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Luminal and parenteral TFF2 and TFF3 dimer and monomer in two models of experimental colitis in the rat

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Abstract

Background: Peptides of the trefoil factor family (TFF1, TFF2 and TFF3) are cosecreted with mucus from mucus-producing cells in most organ systems and are believed to interact with mucus to form high-viscosity stable gel complexes. In the gastrointestinal tract, they sustain the mucosal barrier, and both injected and orally administered TFF peptide have protective and healing functions in the gastric mucosa.
Aim: To investigate the possible treatment effect of luminally and parenterally administered TFF peptides in experimental colitis in rats.
Methods: Colitis was induced by administration of 5% dextran sodium sulphate in the drinking water or by one intraperitoneal injection of mitomycin C, 3.75 mg/kg. TFF peptides were administered as subcutaneous injections or directly into the lumen via a catheter placed in the proximal colon. Treatments were saline, TFF2, TFF3 monomer or TFF3 dimer 5 mg/kg twice per day throughout the study [dextran sulphate sodium (DSS)] or from day 4 to 7 (mitomycin C). Colitis severity was scored in a stereomicroscope and histologically.
Results: Luminal treatment with TFF3 in its dimeric form significantly improved the colitis score in both colitis models, whereas TFF2 had positive effect only in DSS-induced colitis. The TFF3 monomer was without any effects in both models. Treatment effect was most pronounced in the middle part of the colon, closest to the tip of the catheter. Injected TFF peptides, especially the TFF3 monomer, aggravated the colitis score in both colitis models.
Conclusions: Intracolonic administration of TFF3 dimer and TFF2 improves experimentally induced colitis in rats. The TFF3 monomer has no effect. Parenteral administration of TFF peptides aggravates the colitis especially the TFF3 monomer.

Keywords: TFF; Mitomycin C; Dextran sulphate sodium

1. Introduction

The trefoil factor family (TFF) consists of three peptides, TFF1, TFF2 and TFF3, which are cosecreted with mucus from mucus-producing cells and which by binding to mucus and by complex formation are able to influence the viscosity of mucus. They contain one or two trefoil domains defined as a sequence of 38 or 39 amino acid residues which by means of three disulphide bonds create a characteristic structure consisting of three loops. TFF2 has two trefoil domains, whereas TFF1 and TFF3 have one, but both are able to form dimers [1–3]. TFF1, previously known as pS2, breast-cancer-associated peptide, and TFF2, previously known as SP, spasmolytic polypeptide, are primarily localized to the stomach [4–6], whereas TFF3, known as intestinal trefoil factor (ITF), is more generally distributed to mucus-secreting cells and glands in all organ systems, including the goblet cells of the intestines [7–13]. Under pathophysiological conditions, all three TFF peptides can be up-regulated, and, in the GI tract, they are found in the ulcer-associated cell line (UACL) cells, which are cells that

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arise in areas with ulceration or inflammation [14–16]. A main function of the TFF peptides is considered to be regulation or modification of the viscosity of mucus-containing exocrine secretions on mucosal surfaces [17–20]. Recent investigations have disclosed that the three TFF peptides differ in respect to tertiary structure and surface charge [21–23]. TFF2 has a very compact structure in comparison to the others, and the highest viscosity is obtained following interaction between mucus and TFF2, whereas TFF3, in its dimeric form, produces a mucus/TFF complex with lower viscosity and elasticity, and TFF3 monomer is without any direct effect on the viscosity [20].

A receptor-like activity to the trefoil peptides has been demonstrated throughout the gastrointestinal tract [24–27]. In several experimental studies, it has been demonstrated that both luminally administered and injected TFF2 and TFF3 in the dimeric form increase the resistance of the mucosa of the stomach and accelerate the healing of gastric ulcers [28–33]. A similar effect might be expected in the intestinal system, but the results of experimental studies have been less consistent and convincing there than in the upper gastrointestinal tract. TFF3 knockout mice had increased sensitivity to dextran sulphate sodium (DSS) colitis [34], and luminal treatment with TFF2 increased the healing of DNBS-induced colitis in rats [35]. Recently, Soriano et al. [36] found positive effects on clinical parameters following both luminal and systemic pretreatment with TFF2 in DSS-induced colitis, but there was no effect on the histological parameters and no effect on existing colitis. In the present study, we have investigated the effect of both systemic and luminal treatment with TFF2 and TFF3 monomer and dimer in two rat models of experimental colitis. In order to obtain adequate luminal treatment, we have introduced an experimental model, in which rats are fitted with a proximal colonic catheter for direct intraluminal administration. We demonstrate an effect of luminal treatment with TFF2 and TFF3 dimer in both rat models, and a highly significant aggravation following systemic treatment, especially following the monomer.

2. Material and methods

2.1. Animals

The experimental studies were approved by the Danish National Committee of Animal Studies. One hundred twenty-eight female Wistar rats weighing approximately 200 g were used in the study. They were maintained throughout the course of the experiment on water and chow (no. 1314, Altromin, Lage, Germany) ad libitum in the animal facilities of the Panum Institute, University of Copenhagen, Copenhagen, Denmark, with temperature (21 °C) and humidity (55%) controlled rooms with a light–dark cycle of 12 h each.

2.2. Induction of colitis

Two models of experimental colitis were used in the study, that induced by dextran sulphate sodium (DSS) and that induced by mitomycin C. DSS colitis was induced by giving 5% DSS (cat. no. 160110, ICN Biomedicals, OH, US) in the drinking water for 10 days. The rats were kept in single cage, and their water intake was monitored each day to secure that there were no differences in DSS intake between the groups. Treatments were given from the day before initiation of DSS exposure until sacrifice on day 10.

Mitomycin C colitis was induced by one single intraperitoneal injection of mitomycin C (cat. No. 100498, ICN Biomedicals), 3.75 mg/kg. Treatment was given for 4 days from day 4 to day 7 inclusive, where the rats were sacrificed.

The rats were sacrificed by an overdose of methohexital (Brietal, Lilly, USA). The abdomen was opened by a midline incision, and the colon was fixed in situ by intraluminal injection of ice-cold 0.1 mol/l phosphate buffer, pH 7.4 with 4% paraformaldehyde to slightly distend the colon to avoid mucosal foldings. After 5 min, the colon, including the anal canal, was taken out, cut open antimesenterially and suspended on a polyethylene plate. After fixation for a further 24 h, the specimens were rinsed in tap water and surface-stained as whole mounts with Alcian Green 3BX for 30 min. The colonic specimens were examined using a Wild Photomacroscope, and the extent of disease in the colon was quantified in a blinded way. Mitomycin colitis results in a granulated surface with small shallow ulcerations, and the DSS colitis in areas with flat erosions, both easily distinguished from the normal mucosa (Fig. 1A–C).

For histological analysis, specimens, approximately 2 -m cut in the longitudinal direction, were taken out in a blinded way from the middle and distal colon. Histological sections of 5 μ were stained with PAS–hematoxylin–aurentia. In DSS colitis, there is primarily epithelial atrophy, formation of erosions and mucosal inflammation, which progress to the other layers (Fig. 1D–H). The severity of colitis was evaluated in a blinded way by means of a histological scoring technique as published by Williams et al. [37].

2.3. Treatment groups

The rats were divided into groups of eight. In half of the rats, treatments were given as subcutaneous injections twice daily, and, in the other half, they were given directly into the colonic lumen via a polyethylene tube which, 5 days prior to the experiment in anaesthetized rats, was inserted into the lumen of the proximal part of the colon. The catheter, measuring 0.8×1.6 mm (in inner and outer diameter) and approximately 15 cm in length was fitted with a 3-mm piece of a catheter measuring 1.4×2.0 mm (inner and in outer diameter) in each end (4 mm from the ends of the catheter). A little incision was made antimesenterially in the proximal colon, and the end of the catheter with the small outer polyethylene tube was