Teduglutide ([Gly2]GLP-2) protects small intestinal stem cells from radiation damage

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Abstract. Glucagon-like peptide-2 and its dipeptidyl peptidase (DP-IV) resistant analogue teduglutide are trophic for the gastrointestinal epithelium. Exposure increases villus height and crypt size and results in increased overall intestinal weight. As these effects may be mediated through stimulation of the stem cell compartment, they may promote intestinal healing and act as potential anti-mucositis agents in patients undergoing cancer chemotherapy. A study was initiated to investigate the protective effects of teduglutide on the murine small intestinal epithelium following gamma-irradiation using the crypt microcolony assay as a measure of stem cell survival and functional competence. Teduglutide demonstrated intestinotrophic effects in both CD1 and BDF1 mouse strains. In BDF1 mice, subcutaneous injection of GLP-2 or teduglutide (0.2 mg/kg/day, b.i.d.) for 14 days increased intestinal weight by 28% and resulted in comparable increases in crypt size, villus height and area. Teduglutide given daily for 6 or 14 days prior to whole body, gamma-irradiation significantly increased crypt stem cell survival when compared with vehicle-treated controls. The mean levels of protection over a range of doses provided protection factors from 1.3 to 1.5. A protective effect was only observed when teduglutide was given before irradiation. These results suggest that teduglutide has the ability to modulate clonogenic stem cell survival in the small intestine and this may have a useful clinical application in the prevention of cancer therapy-induced mucositis.

INTRODUCTION

The epithelial lining of the intestinal tract is a constantly renewing tissue with rapid rates of cell division. For example, a typical proliferative cell in the mouse small intestine has a cell cycle of 12 h. The cells are generated from lineage ancestor cells, stem cells, located towards the crypt base that have a slower cell cycle time of approximately 24 h. The initial cell divisions of the stem cell generate daughter cells, which mature, divide further and differentiate into various functional cell types. However, if the tissue is damaged, some of these daughter cells may retain

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some stem cell functionality, that is, they are potential stem cells. In the mouse small intestine there are 4–6 linear ancestor stem cells per crypt and a total of approximately 32 cells capable of crypt regeneration [clonogenic (potential) stem cells] and a further (about 120) dividing transit cells with no stem cell attributes (Potten *et al.* 1997b; Potten 1998; Pritchard *et al.* 1998; Marshman *et al.* 2002).

The length of the cell cycle at each of these stages is strictly controlled, as is the number of self-maintaining cell divisions controlling the number of stem cells per crypt. Many growth factors, cytokines and hormones have been either proven or suggested to modulate these parameters. The peptide hormone glucagon-like peptide-2 (GLP-2) is a product of one of the differentiated cell types, epithelial endocrine cells, and exhibits trophic effects on the gastrointestinal (GI) tract (Drucker *et al.* 1996, 1997; Tsai *et al.* 1997a; Xiao *et al.* 1999).

In response to luminal nutrients, GLP-2 is secreted by the endocrine L-cell, primarily in the lower gastrointestinal tract (Drucker *et al.* 1997; Xiao *et al.* 1999). GLP-2 is a member of the glucagon family of peptides, and acts by influencing proliferation rates, the size of individual crypts and villi and the overall intestinal mass, possibly by acting via a neuronal receptor pathway (Bjerknes & Cheng 2001). Over-expression of GLP-2 can induce mucosal hypertrophy (Gleeson *et al.* 1971; Bloom 1972; Stevens *et al.* 1984; Bristol & Williamson 1988; Byrne *et al.* 2001). Similarly, chronic dosing with exogenous GLP-2 increases mucosal mass and intestinal wet weight (Drucker *et al.* 1996, 1997; Tsai *et al.* 1997a,b; Litvak *et al.* 1998). The intestinotrophic effects of GLP-2 are mediated by the GLP-2 receptor (GLP-2R), a member of the superfamily of G-protein coupled receptors. GLP-2R is also most closely related to the GLP-1 and glucagon receptor gene subfamily (Munroe *et al.* 1999).

GLP-2 and teduglutide ([Gly2]GLP-2) promote growth and repair of GI epithelium in models of damage and disease, including enhancing an adaptive response in rats undergoing massive intestinal resection and reducing intestinal hypoplasia resulting from total parenteral nutrition use (Chance *et al.* 1997; Scott *et al.* 1998; Chance *et al.* 2000). Teduglutide also protects against the deleterious effects of dextran-sulphate induced colitis in mice, and reduces NSAID-induced enteritis and chemotherapy-induced intestinal damage (Boushey *et al.* 1999; Drucker *et al.* 1999; Boushey *et al.* 2001). In *ex vivo* studies, teduglutide enhances barrier function across the small intestine by reducing tissue conductance and macromolecule flux (Benjamin *et al.* 2000; Cameron *et al.* 2003).

A major application for any agent that may aid intestinal wound healing is for facilitating repair of the damage after radiation or drug exposure (mucositis). Oral mucositis and gastrointestinal mucositis are common, debilitating, adverse effects of cancer chemotherapy and result in a poor quality of life for patients. Anti-cancer drugs damage normal tissue stem cells, which results in a progressive loss of epithelium leading to ulceration. The symptoms of oral mucositis include significant pain and discomfort, frequently requiring diamorphine for relief. Gastrointestinal mucositis manifests as nausea, anorexia, loss of electrolyte balance, diarrhoea and bacterial infection. If severe, intestinal damage can be lethal. Effective protection of the normal tissue stem cells, or enhanced regeneration from stem cells, would significantly improve quality of life and may enable dose enhancement and, hence, improvement of cure rate.

We have examined the effects of GLP-2 and teduglutide, a peptide analogue which has reduced susceptibility to enzymatic degradation by dipeptidyl peptidase DP-IV on cell turnover (proliferation and apoptosis), crypt and villus size (height, area, cellularity), and we have specifically related these effects on the crucial stem cells. We also examined the effects of GLP-2 and teduglutide on survival of the normal intestinal epithelium and its stem cells following cytotoxic insult. The crypt microcolony assay was used to measure the number of regenerating crypts, and hence functional stem cells, post-irradiation (the cytotoxic model used in this study).

By implication, it is possible that such agents may have effects on the crucial stem cells and may have other potential wound healing applications in the GI tract.

MATERIALS AND METHODS

Animals

Male adult BDF1 or CD1 mice between the ages of 10 and 12 weeks were used and housed under conventional conditions with controlled light, humidity and temperature. A 12-h light cycle was maintained in the rooms, with the lights on at 06.00 h. All experiments were performed within the regulations and guidelines of the Home Office (Scientific Procedures) 1986 Act, UK. CD1 mice were used as a reference model based on previous studies performed using this mouse strain (Drucker *et al.* 1997). BDF1 mice were used for the radiation studies as extensive earlier studies have been performed using this strain. (Potten & Hendry 1985; Potten 1995, 1996; Potten *et al.* 1997a; Farrell *et al.* 1998; Booth *et al.* 2000) BDF1 mice of 10–12 weeks weighed on average 25 g. CD1 mice at 10–12 weeks weighed on average 32.5 g.

Reagents

GLP-2 and teduglutide ([Gly²]GLP-2) were supplied by Allelix Biopharmaceuticals Inc. (Canada), now NPS Pharmaceuticals. Teduglutide was synthesized by NPS Pharmaceuticals (Mississauga, ON, Canada). Purity was assessed by reverse-phase HPLC with a final peptide purity of 98.2%. Rat GLP-2 (Benjamin *et al.* 2000; Potten *et al.* 2001) was synthesized by California Peptide Company (Napa, CA, USA). Amino acid and HPLC analysis confirmed final peptide purity of 100%. Functional activity of both peptides was confirmed by increased *in vitro* cAMP accumulation following rat GLP-2R activation, recombinantly expressed in 293EBNA cells. Estimated EC₅₀ determinations for GLP-2 and teduglutide were a 1.0 ± 0.2 and 1.20 ± 0.06 nM, respectively. The GLP-2 or teduglutide were weighed and dissolved in saline prior to use in any animal experiment. Doses administered to mice varied depending on the nature of the experiment, but for all experiments BDF1 mice were administered GLP-2 or teduglutide by subcutaneous (s.c.) injection. Agents were delivered twice daily (b.i.d.) at 12-h intervals for periods ranging from 4 to 14 days. In all experiments, vehicle control groups received the same volumes of saline as were used to administer GLP-2 or teduglutide. For the histometric studies, the mice received either GLP-2 or teduglutide (0.2 mg/kg/day, twice a day, b.i.d.) for 14 days. Alterations to the standard administration schedule and dose are described in detail for the individual experiments and in Table 1.

Irradiation

A caesium 137 gamma irradiator that delivers whole body ionizing radiation at a dose rate of 3.5 Gy per minute was used. Radiation was used as the cytotoxic model, as dose delivery throughout the tissue is known. Intestinal responses are rapid and hence few side-effects resulting from whole body irradiation are seen within the time frame of a crypt microcolony assay. BDF1 mice were irradiated two at a time in a chamber that passes through the caesium source. The mice received air pumped to the chamber during the course of the irradiation. A range of doses between 11 and 16 Gy was delivered in order to generate full clonogenic cell (crypt) survival curves (see Withers & Elkind 1970; Potten & Hendry 1985; Roberts 1990) and are described in detail for the individual experiments. For each radiation dose a minimum of six mice was used. In some experiments, groups of animals were repeated and, where this occurred, the data have been pooled, giving in some cases experimental groups consisting of up to 18 mice.

Table 1. Treatment schedule and range of doses (six mice per time point and dose)

Treatment groups	Dose regimen (mg/kg/per injection)	Duration of administration relative to irradiation	Radiation doses (Gy)
None		Irradiation only	11, 12, 14, 15, 16
Saline		4 days post-irradiation	14
Saline		6 days pre-irradiation	
Saline		6 days pre-treatment and 4 days post-treatment	
Saline		6 days pre-irradiation	14
Saline		4 days post-irradiation	14
Teduglutide	0.1, b.i.d.	6 days pre-treatment	11, 12, 14, 15, 16
Saline			
Teduglutide	0.1, b.i.d.	4 days post-treatment	11, 12, 14, 15, 16
Saline			
Teduglutide	0.1, b.i.d.	6 days pre-treatment and 4 days post-treatment	11, 12, 14, 15, 16
Saline			
Teduglutide	0.1, b.i.d.	14 days pre-treatment	11, 12, 14, 15, 16
Saline			
Teduglutide	0.1, b.i.d.	14 days pre-treatment and 4 days post-treatment	11, 12, 14, 15, 16
Saline			

The injection protocols for teduglutide were tested using a range of radiation doses to generate full crypt survival curves as summarized in Table 1. Intestinal material was fixed in Carnoy's fixative 4 days after irradiation.

Tissue preparation and crypt microcolony assay

Irradiation was performed in the morning and animals were killed 4 days later at the same time of day. The whole small intestine was excised, the contents removed and weighed (g). The tissue was then fixed intact in Carnoy's fixative for 30 min and stored in 70% alcohol prior to paraffin embedding and sectioning as 'gut bundles' as described previously (Potten & Hendry 1985). The number of regenerating crypts was counted in each of the 10 intestinal circumferences randomly distributed along the entire ileum from each mouse. An intestinal circumference is a convenient and relatively standard unit of length, and hence the number of surviving or regenerating crypts per circumference could be used to generate a crypt survival curve, by expressing the surviving crypts per circumference as a percentage or fraction of the control value.

Crypts that contain surviving clonogenic stem cells are easily recognizable and often somewhat larger than the control crypts. As the probability of detecting a surviving crypt in a histological section depends on the size of the crypt, the data were adjusted for crypt size. This was done by measuring the crypt diameter in 15 longitudinal sections of crypts from each mouse and determining the widths of the crypts in the control and treated groups and adjusting the counts accordingly (Potten *et al.* 1981).

This method of crypt microcolony assay is a well-established, and records the number of regenerating crypts per unit length. Data are generally expressed as a crypt surviving fraction, that is the experimental irradiated value for crypt survival expressed as a percentage or fraction of the control value.

Histometric analysis

Measurements were performed on small intestinal crypts and villi using a Zeiss AxioHOME™ microscope (Potten *et al.* 2002b). For each individual mouse, the parameters were measured in

10–15 separate crypts and villi. Where possible, the crypts and villi measured were continuous indicating a true longitudinal section. Crypt and villus height (μm) and area (μm^2) were measured and the data presented as group mean percentage change in size from the vehicle control. Each treatment group contained six animals.

Data analysis

The data are either presented as the number of surviving crypts per circumference, or as a ratio of the treated sample to the saline control, that is the protection factor. Values greater than 1 indicated the factor by which crypt survival increased in the teduglutide-treated group. These curves are defined by the size of the shoulder on the survival curve, which is expressed as the extrapolation number (N), the value obtained by extrapolating the exponential portion of the curve back to zero dose of radiation, and by the reciprocal of the slope of the exponential region of the curve (D_0) or mean lethal dose, which is a measure of the sensitivity of the clonogenic stem cells. This sensitivity is determined, to some extent, by the ability of the colonogenic stem cells to undertake processes like repair of DNA damage. The shoulder region of the curve has been interpreted to represent a cell multiplicity factor (the number of clonogenic cells in each crypt that have to be sterilized before the crypt itself will be sterilized and disappear) and an element attributable to repair capacity of individual cells, that is, the amount of repair that can be achieved before the levels of damage overwhelm the cell, so that it no longer survives.

Statistical analysis

The survival curves were fitted using the DR FIT program (Roberts 1990) and an iterative program to determine convergence. Once fitted, the parameters defining the curves were calculated along with their associated standard errors. An analysis of variants F -test was used to compare any pair of fitted curves which then gave the statistical significance of any differences. For each of the treatment schedules shown in Table 1, the survival curves are presented with their respective vehicle-treated curves.

RESULTS

The current study supports previous reports regarding the intestinotrophic effects of GLP-2 and confirmed a similar action of teduglutide. Protracted administration of either GLP-2 or teduglutide (0.2 mg/kg/day, b.i.d.) for 14 days resulted in an increase in villus and crypt height and area, as well as an increase (30%) in small intestinal wet weight (Figs 1 and 2). In BDF1 mice, the height and area of the crypts increased 11 and 24% following GLP-2, and 25 and 32% following teduglutide administration. Villus height and areas increased 8 and 15% following GLP-2 and 23 and 36% following teduglutide administration. In CD1 mice, the crypt heights and areas increased 7 and 15% following GLP-2 and 5 and 14% following teduglutide treatment. Villus height and areas were similarly increased following GLP-2 (15 and 14%) and teduglutide (17 and 12%) administration. These data are illustrated in Figs 1 and 2. We made the comparison between CD1 and BDF1 mice because the former have been studied before (Drucker *et al.* 1996, 1997; Tsai *et al.* 1997b; Drucker *et al.* 1999) and BDF1 mice were used here for the crypt survival study and have been used for many earlier radiation studies (Potten & Hendry 1985; Potten 1995, 1996; Potten *et al.* 1997a; Farrell *et al.* 1998; Booth *et al.* 2000). Similar trends in tissue morphometry have been observed in earlier published data (Drucker *et al.* 1996, 1997; Tsai *et al.* 1997b).

Figure 1. Effects of GLP-2 and teduglutide on small intestinal weight in CD1 and BDF1 mice. Effects on small intestinal weight in CD1 and BDF1 mice following administration of teduglutide or GLP-2 by s.c. injection (0.1 mg/kg/injection, G.i.d.) for 14 days. Values represent the per cent change compared with saline-treated controls.

Figure 2. Morphological effects of GLP-2 and teduglutide in CD1 and BDF1 mice. The effects on villus area (a), villus height (b), crypt area (c) and crypt height (d) in CD1 mice and BDF1 mice following treatment with either teduglutide or GLP-2. The data were obtained by determining the changes in the parameters relative (percentages) to saline-treated control animals. Teduglutide or GLP-2 was administered by s.c. injection (0.1 mg/kg/injection, G.i.d.) for 14 days.

Figure 3. Crypt survival following irradiation in vehicle-treated and control animal groups. Crypt survival curves determined on day 4 following 14-day pre-treatment, 14-day pre-treatment and 4-day post-, 4 day post-irradiation (a), 6-day pre-treatment, 6-day pre-treatment and 4-day post-irradiation (b) with saline (i.e. vehicle controls) and for animals receiving no treatment at all (irradiation alone). Each point represents mean $\pm$ SEM of six mice. The parameters defining these survival curves are presented in Table 2.

Despite the morphological changes observed, there was no significant change in the number of S-phase cells (as determined by tritiated thymidine labelling index cell position distributions in the crypt, see Potten *et al.* 1990), or the number of cells expressing the Ki67 proliferation marker. Only higher doses of GLP-2 (0.8 mg/kg per injection) resulted in an increase in the fraction of S-phase cells throughout the crypt and a slight increase in the Ki67 labelling index distribution (data not shown).

Using the crypt microcolony assay, survival curves were generated for the number of surviving crypts against a range of radiation doses. Each teduglutide-treated group was compared with its vehicle control group. Figure 3 shows the reproducibility of each of the control (vehicle only) crypt survival curves generated in the different treatment protocols. There was no significant difference between the groups, with the exception of the animals that received vehicle for 6 days prior to irradiation and for 4 days post-irradiation (that is until the sampling time). Here, there was a small change in sensitivity and a small reduction in the size of the shoulder on the survival curve. However, there was no significant difference in these curves compared with a crypt survival curve from animals irradiated with no other treatment.

When teduglutide was delivered for 14 days prior to irradiation, there was a statistically significant change ($P < 0.001$) in the crypt survival curve with a higher D_0 value, that is an increased resistance or increased crypt survival, as illustrated in Fig. 4. D_0 values were 1.37 ± 0.91 with an extrapolation number of 1052 ± 604 for the vehicle group and 1.69 ± 0.88 and 249 ± 93 for the teduglutide-treated group (see Table 2). The effect was on both the D_0 or sensitivity and the extrapolation number or shoulder (N) of the survival curve.

A comparable result was observed in animals that were pre-treated with teduglutide for 6 days prior to irradiation. The survival curve in Fig. 5 shows a highly significant shift to the right for the teduglutide group ($P < 0.001$). In this case the D_0 was 1.41 ± 0.80 with an extrapolation number of 721 ± 342 for the vehicle-treated groups and a D_0 of 1.40 ± 0.68 with an extrapolation number of 1046 ± 429 for the teduglutide-treated group. The effects here were

Figure 4. Crypt survival following 14-day treatment with teduglutide prior to irradiation. Crypt survival curves in BDF1 mice administered teduglutide by s.c. injection (0.2 mg/kg/day) b.i.d. for 14 days prior to exposure to doses of irradiation ranging from 11 to 16 Gy. Teduglutide significantly increased crypt stem cell survival compared with vehicle controls ($P < 0.001$). Each point represents mean $\pm$ SEM of six mice. The D_0 and N -values for these curves are shown in Table 2.

Table 2. D_0 and extrapolation numbers for the crypt survival curves for teduglutide-treated and saline-treated groups

Figure	Treatment regimen	Agent	D_0	Extrapolation no.	P -value	Sensitivity or shoulder change
4	14 days pre-irradiation	Saline	1.369 ± 0.9083	1052 ± 603.6	< 0.001	Sensitivity (D_0) and shoulder (N)
		Teduglutide	1.694 ± 0.8832	249.3 ± 92.71		
5	6 days pre-irradiation	Saline	1.409 ± 0.7969	720.6 ± 342.0	< 0.001	Shoulder (N)
		Teduglutide	1.401 ± 0.6792	1046 ± 429.1		
6	6 days pre- & 4 days post-irradiation	Saline	1.761 ± 0.1524	123.2 ± 73.26	< 0.001	Shoulder (N)
		Teduglutide	1.803 ± 0.1334	149.4 ± 74.47		
7	14 days pre- & 4 days post-irradiation	Saline	1.225 ± 0.6958	4056 ± 2207	< 0.31	No effect
		Teduglutide	1.287 ± 0.6332	2833 ± 1276		
8	4 days post-irradiation	Saline	1.384 ± 0.7009	1316 ± 574.7	< 0.69	No effect
		Teduglutide	1.356 ± 0.9830	1570 ± 999.6		

smaller than in the previous experiment and were predominantly effects on the shoulder region of the curve, that is the extrapolation number.

Fig. 6 illustrates a similar result for animals that received a 6-day pre-treatment and 4-day post-treatment with teduglutide following exposure to irradiation. Again, there was a significant

Figure 5. Crypt survival following 6-day treatment with teduglutide prior to irradiation. Crypt survival curves in BDF1 mice administered teduglutide by s.c. injection (0.2 mg/kg/day, b.i.d.) for 6 days prior to exposure to doses of irradiation ranging from 11 to 16 Gy. Teduglutide significantly increased crypt stem cell survival compared with vehicle controls ($P < 0.001$). Each point represents mean $\pm$ SEM of six mice. The D_0 and N -values for these curves are shown in Table 2.

shift in the survival curve to the right, indicating more radioresistance, but the effect here was smaller. However, the curves were still highly significantly different ($P < 0.001$) and the relevant parameters defining the curves were 1.76 ± 0.15 for the D_0 and 123 ± 73 for the extrapolation number for the vehicle-treated group and 1.80 ± 0.13 and 149 ± 74 for the teduglutide-treated group (see Table 2). Here, the effect was one predominantly on the extrapolation number (N), an effect on the shoulder of the curve.

Fig. 7 shows the crypt survival curves for animals that received teduglutide for 14 days pre-irradiation and for 4 days post-irradiation. The D_0 and extrapolation numbers were 1.23 ± 0.70 and 4056 ± 2207 for the vehicle-treated group and 1.29 ± 0.63 and 2833 ± 1276 for the teduglutide-treated group. These curves were not significantly different ($P < 0.31$), even though the overall average protection factor was 1.3, which was strongly influenced by the level of protection at the highest dose.

When teduglutide was delivered for only 4 days post-irradiation there was no significant effect on the survival curve (Fig. 8), with the D_0 and N -values being very similar ($P < 0.69$). The D_0 and extrapolation numbers were 1.38 ± 0.70 and 1316 ± 575 for the vehicle and 1.36 ± 0.98 and 1570 ± 1000 for the teduglutide-treated group.

The D_0 and extrapolation numbers for each set of data are summarized in Table 2 while the protection factors for the various doses are listed in Table 3, together with the group mean

Figure 6. Crypt survival following 6-day pre-treatment and 4-day post-irradiation treatment with teduglutide. Crypt survival curves in BDF1 mice administered teduglutide by s.c. injection (0.2 mg/kg/day, b.i.d.) for 6 days prior to and 4 days following exposure to doses of irradiation ranging from 11 to 16 Gy. Teduglutide significantly increased crypt stem cell survival compared with vehicle controls ($P < 0.001$). Each point represents mean $\pm$ SEM of six mice. The D_0 and N -values for these curves are shown in Table 2.

Table 3. Protection factors following various teduglutide treatment regimens at doses of irradiation ranging from 11 to 16 Gy

Dose (Gy)	14 days pre-treatment	6 days pre-treatment	6 days pre-treatment & 4 days post-treatment	14 days pre & 4 days post-treatment	4 days post-treatment
11	0.99	1.45	1.23	1.04	0.98
12	1.60	1.04	1.64	1.31	0.96
14	1.65	1.37	1.58	1.15	1.18
15	1.75	2.24	1.54	0.84	0.83
16	1.15	1.23	1.46	2.02	0.35
Mean	1.428	1.466	1.490	1.272	0.860
Figure	4	5	6	7	8

Values were determined from the ratio of crypt stem cell survival between teduglutide (0.2 mg/kg/day, b.i.d.) and saline-treated groups for each radiation dose.

Figure 7. Crypt survival following 14-day pre-treatment and 4-day post-irradiation treatment with teduglutide. Crypt survival curves in BDF1 mice administered teduglutide by s.c. injection (0.2 mg/kg/day, b.i.d.) for 14 days prior to and 4 days following exposure to doses of irradiation ranging from 11 to 16 Gy. Teduglutide did not increase crypt stem cell survival compared with vehicle controls ($P < 0.31$). Each point represents mean $\pm$ SEM of six mice. The D_0 values for these curves are shown in Table 2.

protection factors. The overall impression is that teduglutide treatment influences the size of the shoulder region of the survival curves with some effect on the slope for the 14-day pre-treatment group. Post-treatment had little effect. Although the 14-day pre- and 4-day post-treatment study shows no statistically significant effect, there is a trend in reduction of extrapolation numbers as was seen with the 14-day pre-treatment group.

DISCUSSION

Previous studies have demonstrated similar intestinotrophic responses to exogenous administration of GLP-2. Small intestinal weight and villus height increased 1.5–2-fold and 1.2–1.5-fold, following GLP-2 administration by s.c. injection, b.i.d. in 6–8-week-old female CD1 mice at doses ranging from 12.4 μ /day to 87.5 μ /day (Drucker *et al.* 1996). Similar increases in small bowel weight and mucosal crypt-plus-villus height were observed in male and female CD1 mice up to 16 weeks of age, and female C57BL/6 mice ranging from 6 to 24 months following GLP-2 administration for 14 days at a dose of 5.0 μ g/day (Tsai *et al.* 1997b). Here, morphological changes were predominantly observed in jejunum as increased villus height.

Initial studies first described the structural effects of GLP-2 administration on the gastrointestinal epithelium, specifically showing increased small bowel weight as a result of increased

Figure 8. Crypt survival following 4-day treatment with teduglutide following irradiation. Crypt survival curves in BDF1 mice administered teduglutide by s.c. injection (0.2 mg/kg/day) b.i.d. for 4 days following exposure to doses of irradiation ranging from 11 to 16 Gy. Teduglutide did not increase crypt stem cell survival compared with vehicle controls ($P < 0.69$). Each point represents mean $\pm$ SEM of six mice. The D_0 values for these curves are shown in Table 2.

mucosal mass and villus height. This identified a biological role for GLP-2 (Drucker *et al.* 1996; Tsai *et al.* 1997b). Additional studies have shown that GLP-2 and its analogues also decrease mortality and improve disease-related histopathology associated with chemically induced damage to the intestine, including dextran-sulphate-induced colitis, NSAID-induced enteritis and chemotherapy-induced mucositis (Boushey *et al.* 1999; Drucker *et al.* 1999; Boushey *et al.* 2001). These effects in part may be attributed to the repair of damaged intestinal epithelium, a reduction in cytokine levels and changes in barrier function. In *ex vivo* studies, GLP-2 analogues enhance barrier function across the small intestine by reducing tissue conductance and macro-molecule flux in both normal and food allergy-induced animals (Benjamin *et al.* 2000; Cameron *et al.* 2003), GLP-2 has been shown to acutely increase intestinal blood flow, although the implications for intestinal damage and repair are as yet unknown (Burrin *et al.* 2000). Therefore, multiple physiological effects other than increases in mucosal mass may contribute to the overall reparative or protective effects of GLP-2 and teduglutide.

Crypt survival is dependent on clonogenic stem cell survival. At least one clonogenic stem cell in the crypt must survive in order for the crypt to survive. If too many crypts are sterilized (no surviving clonogenic cells), ulceration of the intestine will ensue and if extensive ulceration occurs the animal will die within 3–10 days. Such sterilized crypts disappear over the first 2 days post-irradiation. Crypts that contain one or more surviving clonogenic stem cells regenerate to form a crypt structure at 3–4 days post-irradiation. The results from this study clearly demonstrate

that pre-treatment with teduglutide is highly effective in protecting the clonogenic stem cells in the crypts of the small intestine, ensuring that more stem cells survive, which can then contribute to epithelial regeneration. However, delivery of teduglutide following irradiation, had no effect. Consistent with this observation is the fact that administration of teduglutide pre- and post-irradiation afforded no greater protection than the pre-treatment alone strategy.

The level of protection achieved by teduglutide pre-treatment ranged from essentially no protection (that is a protection factor of about 1.0) to a protection factor of 2.24 depending upon the treatment regimen and the radiation dose. In the best circumstances, teduglutide resulted in 2.24 times more crypts surviving than in the comparable vehicle control group in this model (Table 3). Although the levels of protection are not large, small differences in the number of surviving crypts can result in dramatic differences in the long-term regenerative capacity for the epithelium, and ultimately for animal survival. The average protection factor for all doses ranged from 1.3 to 1.5 in all the groups where teduglutide was given before irradiation (whether or not there was also some post-treatment). There were indications that the sensitivity of the clonogenic stem cells changed by 14 days teduglutide pre-treatment, that is, the slope of the survival curve altered (the D_0 value). However, using the shorter pre-delivery protocol, the sensitivity of the clonogenic cells remained constant, but the size of the extrapolation number was altered, which could indicate a change in the number of clonogenic cells per crypt and/or their ability to repair sublethal damage.

These results indicate that the GLP-2 analogue teduglutide may have clonogenic stem cell modulatory effects, and may have useful clinical applications in the prevention of cancer therapy-induced mucositis.

Further experiments are needed to determine the optimum treatment and dosing regimen for maximizing stem cell protection and functional capacity (for example, severity of diarrhoea or electrolyte transport) in the intestine, and that the effects are observed in terms of an end-point such as animal survival.

Although intestinal weight increased by approximately 30%, and slightly smaller increases in crypt and villus height and area were observed, there was no direct evidence of an increase in crypt cellularity (except after high dose administration 0.8–2.0 mg/kg/day) as determined from cell counts along the crypt. Thus, the mechanisms underlying the protection remain to be resolved.

Mechanisms of action for the protection afforded by teduglutide (or GLP-2) of intestinal stem cells remain unclear but include changes induced in: stem cell numbers prior to insult; the proliferative status of stem cells; the susceptibility to apoptosis or differentiation; or the environmental signalling for the stem cells. Within the context of the latter, we have reported earlier (Potten 2004) that, at the relevant time scales, we have failed to detect the endothelial apoptosis evoked by some to be an important contributor to such mucosal damage (Paris *et al.* 2001). Further studies are clearly needed to address the issue of mechanisms of action.

A variety of growth factors and cytokines have been reported to reduce adverse effects to cytotoxic exposure and therefore may be useful as potential anti-mucositis agents to improve the quality of life for cancer therapy patients. These include transforming growth factor (TGF) β 3 (Potten *et al.* 1997a; Booth *et al.* 2000), transforming growth factor α (Romano *et al.* 1992), fibroblast growth factor (Paris *et al.* 2001), keratinocyte growth factor (Farrell *et al.* 1998; Potten *et al.* 2001; Potten *et al.* 2002b; Zeeh *et al.* 1996) and interleukin-11 (Du *et al.* 1994; Orazi *et al.* 1996; Potten 1995, 1996). Several studies examining these agents have used the crypt microcolony assay as a test for stem cell survival (Potten 1996; Farrell *et al.* 1998; Paris *et al.* 2001; Potten *et al.* 1997a). The study on TGF β 3 (Booth *et al.* 2000) demonstrated that protection seen in the crypt microcolony assay is reflected both in a dramatic reduction in diarrhoea levels (a measure

of functional impairment) and also in whole animal survival (a surrogate for patient well-being). Studies using keratinocyte growth factor suggested stem cell protection might be afforded by increasing the number of stem cells prior to an insult (Potten *et al.* 2001) while transforming growth factor $\beta 3$ probably protected intestinal stem cells by switching them out of cycle (Potten *et al.* 1995; Booth *et al.* 2000). The levels of protection (protection factor) varied with growth factor, radiation dose, and dose and delivery protocol for the growth factor. For teduglutide, these elements have not been optimized, however, the protection factors seen in the present experiments are similar to those observed with many other growth factors, but lower than those seen with some growth factors using a variety of dose and delivery protocols.

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