An Experimental Model of Prolonged Esophagitis With Sphincter Failure in the Rat and the Therapeutic Potential of Gastric Pentadecapeptide BPC 157

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Abstract. We report a simple novel rat model that combines prolonged esophagitis and parallel sphincters failure. The anti-ulcer gastric pentadecapeptide BPC 157, which was found to be stable in gastric juice, and is being evaluated in inflammatory bowel disease trials, is an anti-esophagitis therapy that recovers failed sphincters. Twelve or twenty months after the initial challenge (tubes sutured into sphincters for one week and then spontaneously removed by peristalsis), rats exhibit prolonged esophagitis (confluent hemorrhagic and yellowish lesions, thinner epithelium and superficial corneal layer, with stratification derangement); constantly lowered pressure of both sphincters (assessed by using a water manometer connected to the drainage port of a Foley catheter implanted into the stomach either through esophageal or duodenal incision); and both lower esophageal and pyloric sphincter failure. Throughout the esophagitis experiment, BPC 157 was given at either 10 µg/kg, i.p., once a day (last application 24 h before assessment) or alternatively, it was given continuously in drinking water at 0.16 µg/ml (12 ml/rat). This treatment recovers i) esophagitis (macroscopically and microscopically, at either region or investigated time period) and ii) pressure in both sphincters (cmH₂O). In addition, BPC 157 (10 µg/kg) or saline (1 ml/rat, 5 ml/kg) was specifically given directly into the stomach; pressure assessment was performed at 5 min thereafter. The effect of BPC 157 is specific because in normal rats, it increases lower esophageal sphincter-pressure, but decreases pyloric sphincter-pressure. Ranitidine, given as the standard drug using the same protocol (50 mg/kg, i.p., once daily; 0.83 mg/ml in drinking water; or 50 mg/kg directly into the stomach) had no effect.

Keywords: BPC 157, esophagitis, sphincter (rat)

Introduction

Surprisingly, to date, there has been no suitable model for esophagitis disorders with existing but functionally defective sphincter(s). Apart from clinical observations, no approach combining maintained esophagitis mucosa and sphincters tone has been developed experimentally. Using a new suitable rat model for this purpose, we focus on the therapeutic effects of the gastric pentadecapeptide BPC 157 on esophageal mucosa integrity and the lower esophageal and pyloric sphincter.

BPC 157 is a stable anti-ulcer peptide (1 – 7), particularly in human gastric juice (8), with no toxicity reported so far (9). The gastric pentadecapeptide BPC 157 is now in trials for the treatment of inflammatory bowel disease (coded PL-10, PLD-116 by Pliva, Croatia) and wound healing (5, 6) (including muscle healing after complete transection) (10). It is more effective than standard agents (1, 4, 7, 11), and it also protects the esophageal mucosa (7) and mediates salivary gland function (4). It has been recognized as a peptide basal protectant in saliva and gastric juice
(4, 12), and this pentadecapeptide has recently been suggested to be the answer to the longstanding Pavlov’s gastric juice physiology (12). Encouragingly, it alleviated rat esophago-jejunal anastomosis-esophagitis (7) when it was given in drinking water for one month, unlike standard agents (ranitidine, sucralfate, cholestyramine) that did not. This stable peptide induces esophageal mucosal healing promptly and then maintains the healing for the whole period of our study, counteracting an otherwise aggressive esophagitis.

More attention should probably be focused on unresolved esophagitis disorders with existing, but failed, sphincter(s). Regardless of being harmful, an atypical gastrointestinal tract with diverse mutilations and/or reconstructions, or without sphincter(s) (13–24), could not solve this problem. Besides, only a few studies have investigated this correlation in the rat (15), the commonly used species in esophagitis studies. Furthermore, procedures on the rat, like esophageal myectomy or crural myotomy that are used to decrease esophageal lower sphincter pressure, could not produce esophagitis for a period of one month (15).

In this study, the esophagitis rats with failure of both sphincters can not spontaneously heal. However, they positively respond following adequate therapy. In this procedure, we insert one tube in the lower esophageal sphincter, another tube in the pyloric sphincter, provoking in this way a stretch-muscle injury in both sphincters, and loss of their dynamic function. After one week, both tubes are naturally evacuated by peristalsis. Initially, multiple erosions appear with occasional ulceration and yellowish exudates. Within 24 h, edema and thickening of the submucosal layer develop, with notable changes of the superficial epithelium, and a significant decrease of pressure in both sphincters (25).

Unlike peroral ranitidine or sucralfate, pentadecapeptide BPC 157 is beneficial given in drinking water. Interestingly, this simple novel rat model allows combining prolonged esophagitis with sphincters failure. Bearing in mind the considerable healing potential of gastric pentadecapeptide BPC 157 (1–12, 25, 26), we examine whether its protection of esophageal mucosa corresponds with the maintained sphincters pressure throughout the 12–20-month period. We also hypothesize that pentadecapeptide BPC 157 could instantly recover lower esophageal and pyloric sphincter pressure disturbed in rats after 12–20 months of untreated esophagitis. Assuming the distinctiveness of sphincteric failure in esophagitis rats, its effect is substantiated in normal rats as well. Ranitidine, an H₂-receptor antagonist, is used as the standard agent.

Materials and Methods

Animals

Wistar Albino female rats (200 g b.w.) were randomly assigned in the experiments, and all experiments were approved by the Local Ethic Committee. All experiments were carried out under anesthetic protocol, and the effect was assessed by examiners who were completely unaware of the given protocol.

Drugs

Pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, M.W. 1419), (Diagen, Ljubljana, Slovenia) dissolved in saline was used in all experiments. The peptide BPC 157 is part of the sequence of human gastric juice protein BPC, and it is freely soluble in water at pH 7.0 and in saline. It was prepared as described previously (1–7, 25–39), with 99% high pressure liquid chromatography (HPLC) purity, having 1-des-Gly peptide as an impurity. Ranitidine (Sigma, St. Louis, MO, USA) dissolved in saline was accordingly used (1, 4, 7, 28, 30).

Esophagitis induction, therapy protocol, and assessment

To provoke esophagitis in an intact gastrointestinal tract, using the reflux of gastroduodenal contents, one tube was placed and sutured into pylorus, and another tube was placed into the lower esophageal sphincter. The procedure was carried out under deep anesthesia. The tubes were naturally evacuated by peristalsis by day 7. The assessment was performed 12 or 20 months thereafter.

Gastric pentadecapeptide BPC 157 (10 µg/kg), ranitidine (50 mg/kg), or 0.9% NaCl (5 ml/kg) was given i.p., once a day, with the first application 30 min following surgery and the last application, 24 h before sacrifice. Alternatively, gastric pentadecapeptide BPC 157 (0.16 µg/ml), ranitidine (0.83 mg/ml), or nothing was given in drinking water (12 ml/rat) until the sacrifice.

Macroscopical assessment was done accordingly with scores of 0–4 using direct esophagus scanning (ScanMaker i900; Microtek, Willich, Germany) as described previously (23): normal glistening mucosa (score 0), edematous mucosa with focal hemorrhagic spots (score 1), multiple erosions with hematomas attached (score 2), tiny esophagus with hemorrhagic and linear yellowish lesions (score 3), tiny esophagus with coalesced hemorrhagic and yellowish lesions (score 4).

Thereafter, microscopical analysis (using the special
program ISSA; VAMSTEC-Software Company, Zagreb, Croatia) was carried out (7). Briefly, after evisceration, the esophagus, stomach, and duodenum were spread out on a styropore board and fixed for 24 h in 10% neutral buffered formalin. The lateral margins were inked, and the esophagus was split longitudinally and divided into three segments (proximal, medial, and distal including a segment of the stomach). These segments were embedded in paraffin so that the whole length of the segment could be sectioned. The blocks were cut on a sliding microtome at 4-µm thickness and stained with hematoxylin-eosin. Consecutive fields of each segment were scanned using a video camera (CTK1270; Sony, Tokyo) and put in a database (ISSA, VAMSTEC). For each segment, an overview image was first created and then each segment was scanned with a 6.3 objective. These images were used for morphometrical assessment of epithelial thickness, keratosis layer thickness, and deranged structure of the epithelium presentation (scored 1–3: 1, less than 10% of epithelium length has visible nuclei throughout whole epithelium thickness; 2, 10%–50% of epithelium length has visible nuclei throughout whole epithelium thickness; 3, over 50% of the epithelium length has visible nuclei throughout the whole epithelium thickness).

Lower esophageal sphincter and pyloric sphincter pressure

To directly assess the lower esophageal sphincter and the pyloric sphincter pressure, healthy rats or those with prolonged esophagitis (for 12 or 20 months) that received once daily saline (5.0 ml/kg) or pentadecapeptide BPC 157 10 µg/kg (last application 24 h before assessment), i.p. randomly underwent an operation under deep anesthesia to implant a Foley catheter into the stomach, through the esophageal (lower esophageal sphincter pressure assessment) or the duodenal incision (pyloric sphincter pressure assessment). Gastric pentadecapeptide BPC 157 (10 µg/kg), ranitidine (50 mg/kg), or 0.9% NaCl (5 ml/kg) was given directly into the stomach (1 ml/rat). After 5 min, a manometrical evaluation (cmH₂O) was performed with a water manometer connected to the drainage port of the Foley catheter. The proximal side of the esophageal or distal side of the duodenal incision was ligated to prevent regurgitation.

Statistical analyses

Statistical analysis was performed by non-parametric Kruskal-Wallis ANOVA and subsequent Mann-Whitney U-test to compare groups. Values of P<0.05 were considered statistically significant.

Results

The long lasting result of the initial procedure (insertion of tubes for only a week to provoke sphincter failure) is permanently decreased sphincters pressure, assessed after 12 or 20 months of prolonged esophagitis. On the contrary, when pentadecapeptide BPC 157 is given throughout the experiment, the sphincter pressure is stable. This effect is apparently not random since it was seen in both sphincters, after either 12 or 20 months. It is also present during 24 h following application of BPC 157 (Fig. 1) in rats treated daily with BPC 157, with the last application at 24 h prior to the saline application before the sphincter pressure assessment. Importantly, the sphincter pressure was maintained at normal values in rats treated with pentadecapeptide and this corresponded to the attenuation of esophagitis (Figs. 2 – 4).

Considering esophagitis attenuation, BPC 157 is effective by either intraperitoneal application or when it is given in drinking water. Macroscopically and microscopically esophageal lesion attenuation includes all regions and all investigated time intervals. Grossly, advanced esophagitis in controls characterized by the regular confluent hemorrhagic and yellowish lesions that appear in advanced esophagitis (Figs. 2 and 4) are strongly counteracted by treatment with BPC 157. Microscopically, controls present more pronounced subepithelial and muscular edema, mononuclear infiltration, with thinner epithelium and superficial corneal layer than naive healthy or BPC 157-treated esophagitis animals. Controls have a stratification derangement in contrast to healthy or BPC 157-treated esophagitis animals (Figs. 2 – 4).

Pentadecapeptide BPC 157 shows a prolonged effect manifesting as a stable sphincter pressure for at least 24 h after its last application. It also has an immediate effect on the sphincter pressure. This could be seen when assessed at 5 min after its direct application in the stomach in esophagitis rats treated daily with saline, with the last application at 24 h before sphincter pressure assessment, and with decreased pressure of both sphincters. BPC 157 consistently recovers otherwise decreased pressure of both lower esophageal sphincter and pyloric sphincter. Notably, given in the condition of normal sphincter pressure, in healthy rats, pentadecapeptide increases lower esophageal sphincter pressure and decreases pyloric sphincter pressure (Fig. 1).

Values obtained after ranitidine application, given as the standard agent and using the same protocol, are not different from values seen in the controls (Figs. 1, 3, and 4).
Discussion

BPC 157 decreases dysfunction of lower esophageal and pyloric sphincters, in this way preventing regurgitation of gastroduodenal content into the esophagus, and apparently attenuates esophagitis. The positive effects are the same after 12 or 20 months of esophagitis [as well as at the earlier intervals (25, 26)]. Therefore, they are not random, particularly since ranitidine, a potent anti-secretory H₂-receptor blocker, is not effective. Interestingly, in normal rats, this peptide acts on these sphincters by increasing the lower esophageal sphincter and decreasing the pyloric sphincter pressure. Such effects of pentadecapeptide BPC 157 on the pyloric sphincter could suggest a particular anti-reflux mechanism and sphincter balance in normal or pathological conditions. This seems to be supported by the other results presented in this study.

Generally, we note in control esophagitis rat sphincters pressure values that are consistently lower than those in the healthy rats, at both shorter (25, 26) and longer investigated time intervals. They could not heal without therapy, a situation similar to that in esophagitis patients; healthy individuals have protection against changes in sphincter pressure, which is deficient in individuals with esophagitis (40). Because of that, a therapy is only beneficial if it improves mucosal integrity and promotes recovery of failed pressure of the sphincters. Thus, in animals with severe esophagitis, the best indication of their recovery should be regaining of sphincter pressure levels to near the healthy levels. Indeed, in esophagitis rats treated with pentadecapeptide BPC 157, the values are similar to those in the healthy ones. Furthermore, a consistent positive outcome with pentadecapeptide BPC 157, both in esophagitis rats and in healthy rats (prevention of ulcer development, corresponding prophylactic and therapeutic effect (1 – 7, 10, 11, 25 – 39)] is noted. This goes with its unusual capability of

Fig. 1. Lower esophageal sphincter and pyloric sphincter pressure (cmH₂O). Min/Med/Max. Gastric pentadecapeptide BPC 157 (10 µg/kg) (B), ranitidine (50 mg/kg) (R), or 0.9% NaCl (5 ml/kg) (S) given directly into the stomach (1 ml/rat) 5 min before direct assessment of lower esophageal sphincter and pyloric sphincter pressure in rats with prolonged esophagitis (for 12 or 20 months) (esophagitis rats) (left) that had been treated i.p. once daily with saline (5 ml/kg), pentadecapeptide BPC 157 (10 µg/kg), or ranitidine (50 mg/kg) (last application 24 h before assessment) or in healthy rats (right). There are 10 rats per experimental group. *: gastric pentadecapeptide BPC 157 (0.9% NaCl + BPC 157, BPC 157 + 0.9% NaCl, BPC 157 + BPC 157) vs control (0.9% NaCl + 0.9% NaCl) or gastric pentadecapeptide BPC 157 vs control (0.9% NaCl), P<0.01, at least.
decreasing (seen in normal) or increasing (seen in esophagitis) pyloric sphincter pressure. Otherwise, without an active anti-reflux mechanism for the pyloric sphincter, the esophagitis would be aggravated because of exaggerated reflux due to further decline of the already diminished pressure values. With an over-increased pyloric sphincter tone, the damage should also appear in previously healthy rats. Likewise, this capability of pentadecapeptide BPC 157 could be important for its activity against other ulcers. For instance, cysteamine damages strongly correlate with delayed gastric emptying (41) and BPC 157 strongly reduces cysteamine lesions (1, 4).

Its ability to exert esophageal protection targets pyloric sphincter and duodenogastroesophageal reflux, which is potentially more damaging than pure acid reflux (42) and can explain ranitidine inefficacy in this study. It is also known that the role of gastric acid (20, 43) and gastric juice in inducing esophagitis (44) contrast with esophagitis after gastrectomy (7, 16). BPC 157 is also advantageous over famotidine in the inhibition of gastric lesions in pylorus-ligated rats (11), but it has no effect on gastric acid secretion or gastric juice volume in rats with pylorus-ligation or gastric-fistulas (27, 45, 46). Therefore, it is obvious that pentadecapeptide BPC 157 induced protection of esophagus (7, 25, 26) stomach, duodenum, and intestine (1 – 6, 9, 11, 28 – 30, 36 – 39, 47 – 49) through other non-secretory mechanisms (Takeuchi, personal communication).

BPC 157 also affords cytoprotection (an effect independent from gastric acid) (28) and protection against indomethacin or aspirin lesions (11, 29). The ability of BPC 157 to heal gastric lesions induced by chronic alcohol drinking (30) or acetic acid (11, 45) may be relevant for the mechanism of chronic esophagitis attenuation. Concurrent administration of indomethacin, which suppressed PGE\(_2\) generation, almost completely reversed the acceleration of ulcer healing and the accompanying increase in gastric mucosal blood flow at the ulcer margin otherwise evoked by this peptide (45). Pentadecapeptide BPC 157 also controls salivary gland function and maintains gastrointestinal mucosal integrity after sialoadenectomy (4), unlike non-effective ranitidine and other standard anti-ulcer agents. These beneficial effects should also attenuate esophago-jejunal anastomosis-esophagitis (7) when BPC 157 is given in drinking water for a one-month period.

Moreover, besides the described cytoprotective effect relevant for both esophageal mucosa and sphincters integrity, several lines of further evidence support the crucial involvement of pentadecapeptide BPC 157. Firstly, the consistently improved dysfunction of lower
Fig. 3. Epithelial thickness and keratosis layer thickness, µm. Min/Med/Max. Controls exhibit a thinner epithelium and superficial corneal layer than naive healthy or BPC 157-treated esophagitis animals. Gastric pentadecapeptide BPC 157 (10 µg/kg) (B), ranitidine (50 mg/kg) (R), or 0.9% NaCl (5 ml/kg) (C) was given intraperitoneally, once daily, first application 30 min following surgery, last application 24 h before sacrifice, or pentadecapeptide BPC 157 (0.16 µg/ml) (B), ranitidine (0.83 mg/ml) (R), or nothing (C) was given in drinking water (12 ml/rat), until the sacrifice. There were 10 rats per experimental group. *: gastric pentadecapeptide BPC 157 vs control, $P<0.01$, at least.

Fig. 4. Scoring of presentation of the deranged structure of epithelium (scored 1–3) and the gross esophageal lesion (scored 0–4). Min/Med/Max. The deranged epithelium stratification was advanced in controls and gastric pentadecapeptide BPC 157-treated rats showed less esophageal gross lesions. Gastric pentadecapeptide BPC 157 (10 µg/kg) (B), ranitidine (50 mg/kg) (R), or 0.9% NaCl (5 ml/kg) (C) was given intraperitoneally, once daily, first application 30 min following surgery, last application 24 h before sacrifice (upper), or pentadecapeptide BPC 157 (0.16 µg/ml) (B), ranitidine (0.83 mg/ml) (R), or nothing (C) was given in drinking water (12 ml/rat), until the sacrifice (lower). There were 10 rats per experimental group. *: gastric pentadecapeptide BPC 157 vs control, $P<0.01$, at least.
esophageal and pyloric sphincters was associated with pentadecapeptide BPC 157 given directly into the stomach lumen. That BPC 157 rapidly affects sphincters pressure is not surprising considering its long stability in human gastric juice (8) and rapid anti-ulcer effect against ethanol-necrotizing lesions when given intragastrically (1, 28). Given systemically, it maintains the pressure of both sphincters for at least 24 h. In addition, the role of this peptide is supported by the interactions with sphincter controlling systems (2, 3). Commonly, neural input controls lower esophageal sphincter relaxation as a function of NANC innervation, with significant influence of nitric oxide (NO) (50) and the gut sphincters richly innervated by the peptidergic nerves (51), like the rest of the gastrointestinal tract, with the contribution of central mechanisms (50). Importantly, the pentadecapeptide BPC 157 has an anti-inflammatory effect (29), and its amelioration of esophago-jejunal anastomosis-esophagitis corresponds to the reduction of inflammatory cells (7) and reduced LTB4, TXB2, and MPO in serum and inflamed tissues (47). All these together could significantly influence afferent nerve function (52): as seen in capsaicin studies, it interacts and recovers somatosensory neurons system function, in both adult and new born rats (2). It considerably affects the NO-system: in different species (3, 31, 53), both in vivo and in vitro, it modulates NO-synthesis, as well as NO-agonist and NO synthase (NOS)-blocker effects (3, 31, 53, 54). BPC 157 has a strong angiogenic effect (39), directly protects endothelium (1), and counteracts endothelin over-expression (32). Because of the central nervous system (CNS)/gastrointestinal tract interactions, pentadecapeptide BPC 157 given peripherally could affect sphincter function through serotonin (33, 34) and dopamine (35, 36) systems. It may also prevent/reverse catalepsy or stereotypes due to central dopamine system failure (35, 36). Of note, all concomitant gastro-intestinal lesions, induced by haloperidol (36), reserpine (37), or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (38), are strongly antagonized with pentadecapeptide BPC 157 used in the same dose-range.

Exhibiting a defined peptidergic activity, BPC 157 is regularly given without a carrier. Therefore, the positive effects of BPC 157 are a direct consequence of its own peptidergic activity (1 – 7, 25 – 39, 53). It is suitably stable (1 – 12, 25 – 39, 47 – 49, 53) because it has intact presence in gastric juice for more than 24 h, unlike the labile standard peptides like EGF or TGF-β that are degraded within minutes (8). Unstable standard peptides, like EGF (55), which are supposed to protect esophageal mucosa, need carrier(s) addition, forming peptide + carrier(s) complex, and raising unavoidable methodology/activity dilemmas (for reviews, see refs. 56 and 57).

An additional benefit of pentadecapeptide BPC 157 is its effect in muscle healing (10). Given systemically, after complete transection of the rat quadriceps muscle, peptide BPC 157 leads to full muscle healing (evaluated functionally, biomechanically, macroscopically, microscopically, and immunohistochemically) (10). Likely, the earliest function improvement (10) may be analogous to the sphincter function improvement noted in the present study.

In summary, we propose that pentadecapeptide BPC 157, presently in trials for inflammatory bowel disease (9), could be successful for treatment of esophagitis disorders. As a potent cytoprotective anti-ulcer agent (1 – 7, 9, 11, 25 – 39, 45 – 49, 53) that promotes wound healing (5, 6), including healing of the transected major muscle (10), the stable gastric pentadecapeptide BPC 157 could have combined strong effects on mucosal and muscle recovery. It interacts with the function of the controlling systems [somatosensory neurons system/NOS-system, central serotonin (33, 34) and dopamine (35, 36) system]; and particularly, it could be worthy as the recognized stable peptide-protectant in the saliva and basal gastric juice and the answer to the longstanding Pavlov’s gastric juice physiology (4, 12).

References


