The effects of keratinocyte growth factor in preclinical models of mucositis


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Abstract. The epithelium of the oral cavity and small intestine of the gastrointestinal tract have a high rate of cell renewal and as such, are sensitive to cytotoxic therapies that kill rapidly dividing cells. Mucositis is a complication of cancer therapy where impairment of the regenerative capacity of the epithelium leads to atrophy, ulceration and a loss of barrier function. Keratinocyte growth factor (KGF) is an epithelial cell-specific growth and differentiation factor that is trophic for the mucosal epithelium of the gastrointestinal tract. In this study, KGF in normal animals caused epithelial thickening in the squamous epithelium of the oral cavity and increased crypt depth and villus height of the small intestine. It also appeared to regulate gene expression in these tissues including that of some antioxidant enzymes and intestinal trefoil protein. KGF has been shown to be efficacious in several preclinical models of mucositis where KGF pretreatment reduced weight loss typically seen during and after the course of therapy and significantly improved survival. At a tissue level KGF reduced atrophy, accelerated regrowth, and decreased ulcer formation of the oral epithelium after irradiation, and improved crypt survival and prevented villus atrophy in the small intestine of irradiated or chemotherapy-treated mice. Preliminary studies suggest that its efficacy may be partly a consequence of the growth and differentiation effect, and also partly due to regulation of the expression of genes that play a role in mucosal protection. These data suggest that KGF may be useful for the prevention or treatment of mucositis in patients treated with regimens of cancer therapy that have gastrointestinal toxicity.

Keywords: KGF, mucositis, epithelium, radiation, gastrointestinal tract.

KERATINOCYTE GROWTH FACTOR: IN VIVO ACTIVITIES

Effects in the gastrointestinal tract in normal animals

KGF is a growth factor for which epithelial cell specificity was initially identified in in vitro assays (Rubin et al. 1989; Finch et al. 1989). Subsequent studies in normal animals showed that it had growth and differentiating activities in a variety of epithelial tissues in vivo including the gastrointestinal tract. These activities included a trophic effect on the glandular epithelium...
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of the gastrointestinal tract including stomach and large and small bowel, a trophic effect on the
squamous forestomach (Housley et al. 1994), stimulation of hepatic growth (Housley et al. 1994)
and lipid metabolism (Nonogaki et al. 1995), and ductal proliferation in the pancreas (Yi et al.
1994). These activities are probably direct effects of KGF in these tissues because transcript for
the receptor, a splice variant of the fibroblast growth receptor II, is expressed in the epithelium
along the length of the gastrointestinal tract (Housley et al. 1994). KGF is also expressed in
the same tissues (Housley et al. 1994) and in situ hybridization studies have shown that the transcript
is found in cells adjacent to the epithelium during development and in adults, suggesting that
this growth factor–receptor ligand system functions in a paracrine manner to stimulate tissue
growth during development and to maintain function in adults (Werner 1998).

More detailed analysis of the effects of administration of pharmacological doses of KGF to
animals shows striking activities in the upper aerodigestive tract and the small intestine. These two
regions of the gastrointestinal tract are lined by a continuously renewing epithelium where rapid
proliferation of stem or germinal cells is followed by differentiation, migration and subsequent
loss of postmitotic cells. The upper aerodigestive tract has a squamous epithelium composed of
keratinocytes organized into layers; a basal germinal layer, the spinous layer, and the upper granular
layer, all of which in rodents are protected by a keratinized surface layer. The epithelium of the upper
aerodigestive tract responds robustly to KGF; this was shown in studies where 5 mg/kg KGF was
administered daily to mice for 3 days, the tongues resected 24 h after the last dose and processed
for light and electron microscopic morphometry. Examination of the toluidine blue-stained semi-
thin sections showed a marked increase in the overall epithelial thickness. In addition to this increased
cellularity, there was a significant augmentation of the keratohyalin granule component of the
granular layer, with a commensurate increase in the density of the keratinized layer (Fig. 1).

In these tongues, measurement showed a two-fold increase in thickness of the nucleated layers
of the ventral epithelium as well as a four-fold increase in the keratohyalin granule area (Fig. 2). This
increase in area was due to increased numbers of individual granules in combination with an increase
in the average size of the granules. In similar experiments, animals were injected with bromodeoxy-
uridine (BrdU), a marker of proliferation, 1 h before necropsy, and processed for BrdU immuno-
staining (Housley et al. 1994) and haematoxylin and eosin staining of paraffin sections. It was
seen that the KGF-treated mice also had increased numbers of BrdU positive cells and a correlated
increase in mitotic figures (Fig. 2), both of which were confined to the basal layers of the epithelium.

Studies by Frank et al. (1997) showed that KGF specifically upregulated the expression of a
non-selenium-dependent glutathione peroxidase (nsGPX) in human keratinocytes in culture. To

Figure 1. Semi-thin sections of the ventral surface of the mouse tongue in normal, control mice and mice that were
treated with 5 mg/kg KGF for 3 consecutive days. Increased cellularity as well as increased granule size and number
are evident.

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determine whether KGF had effects on antioxidant enzymes in vivo, in situ hybridization (Wilcox 1993) was performed with probes for the mRNA of nsGPX and glutathione-S-transferase (GST). In mice treated with 5 mg/kg/day of KGF for 3 days, the hybridization studies showed increased signal for the transcript of both genes (Fig. 3). Gene regulation of other antioxidant enzymes...
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The above observations are interpreted to mean that KGF has proliferative effects on oral epithelium as well as effects on postmitotic cells, and suggests that there are multiple mechanisms that account for the increase in thickness measured in this epithelium in response to KGF treatment. The rate of tissue generation appears to be increased as more cells are proliferating, but also the rate of cell loss due to desquamation at the surface may be decreased by the KGF treatment due to enhanced desmosomal attachment (Farrell et al. 1999) and keratohyalin protection. Additionally, KGF appears to regulate antioxidant genes in vivo, suggesting that oxidative tissue stress induced by irradiation may be abrogated by KGF treatment.

The small intestine is also sensitive to KGF and, like the squamous mucosa, KGF has growth and differentiating effects in this segment of the gastrointestinal tract. Housley et al. (1994) showed that administration of KGF to rats causes increases in duodenal crypt depth and increased numbers of goblet cells. To characterize the tissue response further, mice were treated with 5 mg/kg of KGF for 3 days, and injected with BrdU 1 h prior to necropsy, when the small intestine was removed and divided into segments that were flushed with saline, blotted and weighed. Tissues were processed for light and electron microscopy, as well as for immunostaining and morphometry; the data are presented in Table 1. Wet weights of the intestinal segments were increased by the KGF treatment and morphometric measurement showed that both crypt depth and villus height were also increased (Farrell et al. 1998). Further morphometric analysis of villus features such as microvillus length showed that simultaneous changes in these parameters in response to the KGF treatment lead to a 45% increase in total absorptive surface area. These morphometric measures are consistent with the reported increases in brush border enzyme activities in KGF-treated mice (Russell et al. 1997).

**Table 1.** Microvillus measurements were made on electron micrographs. The total absorptive area of the jejunum was calculated as the product of microvillus surface area and density on enterocytes, and of the villus surface area and density in a mm$^2$ of en face jejunum. The final product is expressed as mm$^2$ absorptive area/mm$^2$ jejunum wall.

<table>
<thead>
<tr>
<th>Tissue wet weight</th>
<th>Control (mean ± SEM)</th>
<th>KGF (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum (g)</td>
<td>0.31 ± 0.01</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>Jejunum (g)</td>
<td>0.52 ± 0.03</td>
<td>0.62 ± 0.03</td>
</tr>
<tr>
<td>Ileum (g)</td>
<td>0.18 ± 0.01</td>
<td>0.21 ± 0.01</td>
</tr>
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<table>
<thead>
<tr>
<th>Jejunal villi</th>
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<tbody>
<tr>
<td>Length (µm)</td>
<td>473 ± 38</td>
<td>538 ± 27</td>
</tr>
<tr>
<td>Maximum diameter (µm)</td>
<td>160 ± 15</td>
<td>194 ± 32</td>
</tr>
<tr>
<td>Minimum diameter (µm)</td>
<td>99 ± 10</td>
<td>98 ± 11</td>
</tr>
<tr>
<td>Density (villi/mm$^2$)</td>
<td>35 ± 5</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>Enterocytes (/mm villus)</td>
<td>167 ± 23</td>
<td>169 ± 16</td>
</tr>
</tbody>
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<tr>
<th>Enterocyte Microvilli</th>
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<tbody>
<tr>
<td>Microvilli density (/µm$^2$ enterocyte)</td>
<td>48 ± 7</td>
<td>49 ± 3</td>
</tr>
<tr>
<td>Diameter (nm)</td>
<td>122 ± 7</td>
<td>122 ± 6</td>
</tr>
<tr>
<td>Length (nm)</td>
<td>1180 ± 251</td>
<td>1489 ± 319</td>
</tr>
<tr>
<td>Total absorptive area (mm$^2$)</td>
<td>223</td>
<td>320</td>
</tr>
<tr>
<td>Goblet cells (/mm villus)</td>
<td>9 ± 2</td>
<td>22 ± 2</td>
</tr>
</tbody>
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As in the rat, there were also more goblet cells in KGF-treated mice than in saline-treated controls. The increase was apparent at all levels of the intestine and counts made in cross-sections at the level of the jejunum showed a threefold increase (Table 1). Goblet cells produce mucins that are thought to function as a luminal barrier in the small and large intestines. They also produce intestinal trefoil protein (ITF), which is a member of a family of small protease-resistance proteins shown to contribute to gastrointestinal defence and repair (Playford et al. 1996). In studies where rats were treated with 5 mg/kg/day of KGF for 6 days, in situ hybridization (Wilcox 1993) showed that the signal for ITF was considerably higher than in control, saline-treated rats (Fig. 4), presumably due to the increased numbers of goblet cells, although a concomitant increase in individual cell production of ITF can not be ruled out. These growth and differentiating effects of KGF, including surface area increase and elevated expression of mucin and related proteins, suggest that KGF may enhance the absorptive and barrier functions of the small intestine.

Effects in the upper aerodigestive tract after radiation injury
Under normal physiological conditions, steady state is maintained in squamous epithelium by continuous production of cells in the germinal layers as the surface layers are eroded by desquamation. Radiation disrupts this steady state by impairment of the reproductive capacity of the tissue, leading to atrophy and ulceration. This has been shown also in mouse tongue (Dörr & Kummermehr 1992) where there was a dose-dependent effect of radiation on frequency and confluency of ulceration on the ventral surface. In this model, it was shown that treatment of the mice with KGF resulted in a significant reduction in oral mucosal ulceration (Dörr et al. 2000). In a different mouse model of radiation-induced epithelial atrophy, KGF was able to reverse the trend towards atrophy following single or multiple dose radiation (Farrell et al. 1999). Pre- and post-treatment schedules were evaluated and found to have activity in this model, with the most robust effect observed in the mice that received KGF after irradiation. This was attributed to multiple mechanisms including enhanced proliferation and effects on desmosomes and kerato-hyalin in postmitotic cells. Importantly, it was demonstrated that injured epithelium retained the ability to respond to KGF. Further studies are underway to evaluate the role of antioxidant enzymes as preliminary studies have shown that KGF does regulate the expression of some of

Figure 4. In situ hybridization of rat small intestine showing mRNA expression for ITF. These photomicrographs show ITF that is normally expressed in the goblet cells of the small intestinal epithelium is apparently up-regulated in response to systemic administration of 5 mg/kg for 6 days.
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Figure 5. The effect of KGF pretreatment on the small intestines of mice irradiated with 12 Gy. While these measures of tissue wet weight, DNA and protein are reduced to less than half of that in normal, unirradiated mice (data not shown), the mice pretreated with KGF show that these measures of growth are improved relative to the radiated group.

Effects in the small intestine after radiation injury
Radiation also kills rapidly dividing cells in the crypts of the small intestine, impairing reproductive capacity and leading to depopulation of the villus. This results in a wide range of symptoms from mild diarrhoea to sepsis. KGF given to mice as a pretreatment before abdominal or total body irradiation has been shown to improve crypt survival and animal mortality (Kahn et al. 1997). In experiments examining tissue effects in the small intestines of irradiated mice, animals were treated with KGF at 5 mg/kg/day for 3 days prior to receiving a single dose of 12 Gy. The tissue wet weight, DNA, and protein were all increased in the KGF-treated animals relative to irradiated controls (Fig. 5), demonstrating that KGF pretreatment spared the small intestine from damage. This correlates with crypt count data showing that crypt survival, and hence growth capacity, was increased in KGF-treated mice compared to control, irradiated mice (Farrell et al. 1998), and suggests that KGF may also impact positively on mucosal functions such as nutrient absorption.

The mechanisms of action of these effects are as yet unknown. It may be a result of the pretreatment trophic effect where increased numbers of crypts and larger villus surface area could enhance reproductive and functional capacity, thereby sustaining the mice through cytotoxic injury. Alternatively, as radiation and chemotherapy kill stem cells via apoptosis, it may be that there is an antiapoptotic effect of KGF. Studies are underway to determine whether KGF affects radiation-induced apoptosis in crypts, and whether KGF influences gene regulation of the apoptosis pathway. An alternative explanation is that KGF given prior to irradiation stimulates stem cells to divide, thus increasing the number of radiation target cells (Potten et al. 2001). With the same level of fractional stem cell killing, a greater number of stem cells will survive in the KGF-treated groups, increasing the efficiency of the regeneration process. KGF has been shown to enhance the glutathione redox state in rat intestinal mucosa during malnutrition (Jonas et al. 1998), and it may be that KGF will reduce radiation-induced tissue oxidative stress also.

CONCLUSION

The rapidly proliferating epithelium of the oral and intestinal mucosae are sensitive to radiation and other cyto-ablative agents that compromise regenerative capacity, leading to nutrition and barrier impairment. The potent growth, differentiation and protective effects of KGF on the gastrointestinal tissues indicate that KGF may be useful for treatment of radiation injury of the gastrointestinal tract. Its effects are directed against epithelial cells where the receptor is expressed, and this specificity for epithelial cells may be an advantage that therapies such as aminothiols, interleukins or TGFβs lack. Data so far suggest that KGF could potentially be used either prior to therapy in order to prevent oral and intestinal toxicity, or after radiation exposure where it has been shown that KGF appears to facilitate the regrowth of the oral epithelium. Further work will be important to understand the mechanisms of the effects of KGF in normal tissues, as well as the interaction of KGF with cyto ablative therapy, so that the clinical application of this growth factor leads to successful treatment of cyto-ablative injury.

REFERENCES


© 2002 Blackwell Science Ltd, Cell Proliferation, 35 (Suppl. 1), 78–85.


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