anti-metabolite, methotrexate [25]. In the current study, IGF-I pre-treatment slightly attenuated this decrease, consistent with results reported for KGF, indicating some protection by both factors from 5-FU chemotherapy [11]. Similarly, IGF-I pre-treatment may have conferred partial protection from intestinal damage by a reduction in the incidence of chemotherapy-induced apoptosis, although the magnitude of this effect was not great. Interestingly, a synergistic relationship between KGF and the chemotherapy drug, methotrexate, has been reported in studies involving tumour-bearing rats [36]. Accordingly, apoptosis would be an important parameter to define in further experimental studies of IGF-I and chemotherapy in the cancer setting.

We conclude that administration of IGF-I prior to chemotherapy only partially attenuated features of intestinal mucositis. Further time-course studies are warranted to determine the (a) optimal dose of prophylactically-administered IGF-I, and (b) optimal time interval between cessation of IGF-I administration and commencement of chemotherapy, required to reduce the severity of intestinal mucositis. Subsequent studies could further address the safety and potential benefits of prophylactic IGF-I therapy by assessing indices of mucositis and tumour growth in tumour-bearing animals.

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