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Efficacy of repifermin (keratinocyte growth factor-2) against abnormalities in gastrointestinal mucosal transport in a murine model of colitis

B. Greenwood-Van Meerveld, K. Venkova and K. Connolly

Abstract

Human keratinocyte growth factor-2 (KGF-2) is a member of the fibroblast growth factor family that promotes healing of experimental small intestinal ulceration and colitis. The aim of this study was to determine whether repifermin, a truncated form of recombinant human KGF-2, reverses abnormalities in colonic mucosal transport in a murine model of dextran sulfate sodium (DSS)-induced colitis. Male Swiss-Webster mice were given 4% DSS in drinking water for 7 days and then normal drinking water for 3 days. Repifermin (5 mg kg^{-1}, i.p.) or vehicle was administered daily for 7 days starting on Day 4 of DSS exposure. On Day 10, net ion transport was measured electrophysiologically in colonic mucosal sheets. Repifermin significantly reduced DSS-induced colonic inflammation measured by tissue myeloperoxidase activity. Concurrently, in colonic tissue taken from mice treated with repifermin, there was a normalization of basal potential difference and short circuit current, and an improvement in the secretory responses to stimulation of muscarinic and ganglionic cholinoceptors. In control mice, repifermin did not interact directly with colonic epithelial cells or intramural neurones to induce immediate changes in net electrogenic transport. The results suggest that repifermin therapy may improve the mucosal electrogenic transport that is impaired during colitis.

Oklahoma Foundation for Digestive Research, Basic Science Laboratories, Veterans Administration Medical Center, Room 151, 921 NE 13th Street, Oklahoma City, OK 73104, USA

Beverley Greenwood-Van Meerveld, Kalina Venkova

Human Genome Sciences Inc., 9410 Key West Avenue, Rockville, MD 20850, USA

Kevin Connolly

Correspondence: Beverley Greenwood-Van Meerveld, Oklahoma Foundation for Digestive Research, Basic Science Laboratories, Veterans Administration Medical Center, Room 151, 921 NE 13th Street, Oklahoma City, OK 73104, USA. E-mail: Beverley-Greenwood@ouhsc.edu

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Introduction

Keratinocyte growth factors (KGFs) are members of the fibroblast growth factor (FGF) subfamily that function as paracrine mediators of epithelial cell proliferation, migration and morphogenesis and are associated with epithelial growth and repair. KGF-2 (also known as FGF-10) and KGF-1 (or FGF-7) are soluble polypeptides secreted by fibroblasts and endothelial cells that act primarily on epithelial cells possessing selective FGF receptors. The structure of recombinant human KGF-2, first reported by Emoto et al (1997), shows 96% homology to the rat FGF-10 protein (Yamasaki et al 1996), 92% homology to mouse FGF-10 (Beer et al 1997), and displays 57% homology to the human KGF-1 protein (Jimenez et al 2000). Both KGF-1 and KGF-2 bind to the FGFR-2iiib receptor isoform, which is expressed exclusively by epithelial cells (Igarashi et al 1998). Unlike KGF-1, KGF-2 also binds to a splice variant of FGFR-1iiib, although with 10-fold lower affinity (Beer et al 2000). The significance of FGF expression is demonstrated in genetically manipulated knockout mice. In contrast to KGF-1-deficient mice that are phenotypically normal, the KGF-2 knockout mouse is a perinatally lethal mutant, lacking lung or limb development (Martin 1998).

Studies in cell culture indicate that KGFs are mitogenically active only on epithelial cells and are produced often by cells of mesenchymal origin, leading to the hypothesis that KGFs function as paracrine mediators of mesenchymal–epithelial communication (Rubin et al 1995). Functional assays, both in organ culture and in-vivo, confirmed a variety of responses to KGF-2 and KGF-1, including proliferation, migration and morphogenesis of epithelial cells (Emoto et al 1997; Igarashi et al 1998; Jimenez et al 2000). The expression of FGF-10 (Tagashira et al 1997) and FGF-7 (Werner 1998) was found to be up-regulated in the dermis of mice after cutaneous wounding. In the

gastrointestinal epithelium, KGF messenger RNA levels are increased during active inflammation associated with inflammatory bowel disease (IBD), suggesting that it may be involved in the alterations of intestinal epithelial cell function in this disease (Finch et al 1996; Bajaj-Elliot et al 1997). When systemically administered in rats, recombinant KGF has been found to induce proliferation of epithelial cells throughout the gastrointestinal tract, confirming its ability to regulate gastrointestinal epithelial populations in-vitro (Housley et al 1994). KGF was also found to have a protective effect against chemotherapy and radiation-induced gastrointestinal injury, and to reduce mortality in mice (Farrell et al 1998). Repifermin is a truncated, recombinant form of human KGF-2 obtained by high-throughput sequencing of prostate and fetal lung cDNA libraries (Ruben et al 2000). Previously, a positive effect of repifermin on epithelial repair in the skin and intestine was found in various animal models (Soler et al 1999; Han et al 2000). Using a murine model of colitis induced by a 7-day exposure to dextran sulfate sodium (DSS), Miceli et al (1999) demonstrated that repifermin treatment significantly reduced weight loss, bloody diarrhoea and colon histopathology. Despite the promising results showing prophylactic activity of repifermin against DSS-induced colitis, the mechanisms that link the protective and healing effect of repifermin on intestinal mucosa to normalization of mucosal function have not been investigated. The overall objective of the present study was to determine whether repifermin has a therapeutic effect in the murine model of DSS-induced colitis, that is whether repifermin treatment is capable of reversing abnormalities in mucosal transport associated with an inflammatory insult. Our specific study objectives were: (i) to investigate the effect of a 7-day treatment with repifermin on the symptoms of colitis and net electrogenic transport in the colon of mice exposed to DSS; and (ii) to determine whether repifermin applied either directly to the isolated mucosal sheets (acute effect) or given intraperitoneally for 7 consecutive days affected the net electrogenic transport in the colon of control mice not treated with DSS. The study describes the effects of repifermin treatment of mice with chronic DSS-induced colitis on the basal parameters of electrogenic colonic transport and secretory responses induced by cholinergic stimulation in isolated colonic mucosal sheets studied in modified Ussing chambers.

Materials and Methods

Test and control materials

Repifermin and the placebo vehicle were supplied by Human Genome Sciences, Inc. (Rockville, MD, USA). Lyophilized repifermin (lot no. HG03411-16-1618-27A) was reconstituted with deionized distilled water to obtain a stock solution containing 2 mg mL^{-1} repifermin. This was aliquoted and stored at $-80°C$. Working dilutions of repifermin were prepared using the vehicle (lot no. 1618-18) and were stored at 4°C for 2–3 days. A dose of 5 mg kg^{-1} repifermin or vehicle control was injected intraperitoneally in a 200-μL volume. Modified Krebs bicarbonate buffer was used in the study of colonic mucosal function, containing (mM): NaCl 120, KCl 6, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 14.4, and glucose 11.5. DSS (MW 40 000–50 000) was obtained from Spectrum Laboratory Products (Gardena, CA, USA). Carbamylcholine chloride, 1,1-dimethyl-4-phenyl-piperazinim iodide (DMPP), and tetrodotoxin were purchased from Sigma-Aldrich Company (St Louis, MO, USA). Reagents were dissolved in distilled water and were added to the baths in volumes of less than 1% of the total bath volume.

Animals

Adult male Swiss-Webster mice (30–35 g bodyweight), supplied by Harlan Laboratories (Indianapolis, IN, USA), were housed four per cage under standard conditions and with free access to rodent chow and water. Animals were assigned to test groups and a 10-day acclimatization period was allowed. The study was approved by the Oklahoma City Veteran's Administration Medical Center Animal Care Sub-Committee in accordance with the provisions of the US Animal Welfare Act and the Guide for Care and Use of Laboratory Animals, ILAR Commission on Life Sciences.

Myeloperoxidase (MPO) activity

Full-thickness colonic tissue samples (approx. 50–75 mg) were excised and immediately frozen in liquid nitrogen. Tissue homogenization and extraction of MPO from the homogenate were carried out in hexadodecyl trimethyl ammonium bromide phosphate buffer (pH 6). MPO activity was assayed in 10-μL samples using the 3,3′,5,5′-tetramethyl benzidine (TMB) Microwell Peroxidase Substrate System (Sigma-Aldrich Company) with horseradish peroxidase as a relative standard. MPO activity in each tissue sample was expressed as equivalent to the activity of the relative standard (ng horseradish peroxidase) converting the same amount of TMB substrate for 10 min at room temperature. Values were normalized per gram of tissue wet weight.

Experimental design

A murine model of DSS-induced colitis was used in these experiments to assess the effect of treatment with repifermin on mucosal transport of electrolytes and water in the colon. Colitis was induced by exposure of Swiss-Webster mice to 4% DSS in the drinking water for 7 days. Thereafter, the animals were provided with regular tap water. Oral administration of DSS results in a time-dependent development of colitis that is fully expressed within the first 4 days of exposure to DSS and persists after the DSS treatment is discontinued. DSS treatment was initiated on Day 1 and continued until Day 7. Repifermin (5 mg kg^{-1}, i.p.) was administered once a day for 7 days from Day 4 through

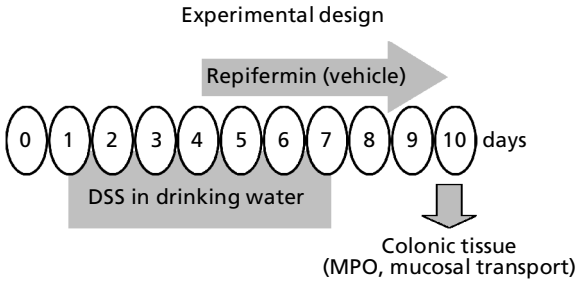

Figure 1 Colitis was induced in male Swiss-Webster mice by exposure to 4% dextran sulfate sodium (DSS) in drinking water from Day 1 to Day 7 of the experiment. Symptoms of colitis were found in all animals on Day 4 of the exposure to DSS and persisted after Day 7, when DSS was no longer added to the drinking water. Starting on Day 4, the mice received repifermin or the vehicle (5 mg kg^{-1}, i.p.) for 7 days. All animals were killed on Day 10 of the experiment and colonic tissue was isolated for in-vitro studies. MPO, myeloperoxidase.

Day 10, as shown in Figure 1. A total of five groups, including DSS-exposed and non-DSS exposed control mice administered either repifermin or vehicle, as well as a naive untreated group were used in the study. The number of animals in each group was as follows: DSS-exposed placebo-treated group, n = 28; DSS-exposed repifermin treated group, n = 24; control placebo-treated group, n = 8; control repifermin treated group, n = 8; and naive group, n = 16. Animals in each group were divided into subsets of four mice that were treated simultaneously and used to provide colonic tissue for each experiment. All animals were killed on Day 10, the colons were collected and used in the study of mucosal electrogenic ion transport and for determination of MPO activity.

In-vivo monitoring of DSS-induced colitis

The course of the disease in this murine model of DSS-induced colitis was followed by daily monitoring of bodyweight loss, faecal occult blood, and stool consistency from Day 0 through Day 10. A composite disease activity index (DAI) was calculated to assess the clinical signs of colitis according to Cooper et al (1993). Briefly, scores between 0 and 4 are given for bodyweight loss, faecal occult blood, and stool consistency (Table 1). Colorectal bleeding was evaluated using a diagnostic screening test for faecal occult blood (Beckman Coulter, Inc., Fullerton, CA, USA). A daily DAI was calculated as the average of the three parameters for each animal. Mean DAI values were calculated within the groups of four animals and averaged to obtain the mean daily DAI values.

Table 1 Parameters of the composite disease activity index (DAI) used to assess the clinical signs of colitis.

Parameter	Change	Score
Weight loss	0	0
	1 to < 5%	1
	5 to < 10%	2
	10 to < 20%	3
	>20%	4
Faecal occult blood	Negative	0
	Positive	2
	Gross bleeding	4
Stool consistency	Normal	0
	Loose	2
	Diarrhoea	4

The DIA is calculated as the mean of the scores given for the three parameters (Cooper et al 1993).

In-vitro electrophysiological measurement of mucosal transport

A subset of four animals within each of the main five experimental groups was killed for each experiment. The colons were harvested, and mucosal sheets stripped from the external muscle layer were placed in modified Ussing chambers (World Precision Instruments, Inc, Sarasota, FL, USA). The luminal and serosal sides of the tissue specimens were bathed with Krebs solution. The solutions were connected via two pairs of agar-KCl-bridge electrodes to a voltage-current clamp apparatus (EVC 4000; World Precision Instruments, Inc.). Potential difference (PD) and short circuit current (Isc) were recorded continuously using a PowerLab data acquisition system (AD Instruments, Grand Junction, CO, USA) by switching between the open circuit PD and Isc modes of the voltage clamp. Tissue conductance was calculated using Ohm's law.

Colonic secretory responses to cholinoceptor agonists

The parameters of basal electrogenic transport were recorded following a 45-min equilibration period. Secretory responses were induced either by carbamylcholine chloride (0.1–500 μM), a muscarinic cholinoceptor agonist, or by DMPP (200 μM), a selective activator of nicotinic cholinoceptors in the myenteric ganglia. These agents were added to the serosal bathing solutions, and the secretory responses were measured in terms of the maximal increase in basal Isc.

Direct effect of repifermin on net electrogenic transport in the colon

A series of experiments was performed to investigate a possible direct effect of repifermin on colonic transport. Transmucosal PD and Isc were recorded following application of either repifermin (1–1000 ng mL^{-1}) or vehicle. To mimic systemic drug application, either repifermin or the vehicle was added to the serosal bathing solution in volumes not exceeding 0.1% (v/v) of the total bath volume. The effect of a single concentration of repifermin (1, 10, 100 or 1000 ng mL^{-1}) was recorded for 120 min in individual preparations. During the treatment period, the recording

system was switched from voltage-clamped to open-circuit mode at specified time intervals (every 5 min from 0 to 20 min, and every 20 min from 20 to 120 min). The values of PD and Isc obtained were used to calculate the tissue conductance during the time-course of treatment.

Data analysis and statistics

Data were presented as the mean ± s.d. The difference between the experimental groups was tested using one-way analysis of variance on numeric values or ranks where appropriate. The difference was considered statistically significant at the 5% level ($P < 0.05$). The concentration of carbachol inducing half the maximal response (EC50) was calculated from the concentration–response curves using regression analysis applied to the linear sections of the curves. The difference between EC50 values was tested statistically by comparing 95% confidence limits (CL) calculated from a summarized regression for all experiments in one group. Statistical analyses were performed using StatView software (SAS Institute, Inc, Cary, NC, USA).

Results

General observations in-vivo

All of the animals exposed to DSS in drinking water had diarrhoea and faecal occult blood or gross colorectal bleeding accompanied by significant weight loss. The symptoms of colitis developed gradually throughout the 7-day exposure to DSS and persisted after cessation of the DSS treatment. As shown in Figure 2, daily treatment with repifermin, administered intraperitoneally at a dose of 5 mg kg^{-1} from Day 4 through Day 10, improved stool consistency and reduced rectal bleeding. In addition to changes in the intensity of the symptoms, diarrhoea and/or faecal blood were observed in only 25% of the animals treated with repifermin, whereas 40% of the placebo-treated mice had diarrhoea and/or blood in the faeces. In animals treated with repifermin, there was no significant reversal in the decline in body weight. Taken together, the daily DAI calculated at the peak of the repifermin response significantly decreased with the treatment (repifermin DAI = 2.4 ± 0.3; placebo DAI = 3.2 ± 0.3).

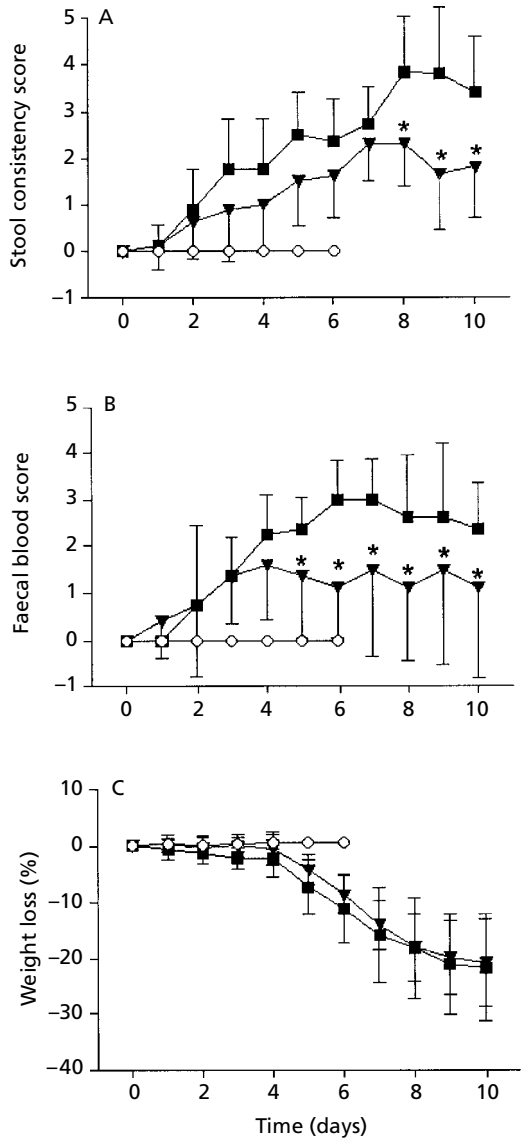

Figure 2 Time-course of the effects of repifermin treatment on stool consistency (A), faecal blood (B), and bodyweight (C). Faecal blood and stool consistency are expressed as the mean daily score (criteria listed in Table 1). Colitis was induced in mice by 4% dextran sulfate sodium (DSS) present in drinking water from Day 1 to Day 7 of the experiment. Repifermin (5 mg kg^{-1}, i.p., ▼) or the vehicle (■) were injected daily from Day 4 to Day 10. Data are mean ± s.d. from 16–28 mice for each group. *$P < 0.05$, significantly different compared with the DSS placebo-treated group (non-parametric analysis of variance followed by Dunn's test). Values measured in naive animals (n = 8) during 7 days are presented as a reference to controls (○).

Effects of repifermin treatment on colonic MPO activity

The MPO activity, indicative of a neutrophil infiltrate in the tissue, was significantly increased in the colons of mice exposed to DSS compared with control mice. MPO activity was 273 ± 150 ng (g tissue)$^{-1}$ in DSS-exposed mice compared with 45 ± 17 ng (g tissue)$^{-1}$ in control mice not exposed to DSS. Administration of repifermin to DSS-exposed mice significantly decreased MPO activity by approximately 40% to 161 ± 117 ng (g tissue)$^{-1}$ (Figure 3). In a different set of experiments, repifermin had no significant effect on colonic MPO activity when administered to naive mice.

Direct effect of repifermin on net electrogenic transport in the colon

The colonic mucosal sheets isolated from control untreated mice had a mean basal transmucosal PD of −1.1 ± 0.2 mV

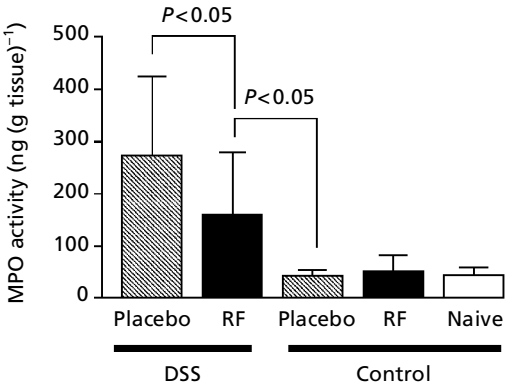

Figure 3 Effects of repifermin or placebo treatment on myeloperoxidase (MPO) activity in the colon of mice with dextran sulfate sodium (DSS)-induced colitis and controls. Colitis was induced in mice by 4% DSS present in drinking water from Day 1 to Day 7 of the experiment. Repifermin (RF) (5 mg kg^{-1}, i.p.) was injected daily from Day 4 to Day 10. All animals, except for naive untreated controls, were killed on Day 10 of the experiment 3 h after the last injection of repifermin. Data are mean±s.d. from 16–28 mice for each group. Significance of differences was assessed using non-parametric analysis of variance followed by Dunn's test.

(n = 12). Exposure of the serosal surface of the colonic epithelium to increasing concentrations of repifermin (1–1000 ng mL^{-1}) had no significant effect on basal PD compared with the effect of the vehicle. Simultaneously, the active electrogenic transport showed a basal Isc of 39±9μA cm^{-2} (n = 12). Repifermin, applied to the serosal bathing solution at concentrations of 1–1000 ng mL^{-1}, had no significant effect on basal Isc values (Figure 4). Basal tissue conductance (35±13 mS cm^{-2}; n = 12) was quantified as an indirect measure of mucosal permeability. Repifermin did not alter the electrical conductance of colonic mucosa (data not shown). In separate experiments (n = 3), repifermin was added to the mucosal bathing solution at a final concentration of 1000 ng mL^{-1} to mimic conditions for topical intraluminal administration of the agent. Under these experimental conditions, neither repifermin nor the vehicle showed any significant effects on basal PD or basal Isc.

Effect of repifermin treatment of mice with DSS-induced colitis on basal colonic transport

The net electrogenic transport across the colonic tissue from mice exposed to DSS and treated with placebo was characterized by lower values of basal PD and Isc as compared with control placebo-treated mice (Table 2). However, this was not associated with a statistically significant change in basal electrical conductance (21±14 mS cm^{-2} vs 29±11 mS cm^{-2}). In mice with DSS-induced colitis, chronic treatment with repifermin significantly in-

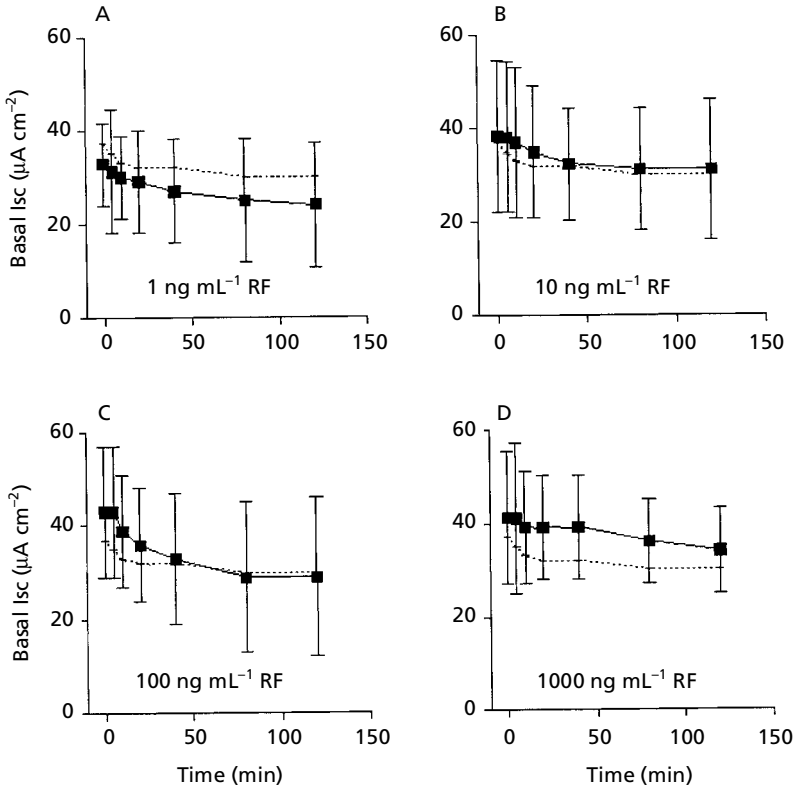

Figure 4 Direct effect following application of increasing concentrations of repifermin (RF) (1, 10, 100 and 1000 ng mL^{-1}) to the serosal bathing solution. Basal short circuit current (Isc) of colonic mucosal sheets isolated from control mice was measured during a 120-min treatment with each concentration. Data represent mean±s.d. from 5–8 mucosal sheets isolated from at least three animals.

creased ($P < 0.05$) basal PD and Isc values compared with the placebo-treated mice with DSS-induced colitis (Table 2). Furthermore, these values did not differ significantly from those in control placebo-treated animals. In control mice, neither basal PD nor Isc showed significant differences between repifermin- and placebo-treated groups.

Effect of repifermin treatment of mice with DSS-induced colitis on carbachol-stimulated colonic secretory responses

Application of increasing concentrations of carbachol (0.1–300 μM) to the serosal side of colonic mucosal sheets from control mice induced a concentration-dependent increase in Isc that was inhibited in the presence of atropine (1 μM). There was no significant difference between the concentration–response curves in mucosal sheets from control mice receiving either placebo or repifermin treatment. Moreover, there was no significant difference ($P > 0.05$) in the maximal increase in Isc between the placebo-treated control mice (79 ± 17 A cm^{-2}; n = 12) or the control mice treated with repifermin (84 ± 12 μA cm^{-2}; n = 10). In colonic mucosal sheets isolated from mice exposed to DSS and treated with placebo, the responsiveness to carbachol was impaired, that is the maximal increase in Isc was suppressed and no concentration–effect relationship was observed compared with the secretory response in the colon of control placebo-treated mice (Figure 5). Treatment of DSS-exposed mice with repifermin induced a significant improvement in the response to carbachol, increasing the maximal Isc and restoring a concentration–response relationship. However, the maximal response to carbachol in the colon of repifermin-treated mice exposed to DSS was lower in amplitude compared with the response in control animals (79 ± 17 μA cm^{-2} vs 29 ± 8 μA cm^{-2}, $P < 0.05$). There was no difference between the EC50 values obtained for repifermin-treated animals (24.7 μM, 95% CL 12–48 μM) and the placebo-treated controls (27.3 μM, 95% CL 18–37 μM).

Table 2 Effects of in-vivo treatment with repifermin (5 mg kg^{-1}, i.p., for 7 days) or placebo on the basal potential difference (PD) and short circuit current (Isc) measured in isolated colonic mucosa.

DSS exposure	Treatment	Basal PD (mV)	Basal Isc (μA cm^{-2})
DSS	Placebo	-0.6 ± 0.4*	12 ± 7[a]
DSS	Repifermin	-1.0 ± 0.5	26 ± 11
Control	Placebo	-1.1 ± 0.5	29 ± 12
Control	Repifermin	-1.1 ± 0.5	32 ± 12

DSS, dextran sulfate sodium. Values are presented as the mean$\pm$s.d. from 22–34 experiments. *$P < 0.05$ compared with placebo-treated controls using one-way analysis of variance followed by Bonferroni test.

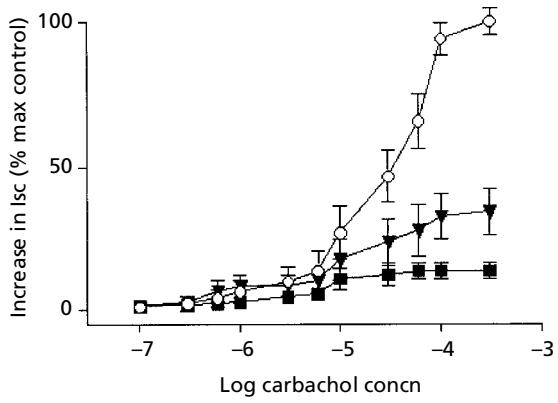

Figure 5 Increase in short circuit current (Isc) in colonic mucosal sheets induced by increasing concentrations of carbachol applied to the serosal bathing solution. Colonic tissue was isolated from control mice receiving placebo (○) or mice with dextran sulfate sodium-induced colitis that received either repifermin (▼) or placebo (■). Repifermin (5 mg kg^{-1}, i.p.) or placebo was injected daily from Day 4 to Day 10. Animals were killed on Day 10 of the experiment 3 h after the last injection of repifermin. Data are mean$\pm$s.d. from 8–12 mice for each group.

Effect of repifermin treatment of mice with DSS-induced colitis on DMPP-induced colonic responses in the colon

This set of experiments was performed to investigate the effect of repifermin treatment on neurally mediated secretory responses in the colon initiated by activation of ganglionic nicotinic cholinoceptors. DMPP, applied to the serosal bathing solution at a concentration of 200 μM, induced a pronounced and reproducible increase in Isc in colonic mucosal sheets obtained from control placebo-treated mice. In colonic preparations isolated from naive mice, the response to DMPP (28 ± 12 μA cm^{-2}, n = 3) was abolished by tetrodotoxin (1 μM), demonstrating activation of enteric neuronal pathways by DMPP. In colons from placebo-treated mice exposed to DSS, the Isc induced by DMPP was significantly inhibited compared with placebo-treated control animals not exposed to DSS. When mice exposed to DSS were treated with repifermin, the colonic response to DMPP increased significantly compared with placebo-treated DSS-exposed mice. However, even with the repifermin treatment, the mean DMPP-induced response was lower in amplitude than that for control placebo-treated mice (Figure 6). There were no significant changes in DMPP-induced increase in Isc in the colons of control (non-DSS-exposed) animals treated with either placebo or repifermin.

Discussion

Normal transport and barrier function of the gastrointestinal mucosa is maintained by continuous regeneration of the epithelial layer from a small number of crypt stem cells. Expression of KGF receptors and KGF mRNA has been detected within the entire gastrointestinal tract, sug-

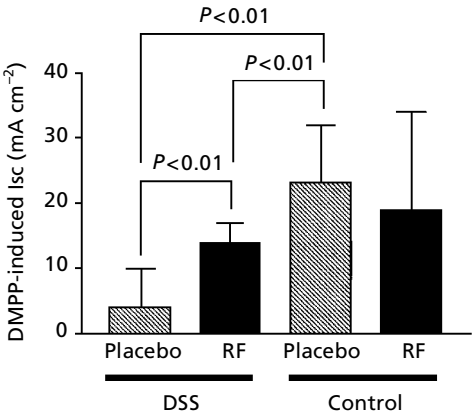

Figure 6 Increase in short circuit current (Isc) in colonic mucosal sheets induced by 1,1-dimethyl-4-phenyl-piperazinimiodide (DMPP; 200 μM) applied to the serosal bathing solution. Tissue was harvested from control mice or mice with dextran sulfate sodium (DSS)-induced colitis. Both control and DSS-exposed mice received either repifermin (RF) (5 mg kg^{-1}, i.p.) or placebo. Data are mean$\pm$s.d. from 7–12 mice for each group. Significance of differences was assessed using one-way analysis of variance followed by Bonferroni (selected pairs) test.

gesting that KGF is likely a normal paracrine mediator of epithelial growth within the gut (Housley et al 1994). However, in patients with IBD, the expression of KGF is increased, possibly as a molecular mechanism promoting repair of the injured mucosa (Finch et al 1996; Bajaj-Elliott et al 1997). Taken together, these findings imply that KGF is an important mediator of mucosal defence and repair and may be of clinical value in the treatment of gastrointestinal mucosal injury. Repifermin, a truncated form of recombinant human KGF-2, has been demonstrated to have therapeutic or protective effects in a variety of models of gastrointestinal mucosal injury (Farrell et al 1998; Soler et al 1999; Han et al 2000; Baatar et al 2002). In previous experiments, repifermin, given as a protective agent before and during the chronic phase of DSS-induced colitis, was found to reduce mortality, decrease colonic inflammation and prevent or improve histological mucosal damage (Egger et al 1999; Miceli et al 1999). In the present study, we focused on the therapeutic action of repifermin administered during the chronic phase of DSS-induced colitis. The model of DSS-induced colitis in the mouse was chosen for a number of reasons: (i) this model induces reproducible mucosal colonic inflammation by an easily administered oral agent that produces lesions that mimic many features of human IBD; (ii) DSS appears to have a primary cytotoxic effect on epithelial cells and is thus particularly suited to the evaluation of mechanisms of epithelial regeneration and the investigation of the potential efficacy of drugs to promote regeneration and healing (Elson et al 1995); and (iii) there was also prior experience with the use of repifermin in the treatment of murine DSS-induced colitis to ameliorate symptoms and reduce histological damage in the intestinal mucosa (Miceli et al 1999).

The present study demonstrates that repifermin treatment applied during the chronic phase of DSS-induced colitis is associated with improvement of the transport function in the colon. Epithelial dysfunction, including abnormal ion secretion, was evident during chronic DSS-induced colitis in the animals treated with the vehicle, that is the basal PD and Isc values were significantly lower compared with controls. In contrast, the basal PD and Isc in the DSS-exposed group receiving repifermin were elevated and did not differ significantly from controls. Since repifermin did not cause changes in Isc or PD when added directly to the bathing solution, the possibility exists that normalization in baseline ion transport results from the "healing" action of repifermin inducing epithelial growth and repair, rather than from a direct interaction with secretory mechanisms in the enterocyte. We thus speculate that the anti-inflammatory effect of repifermin (decrease in colonic MPO activity) in DSS-exposed animals is an indirect consequence of mucosal repair and not a result of repifermin interaction with the immune system. Restoring the integrity of the epithelial lining of the intestine limits the uptake of noxious intraluminal substances and antigens and prevents the release of pro-inflammatory mediators from activated immune cells located in the lamina propria. Such a mechanism of action for repifermin on DSS-induced colitis is supported by the significant improvement in colonic histopathology observed following repifermin (1–10 mg kg^{-1}) treatment reported by Miceli et al (1999).

In the colon of mice exposed to DSS, the active mucosal transport of electrolytes is also significantly impaired. We found that the increase in Isc induced by activation of muscarinic or nicotinic cholinoceptors by carbachol or DMPP, respectively, was significantly reduced in the colon of DSS-treated mice receiving placebo. This finding was similar to the decreased responsiveness of the epithelial layer observed by others in DSS-induced colitis (Diaz-Granados et al 2000) or in different animal models of colonic inflammation (Kachur et al 1995; Asfaha et al 1999). Reduced responses to secretagogues and enteric nerve stimulation are considered relevant to the impairment of intestinal transport in patients with IBD, where a significant inhibition and abolishment of the secretory responses to stimulation of intramural neurons has been observed (Hubel & Renquist 1990). Furthermore, similar to IBD, the DSS-induced model of colitis has a chronic phase and is characterized by the lack of profound therapeutic effects following treatment with a variety of anti-inflammatory agents. For example, DSS-induced colitis is resistant to dexamethasone treatment (Van Meeteren et al 2000) as well as to inhibition of phosphodiesterase activity that is purported to reduce the activity of immune cells by elevation in cAMP (Diaz-Granados et al 2000).

Nevertheless, we established that when mice exposed to DSS were given repifermin, the colonic mucosa responded to carbachol with a concentration-dependent increase in Isc, although the maximal response remained lower in comparison with the control. A similar improvement was observed also in the neurally mediated secretory response to selective activation of ganglionic nicotinic receptors by DMPP. These findings indicate that although repifermin treatment is associated with a significant improvement, there is not complete normalization of colonic mucosal

responsiveness to cholinergic stimuli. Moreover, they strongly suggest that repifermin may have a potential therapeutic effect in IBD, despite the fact that the mechanism(s) and site of action are not precisely understood. Based on current literature data, it is unlikely that repifermin has a direct effect on enteric neurones. Intestinal mucosal inflammation is linked to functional remodelling of the enteric nervous system (Collins 1996), typically manifested as a decrease in the stimulated release of acetylcholine from myenteric neurones (Collins et al 1989; Davis et al 1998; Lourenssen et al 2002). In uninflamed mucosal preparations, impaired cholinergic responses could be mimicked by application of exogenous interleukin-1 or -6, indicating that enteric neurons are affected by the action of pro-inflammatory mediators (Main et al 1993; Xia et al 1999). In addition, in patients with IBD, the intestinal epithelial cells themselves release inflammatory mediators and are targeted by their action (Monteleone et al 2002). Thus, it is most likely that the repifermin-induced improvement in active secretion measured as an increase in Isc is the summarized result of functional improvement in both enteric nerves and enterocytes due to repifermin-activated epithelial growth and repair.

At the time our studies were initiated, it was thought that repifermin exerted its effects purely by stimulating epithelial cell proliferation, differentiation, and migration, thereby promoting the wound healing process, as shown in a topical wound healing model (Soler et al 1999). Subsequently, evidence of anti-inflammatory activity for repifermin has accumulated, as shown by: (i) the up-regulation of cyclooxygenase-2 expression in Caco-2 cells; (ii) the enhanced production of the tissue protective prostaglandin, PGE2; and (iii) the reduced amounts of the proinflammatory cytokine, interleukin-1β, in intestinal tissue from repifermin-treated rats with indometacin-induced intestinal injury (Han et al 2000). In-vitro studies assessing the effect of repifermin on the diffusion of labelled mannitol and sucrose through Caco-2 monolayers (unpublished data on file at Human Genome Sciences, Inc.) suggest a positive effect on preservation of intestinal cell tight junctions. In addition, the possible effect of repifermin on intestinal trefoil factor release from goblet cells is being investigated in DSS-induced murine colitis. In view of these findings, it is believed that repifermin has a complex healing effect and may prove effective in the recovery of the damaged epithelium and normalization of mucosal transport in patients with IBD. Accordingly, clinical trials using repifermin are in progress in patients with ulcerative colitis.

Conclusions

In the present study using a murine model of DSS-induced colitis, a 7-day therapeutic regimen of repifermin (5 mg kg^{-1}, i.p.), begun after 4 days of exposure to DSS, inhibited colorectal bleeding and diarrhoea associated with the colitis, although it did not significantly reduce weight loss over the same time period. It is possible that if the observation period had been extended, a repifermin-mediated rebound in weight gain may have occurred, as has been noted in earlier reports (Miceli et al 1999). Repifermin significantly reduced colonic inflammation (assessed by MPO activity). Assessment of colonic mucosal function indicated that injection of mice with repifermin normalized the spontaneous basal transport of electrolytes and partially restored both muscarinic (carbachol) and nicotinic (DMPP) cholinergically mediated secretion in the DSS-colitis-impaired colon. In naive mice not exposed to DSS treatment, repifermin had no marked effect on colonic electrolyte transport. These data support the hypothesis that repifermin may prove effective in treating mucosal transport abnormalities in patients with IBD. The findings further support the importance of KGF-2 as a mediator of intestinal mucosal defence and repair.

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