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Stable gastric pentadecapeptide BPC 157 heals rectovaginal fistula in rats

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ABSTRACT

Aim: Rectovaginal fistula is a devastating condition providing more than 99% of patients for surgical treatment. We hypothesized that rectovaginal fistula may be healed by therapy with stable gastric pentadecapeptide BPC 157, in consistence with its initial clinical application and effect on external fistulas.
Main methods: BPC 157 (10 μg/kg or 10 ng/kg) was given perorally, in drinking water (0.16 μg/ml or 0.16 ng/ml, 12 ml/rat/day) till sacrifice, or alternatively, intraperitoneally, first application at 30 min after surgery, last at 24 h before sacrifice. Controls simultaneously received an equivolume of saline (5.0 ml/kg ip) or water only (12 ml/rat/day). The assessment (i.e., rectal and vaginal defect, fistula leakage, defecation through the fistula, adhesions and intestinal obstruction as healing processes) was at day 1, 3, 5, 7, 10, 14 and 21.
Key findings: Regularly, rectovaginal fistulas exhibited poor healing, with both of the defects persisting, continuous fistula leakage, defecation through the fistula, advanced adhesion formation and intestinal obstruction. By contrast, BPC 157 given perorally or intraperitoneally, in μg- and ng-regimens rapidly improved the whole presentation, with both rectal and vaginal defects simultaneously ameliorated and eventually healed. The maximal instilled volume was continuously raised till the values of healthy rats were achieved, there were no signs of defecation through the fistula. A counteraction of advanced adhesion formation and intestinal obstruction was achieved. Microscopic improvement was along with macroscopic findings.
Significance: BPC 157 effects appear to be suited to induce a full healing of rectovaginal fistulas in rats.

1. Introduction

Recto-vaginal fistula is a devastating condition. While more than 99% of patients need surgical treatment [1] we hypothesized that rectovaginal fistulas may be healed by the therapy with the stable gastric pentadecapeptide BPC 157, in consistence with its initial clinical application [2–7] and the effects on rat external fistulas' healing [8–11].

BPC 157, GEPPPGKPADDAGLV, M.W. 1419, now in a multiple sclerosis trial [2–7], is originally an anti-ulcer peptide, stable in human gastric juice, designated to be a novel mediator of cytoprotection [6], effective in the whole gastrointestinal tract [2–7], LD1 not achieved, implemented in inflammatory bowel disease trials [2–7], with particular effects in external fistulas' healing [8–11]. These BPC 157's effects were considered as a particular wound healing ability in both internal [7, 12, 13] and external wounds [14–16] as recently reviewed [2–7].

Of note, the evidence is that the fistula's healing is not a simple repeat of separate defects healing but also taking into account all the healing particularities of the fistula healing [8–11]. And thereby, this hampers firm generalization and requires specific demonstration for any fistula healing [8–11]. Especially, it is commonly acknowledged that the healing of the rectovaginal fistulas, experimentally [1, 23–25],and clinically [17–22], represents a particular issue.

A particular hallmark for rectovaginal fistulas healing was most recently, the bio-prosthesis in repairing rectovaginal fistulas, i.e., human amniotic membrane, suggested due to its efficacy as a biologic dressing in burn wound [1]. Thereby, an additional wound healing background would be needed to realize these fistulas' healing and potential agent's efficacy.

Consequently, BPC 157 therapy of the wound-healing process in different tissues (including blood vessels) [2, 26–28] emphasizes in particular burn wound healing [14–16], and likely indicates rectovaginal fistulas healing. Also BPC 157 exhibits an effect on particular genes functions [8, 29–32]. There, in the healing process, BPC-157 regulated the phosphorylation level of extracellular signal-regulated kinases 1 and 2 (ERK1/2) as well as its downstream targets, including c-Fos, c-Jun, and

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egr-1, key molecules involved in cell growth, migration, and angiogenesis [8, 29–32] and affected eNOS-gene function as well [8].

Furthermore, it may be that the healing of the various rat external fistulas [8–11] as an abnormal connection made by surgical anastomosis between the corresponding tissues is alongside with the healing of both deep skin burn wound and gastrointestinal tract lesions, esophageal, gastric, duodenal and colonic [8–11]. And thereby, likely, BPC 157 might synchronize the healing of different tissues [8–11]. In support, as recently emphasized [10], these experimental fistulas, precisely made [8–11] and controlled and real clinical fistulas (for review see i.e., [33]) - whatever pathology background - have in common a marked fistulas leaking, poor or no healing, and even devastating consequences. These were all accordingly counteracted by BPC 157 administration in rats subjected to fistulas creation [8–11]. Consequently, providing the common poor healing background and multifactorial etiology of rectovaginal fistulas (obstetrical trauma and IBD as the most prevalent, and then, colorectal or gynecological surgery, infections, tuberculosis and lymphogranuloma venereum, radiation therapy, cancers or cancers recurrence) [17–22] it seems that all of these rectovaginal fistulas would be commonly suitably approached by these fistulas' disturbances in rats. Thereby, BPC 157 should heal the internal fistulas, and, in particular, those rectovaginal fistulas. Here, rats' rectovaginal fistulas, 5 mm defect versus 2.4 cm vaginal length, ascertain long lasting huge defects and fistula spontaneous patency continuously experienced.

Especially, rectovaginal fistulas are considered complex if they are large (>2.5 cm) [22]. And thereby, that healing in rats fistulas should be experimentally highly relevant, and these rats' fecal leaking through vagina would depict embarrassing symptoms and high healing failure rate of rectovaginal fistulas in patients. By contrast, the rabbits model (used to be a small animal rectovaginal fistula model also made by direct sutured anastomosis between rectum and vagina) has relatively lesser defects (6 mm defect versus 10 cm vaginal length) [23, 24]. Unlike rats, both rabbits [23, 24] and dogs (6 mm defect versus 14 cm vaginal length) [1]), required inserted tube into to the defect to maintain fistula patency [1, 23, 24]. Furthermore, carefully defined defect in surgically made rats' rectovaginal fistulas, and thereby controlling of the defect healing, now introduced also in the rectovaginal fistulas research, may be methodologically useful also compared with the irradiated segment of the rectum of female rats and occasional rectovaginal fistulas observed in animals with severe chronic injury [25].

Recently, pentadecapeptide BPC 157, applied parenterally or perorally, ameliorates the stress urinary incontinence in rat models, improving the otherwise detrimental course of healing after vaginal dilatation and transabdominal urethrolysis, which may be analogous to human injury [34].

Consequently, taking all these data together [2–7], we focused on BPC 157 and its effect on the rectovaginal fistulas healing, as an internal fistula healing (i.e., closure macro/microscopically and biomechanically, no defecation through fistula, reduced passage obstruction, adhesion attenuation). For practical purpose, the stable gastric pentadecapeptide BPC 157 was given daily, intraperitoneally or perorally, in drinking water [2–7], using the previous efficacious regimens in the external fistulas healing [8–11].

2. Materials and methods

2.1. Animals

Wistar Albino female rats (200 g b.w.) were randomly assigned to the experiments (10 animals at least per each experimental group and interval), housed individually during the per-oral (drinking) study period, all of which were approved by the Local Ethic Committee. Furthermore, all experiments were carried out under blind protocol and the effect was assessed by examiners who were completely unaware of the given protocol.

2.2. 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 is freely soluble in water at pH 7.0 and saline. It was prepared as described previously with 99% high pressure liquid chromatography (HPLC) purity, expressing 1-des-Gly peptide as an impurity [8–11].

2.3. Procedure

In deeply anaesthetized rats, rectovaginal fistulas, were created. Longitudinal incision on the posterior wall of the vagina and anterior rectal wall (5 mm) were performed where a precise caliper was used to verify the initial size of the defect. Fistula was created using single-layer suture technique.

2.4. Experimental protocol after surgery

BPC 157 was given perorally, in drinking water (10 μg/kg or 10 ng/kg, 0.16 μg/ml or 0.16 ng/ml 12 ml/rat/day) till sacrifice, or alternatively, 10 μg/kg, 10 ng/kg intraperitoneally once time daily, first application at 30 min after surgery, last at 24 h before sacrifice. Controls simultaneously received an equivolume of saline (5.0 ml/kg ip) or water only (12 ml/rat/day). The assessment was at day 1, 3, 5, 7, 10, 14 and 21, as follows.

Rectal defect, vaginal defect, fistula assessment. Briefly, a precise caliper was used to verify the final size of the defect and the largest diameter of the rectal and vaginal defect was assessed (mm), photographed and further verified using the program ISSA (VAMSTEC Software Company, Zagreb, Croatia) as described before [8–11]. The tissue was processed for further microscopic analysis [8–11].

To assess fistula leakage [8–11] and the closure of the fistula, we assessed the volume (mL) that was sustained before the initiation of the leakage through the fistula. The volume of saline was infused through a syringe-perfusion pump system (Argus 600; Argus Medical A6, Heimberg, Switzerland) at the rate of 1 mL/10 s. The infusion was stopped at the point when the leakage through the external aperture of the fistula started. If there was no leaking till the end of the fifth minute, the fistula was considered to be functionally closed.

Fecal leaking through vagina. All rats were observed for fecal leaking through vagina.

Adhesion. Adhesion presentation was scored 0–7: 0, no adhesion; 1, thin adhesions covering less than one half of anastomosis; 2, more prominent adhesions with more than half of anastomosis covered; 3, exaggerated adhesions with whole anastomosis covered; 4, the mesenterial part of small bowel also included; 5 neighboring small intestine loop also included; 6, many neighboring small intestine loops included; 7, neighboring loops, stomach, liver “packed”, as described before [13].

Intestinal passage obstruction. Likewise, as described before [13], intestinal passage obstruction was scored 0–3, according to the loop diameter ratio, close to the fistula creation. Briefly, if loop diameters at 2 cm orally/loop diameters at 2 cm aborally = 1 passage is normal (score 0), between 1 and 1.33 is the sign of mild obstruction (score 1), between 1.33 and 1.66 is moderate obstruction (score 2), and more than 1.66 is severe obstruction (score 3).

Histopathological findings were scored (1–5) as described before [1], epithelialization (1: none; 2: none; 3: partial; 4: complete,

immature; 5: complete, mature), collagenization (1: none; 2: none; 3: partial; 4: complete, irregular; 5: complete, regular), inflammation (1: severe; 2: moderate; 3: mild; 4: none; 5: none); neovascularization (1: none; 2: none; 3: <5/high power field (HPF); 4: 6–10/HPF; 5: >10/HPF); necrosis (1: extensive; 2: focal; 3: none; 4: none; 5: none); granulation tissue (1: none; 2: immature; 3: mild mature; 4: mod mature; 5: fully mature).

2.5. Statistical analyses

Statistical analysis was performed by a non-parametric Kruskal-Wallis ANOVA and subsequent Mann-Whitney *U* test to compare groups. Fisher's exact probability test for defecation through fistula rate assessment was used. Values of $P < 0.05$ were considered statistically significant.

3. Results

Rats underwent rectovaginal fistulas presented a complex course. Rectal (Fig. 1) and vaginal (Fig. 2) defects, fistula leaking (Fig. 3), adhesions and intestinal obstruction (Fig. 4, Fig 5) were aggravated in controls (i.e., clearly presented rectal and vaginal defect macroscopically (Fig. 6) and microscopically (Fig. 7), fecal leaking through the vagina (Fig. 8)) versus a consistent closure of both defects and fistulas in all BPC 157 rats. Thus, beneficial effect of BPC 157 therapy resulted in healing, either given daily perorally in drinking water or intraperitoneally.

If not treated, rectovaginal fistulas exhibited poor healing (Table 1, Fig.1, Fig. 2, Fig. 3, Fig. 4, Fig. 5, Fig. 6, Fig. 7, Fig. 8), persistence of both defects (Fig. 1, Fig. 2, Fig. 6, Fig. 7, Fig. 8), continuous fistula leakage (Fig. 3, Fig. 8), advanced adhesion formation and intestinal obstruction (Fig. 4, Fig. 5). In rats given BPC 157, perorally or intraperitoneally, in μg- and ng-regimens, there was a consistent improvement of the complete presentation (Fig.1, Fig. 2, Fig. 3, Fig. 4, Fig. 5, Fig. 6, Fig. 7, Fig. 8) (the maximal instilled volume was continuously raised till the values of healthy rats) (Fig. 3), leading to eventual closure of both defects (Fig. 1, Fig. 2, Fig. 6, Fig. 7, Fig. 8), a counteraction of both advanced adhesion formation and intestinal obstruction (Fig. 4, Fig. 5). All control rats had fecal leaking through the vagina till the end of experimental period. By contrast, all BPC 157 rats had no signs of fecal leaking through the vagina (Fisher exact probability test $P < 0.05$, at least vs. control).

Generally, in rectovaginal fistula-rats, the microscopic presentation followed the described macroscopic healing course similarly to the previously described course of other fistula-rats [8–11] (Table 1, Fig. 6, Fig. 7, Fig. 8). BPC 157 treated animals showed closed fistula gap with mild inflammatory infiltration in epithelium and in the stroma, without pronounced edema. By contrast, in controls, the nonkeratinized squamous epithelium of the vagina and columnar rectal epithelium demonstrated a fistula gap with pronounced infiltration of inflammatory cells (mononuclear and polymorphonuclear cells), while stroma showed edema.

Specifically, as evidenced in Table 1, all BPC157 rats (both regimens given daily intraperitoneally or in drinking water) start with epithelialization between 3rd and 5th day, achieve complete epithelialization at 14th day, with maturation during 3rd postoperative week. By contrast, controls presented initial epithelialization since 10-14th day, and partial epithelialization within 21 days.

Alongside was collagenization course: initial collagenization in controls during 10–21 day while in all BPC 157 rats initial collagenization at 5th -7th day resulted with complete irregular collagenization during 10th–21st day (Table 1).

Finally, controls presented severe inflammation during first 7 postoperative days, and moderate inflammation during 2nd and 3rd postoperative week. In all pentadecapeptide BPC157 animals moderate inflammation was noticed during 3rd–7th day with regression to mild to the end of experiment (Table 1).

Commonly, controls exhibited diminished neovascularization process. By contrast, all BPC 157 animals presented neovascularization already at the 5th postoperative day. This results with more than 10

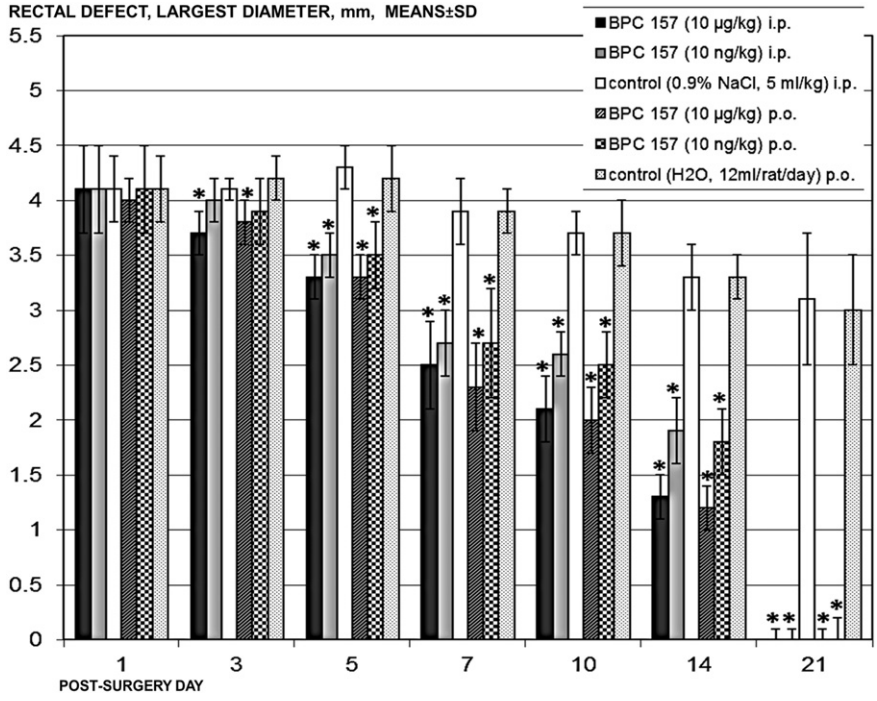

Fig. 1. Rectovaginal fistula in rats, rectal defect presentation, means ± SD, mm. BPC 157 was given perorally, in drinking water (10 μg/kg or 10 ng/kg, 0.16 μg/ml or 0.16 ng/ml 12 ml/rat/day) till sacrifice, or alternatively, 10 μg/kg, 10 ng/kg intraperitoneally, first application at 30 min after surgery, last at 24 h before sacrifice. Controls simultaneously received an equivolume of saline (5.0 ml/kg ip) or water only (12 ml/rat/day). The assessment was at day 1, 3, 5, 7, 10, 14 and 21.*$P < 0.05$, at least vs. control.

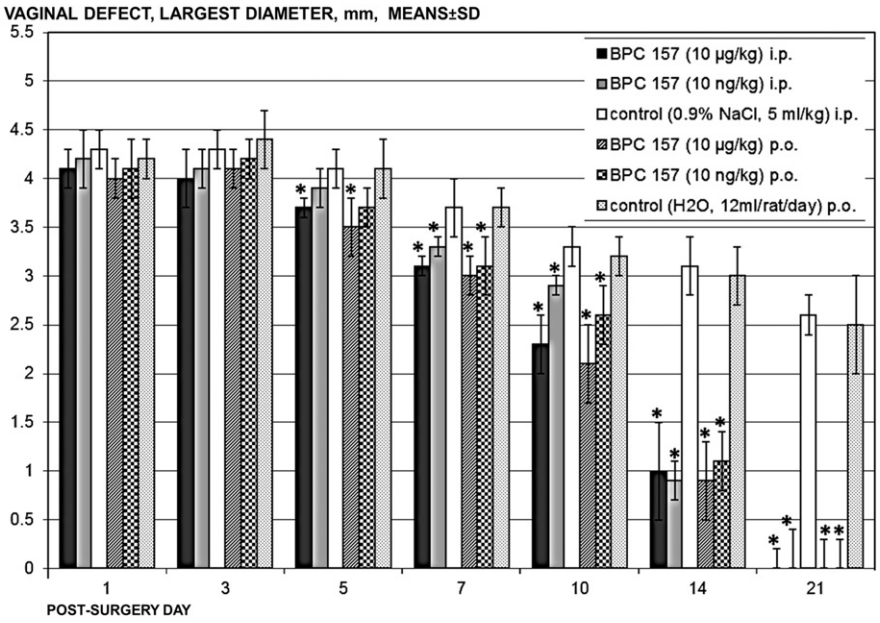

Fig. 2. Rectovaginal fistula in rats. Vaginal defect presentation, means ± SD, mm. BPC 157 was given perorally, in drinking water (10 μg/kg or 10 ng/kg, 0.16 μg/ml or 0.16 ng/ml 12 ml/rat/day) till sacrifice, or alternatively, 10 μg/kg, 10 ng/kg intraperitoneally, first application at 30 min after surgery, last at 24 h before sacrifice. Controls simultaneously received an equivolume of saline (5.0 ml/kg ip) or water only (12 ml/rat/day). The assessment was at day 1, 3, 5, 7, 10, 14 and 21.*$P < 0.05$, at least vs. control.

new formed blood vessels/HPF during third postoperative week (Table 1).

Focal necrosis was noticed in control animals during three postoperative weeks, while no necrosis was seen in pentadecapeptide BPC 157 animals (Table 1).

In controls, granulation tissue was not found till the 7th postoperative day (immature granulation tissue) (Table 1). By contrast, in pentadecapeptide BPC157 animals, a consistent finding was day immature granulation tissue up to 3rd postoperative, and mature granulation tissue since 5th postoperative day.

4. Discussion

We demonstrated that BPC 157, given perorally (in drinking water) or intraperitoneally, successfully healed rectovaginal fistulas in rats. Thereby, this consistent beneficial effect could be hardly disputed. Previously, the resolved various fistulas (esophagocutaneous [8], gastrocutaneous [9], duodenocutaneous [10] and colocutaneous [11]) commonly suggested the external fistulas healing as the BPC 157 successful healing. Consequently, this could be along with its initial clinical application in inflammatory bowel disease trials [2–6], regardless still

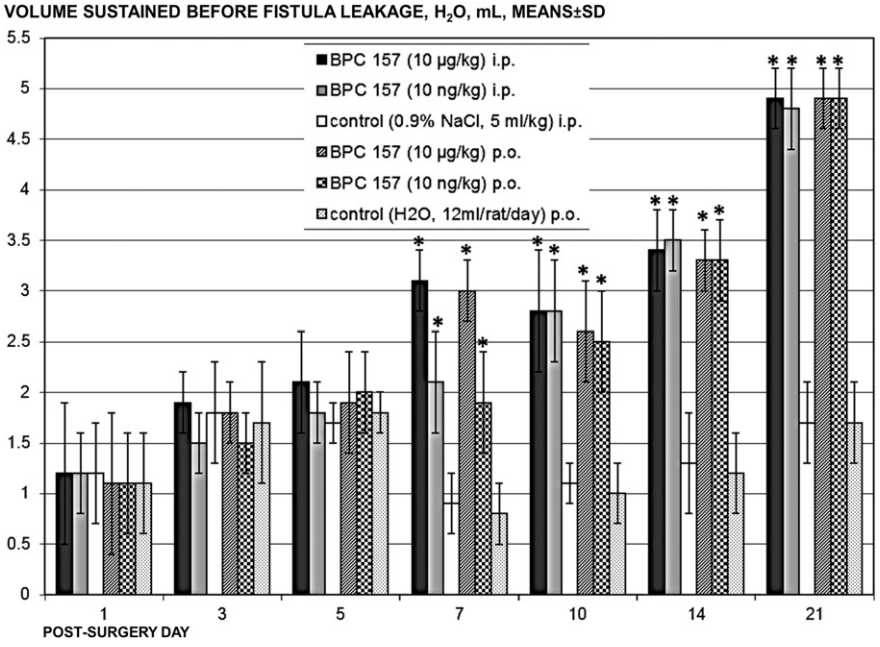

Fig. 3. Rectovaginal fistula in rats. Volume sustained before fistula leakage, means ± SD, ml. BPC 157 was given perorally, in drinking water (10 μg/kg or 10 ng/kg, 0.16 μg/ml or 0.16 ng/ml 12 ml/rat/day) till sacrifice, or alternatively, 10 μg/kg, 10 ng/kg intraperitoneally, first application at 30 min after surgery, last at 24 h before sacrifice. Controls simultaneously received an equivolume of saline (5.0 ml/kg ip) or water only (12 ml/rat/day). The assessment was at day 1, 3, 5, 7, 10, 14 and 21.*$P < 0.05$, at least vs. control.

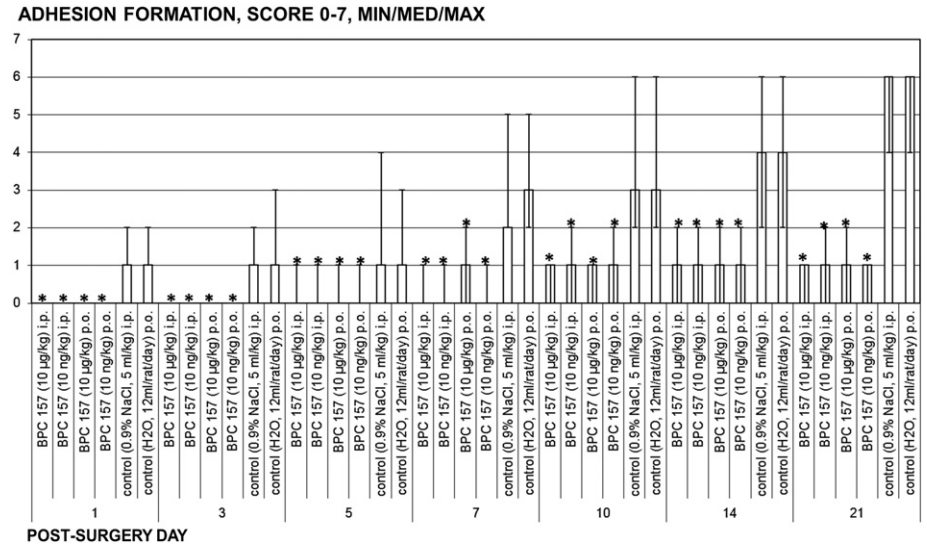

Fig. 4. Rectovaginal fistula in rats. Adhesion formation presentation, scored 0–7, min/med/max. BPC 157 was given perorally, in drinking water (10 μg/kg or 10 ng/kg, 0.16 μg/ml or 0.16 ng/ml 12 ml/rat/day) till sacrifice, or alternatively, 10 μg/kg, 10 ng/kg intraperitoneally, first application at 30 min after surgery, last at 24 h before sacrifice. Controls simultaneously received an equivolume of saline (5.0 ml/kg ip) or water only (12 ml/rat/day). The assessment was at day 1, 3, 5, 7, 10, 14 and 21.*$P < 0.05$, at least vs. control.

limited data. Conceivably, its healing of the rats' rectovaginal fistulas (since spontaneous only poor healing as those in humans [17–22]) could be also a realization of the internal fistula healing concept, an efficient "wound healing-capability" as the therapy of the complicated internal fistula healing.

Thereby, we suggest that the BPC 157 fistulas therapy [8–11] follows an essential healing commonality between the external and internal fistulas. Likewise, it follows some particularities in the rectovaginal fistulas as the internal fistulas healing.

With BPC 157, both defects (internal and external [8–11]; internal and internal in the present study) eventually healed simultaneously. BPC 157 simultaneously initiated the healing of both defects of the external fistulas [8–11]. However, in the rectovaginal fistulas, the BPC 157's healing starts with the rectal defects, and then affecting the vaginal defects. Likely, an already advanced healing of the rectal defect may be required to initiate the vaginal defect healing as well.

Consequently, the rectovaginal fistula healing model (the connected healing, resistant rectal defect healing and even more resistant vaginal defect healing requiring reciprocal healing with fistula closure as the end result of matched healing process(es) in both defects) methodologically resolves the therapeutic success of BPC 157 as a new therapy phenomenon.

Finally, more than an analogy with the largely advocated bioprosthesis efficacy as a biologic dressing in burn wound for next repairing rectovaginal fistulas [1], should be BPC 157 therapy in burn models [14–16, 32]. BPC 157 strongly accelerates the healing of severe burns, topically as a cream, or systemically, also counteracting the severe healing impairment induced by systemic corticosteroid application [14–16]. Thereby, the strong beneficial effects (i.e., skin wound healing better than with the corresponding standard agents (i.e., becaplermin) [14–16, 32]; in the wound and fistulas healing simultaneous healing of the esophageal, gastric, duodenal, colonic, and skin defect [8–11], rectal and vaginal defect in the present study) might be especially related to its molecular effects [8, 28–32]. For instance, prominently stimulated expression of the egr-1 gene includes cytokine and growth factor generation and early extracellular matrix (collagen) formation and blood vessels function, and its repressor naB2 [32]. This very likely provides a particular feedback-process consistently responsible for the simultaneous healing of two different tissues and fistulas healing [8–11].

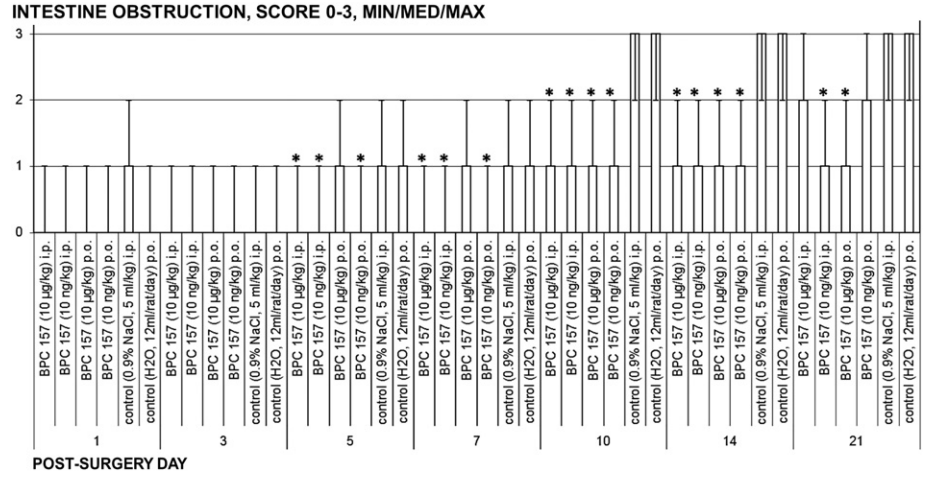

Fig. 5. Rectovaginal fistula in rats. Intestine obstruction presentation, scored 0–3, min/med/max. BPC 157 was given perorally, in drinking water (10 μg/kg or 10 ng/kg, 0.16 μg/ml or 0.16 ng/ml 12 ml/rat/day) till sacrifice, or alternatively, 10 μg/kg, 10 ng/kg intraperitoneally, first application at 30 min after surgery, last at 24 h before sacrifice. Controls simultaneously received an equivolume of saline (5.0 ml/kg ip) or water only (12 ml/rat/day). The assessment was at day 1, 3, 5, 7, 10, 14 and 21.*$P < 0.05$, at least vs. control.

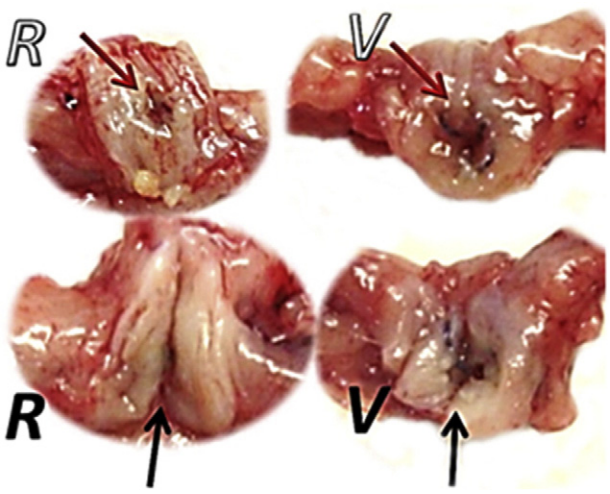

Fig. 6. Rectovaginal fistula in rats. Characteristic gross presentation in controls (upper) and BPC 157-treated rats (low). Characteristic gross presentation at post-surgery day 21, in controls (upper, white) and BPC 157-treated rats (low, black), rectal (R) and vaginal (V) defect, arrows indicate defect presentation, still persistent (control, red arrow) or closed (BPC 157, black arrow).

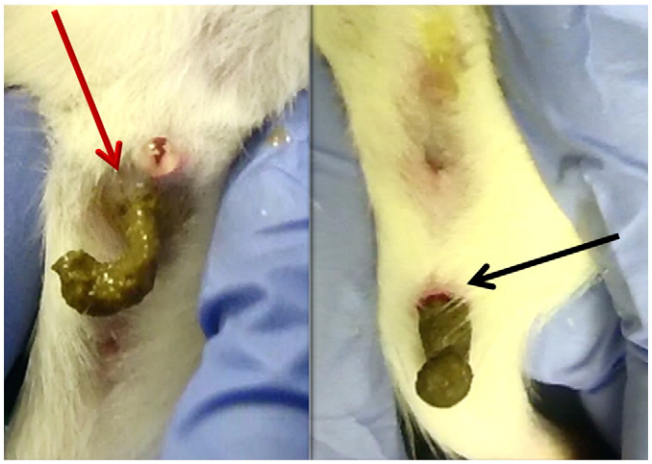

Fig. 8. Rectovaginal fistula at the end of the experiment (day 21) in control rats (left) and in rats who have undergone BPC 157 therapy (right). Defecation through vagina (control, red arrow). Normal defecation (black arrow) in BPC 157 treated rats.

Of note, the additional healing BPC 157's mechanisms in the rectovaginal fistulas healing were not particularly investigated in the present study. However, that advanced healing (and collagen) process [8–16] fairly reflects the biomechanic improvement [8–16], and vice versa. As a consequence, the successful instillation of the maximal volume definitively indicates the rescued fistulas and the healing in BPC 157-rats. Accordingly, the noted attenuated adhesions and passage obstruction, lack of fecal leaking through vagina in the rectovaginal fistulas-rats are along with the advanced biomechanical healing commonly noted in the various wounds, fistulas or remaining intestine function recovery in BPC 157-studies [8–16]. Previously, pentadecapeptide BPC 157, given in the protocol also used in the present study, parenterally or per-orally, ameliorates the stress urinary incontinence in two rat models, vaginal dilatation and transabdominal urethrolysis, that both fairly mimic human injury [34].

The important point for rectovaginal healing should be likely a strong particular BPC 157's angiogenic potential throughout the advanced healing in rats [2–6]. Specifically, it was characterized by upregulating vascular endothelial growth factor (VEGF) expression [35], also in hypovascular and hypocellular conditions [36–38], direct endothelium protection [2, 6, 26–28], influence on the NO-system [3], counteracted over expression of endothelin [39], counteraction of the effects of NOS-inhibitors and NO-precursor [3], what was also seen with external fistula healing [8, 10, 11]. The particular point was unlike VEGF no direct angiogenic effect of BPC 157 on cell cultures [35]. The others also demonstrated that BPC-157 could specifically promote vascular endothelial growth factor expression in wounded skin tissues: BPC-157 enhanced the proliferation of human umbilical vein endothelial cells (HUVECs)and significantly promoted migration of HUVECs, upregulated the expression of VEGF-a and accelerated vascular tube formation in vitro [31]. It was concluded that BPC-157 regulated the phosphorylation level of extracellular signal-regulated kinases 1 and 2 (ERK1/2) as well as its downstream targets, including c-Fos, c-Jun, and Egr-1, which are key molecules involved in cell growth, migration, and angiogenesis [31].

Further demonstration of a particular and modulating effect on angiogenesis that might be essentially important for fistulas (including also rectovaginal) includes corneal ulceration where the stable gastric pentadecapeptide BPC 157 heals corneal ulcerations in rats, allowing them to regain corneal transparency [40].

Admittedly, this study raised several issues, and further studies will elucidate (i.e., growth factor levels and erg-1 studied in situ). However, the essential support is the accurate attribution of the obtained peptide effects. Of note, for skin defect healing other peptides which are given with different carriers have uncertain healing attribution and effect (for review see i.e., [2–6]). Illustratively, BPC 157 is always given alone

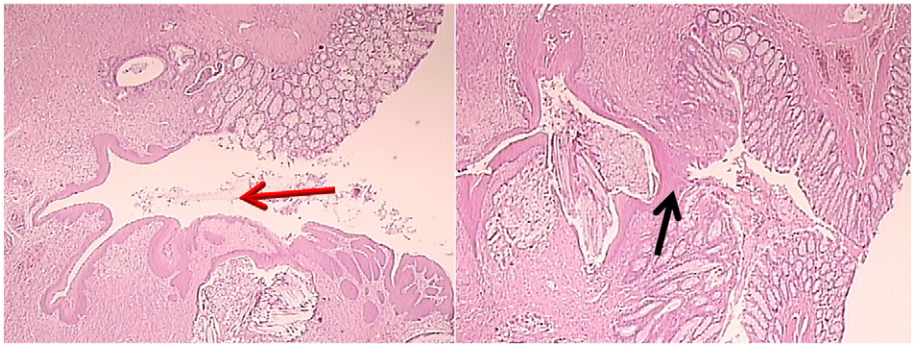

Fig. 7. Rectovaginal fistula in rats at post-surgery day 21. Characteristic microscopic presentation in controls (left) and BPC 157-treated rats (right). Red arrow indicates the regular outcome of surgical fistula creation in controls. Fistula wall is built partially of rectal and partially of vaginal epithelium, an area of granulation tissue, inflammatory cells and edema are present in the stroma, thus, poor fistula healing in control rats. By contrast, unlike less mature granulation tissue, more inflammatory cells and delayed reepithelialization in control rats, pentadecapeptide BPC 157 rats exhibited prominent regeneration of fistulous channel closure (black arrow) with remarked neovascularization, mature granulation tissue, earlier reepithelialization and less inflammatory cells. HEx40.

Table 1
Histopathological findings were scored (1–5) as described before [1], epithelialization (1: none; 2: none; 3: partial; 4: complete, immature; 5: complete, mature), collagenization (1: none; 2: none; 3: partial; 4: complete, irregular; 5: complete, regular), inflammation (1: severe; 2: moderate; 3: mild; 4: none; 5: none); neovascularization (1: none; 2: none; 3: <5/high power field (HPF); 4: 6–10/HPF; 5: >10/HPF); necrosis (1: extensive; 2: focal; 3: none; 4: none; 5: none); granulation tissue (1: none; 2: immature; 3: mild mature; 4: mod mature; 5: fully mature). BPC 157 was given perorally, in drinking water (10 μg/kg or 10 ng/kg, 0.16 μg/ml or 0.16 ng/ml 12 ml/rat/day) till sacrifice, or alternatively, 10 μg/kg, 10 ng/kg intraperitoneally once time daily, first application at 30 min after surgery, last at 24 h before sacrifice. Controls simultaneously received an equivolume of saline (5.0 ml/kg ip) or water only (12 ml/rat/day). The assessment was at day 1, 3, 5, 7, 10, 14 and 21.

Medication	Sacrifice at the post-operative day	Rectovaginal fistulas histopathological findings scored (1–5), min/med/max					
		Epithelialization	Collagenization	Inflammation	Neovascularization	Necrosis	Granulation tissue
0.9% NaCl	1	1/1/1	1/1/1	1/1/1	1/1/1	2/2/2	1/1/2
5 ml/kg	3	1/1/1	1/1/1	1/1/1	1/1/1	2/2/2	1/1/2
Intraperitoneally	5	1/1/1	1/1/1	1/1/1	1/1/1	2/2/2	1/1/2
Once time daily	7	1/1/1	1/1/1	1/2/2	1/1/1	2/2/2	2/2/2
	10	1/1/2	1/1/2	2/2/2	1/1/1	2/2/2	2/2/2
	14	1/2/2	1/1/2	2/2/2	1/1/1	1/2/2	2/2/2
	21	2/2/3	1/1/2	2/2/2	1/1/1	2/2/3	2/2/2
BPC 157	1	1/1/1	1/1/1	1/1/1	1/1/2	*3/3/3**	2/2/2
10 μg/kg	3	*1/1/2**	1/1/1	*1/2/2**	*2/2/2**	*3/3/3**	2/2/2
Intraperitoneally	5	*2/2/3**	*1/2/2**	*1/2/2**	*2/3/3**	*3/3/3**	*3/3/4**
Once time daily	7	*2/3/3**	*2/2/2**	1/2/2	*2/3/3**	*3/3/3**	*3/3/4**
	10	*3/3/3**	*2/3/4**	2/2/3	*3/3/4**	*3/3/3**	*3/3/4**
	14	*3/4/4**	*4/4/4**	2/2/3	*3/4/4**	*3/3/3**	*3/3/4**
	21	*4/5/5**	*4/4/4**	2/2/3	*3/4/5**	*3/3/3**	*3/3/4**
BPC 157	1	1/1/1	1/1/1	1/1/1	1/1/2	*3/3/3**	2/2/2
10 ng/kg	3	*1/1/2**	1/1/1	*1/2/2**	*2/2/2**	*3/3/3**	2/2/2
Intraperitoneally	5	*2/2/3**	*1/2/2**	*1/2/2**	*2/3/3**	*3/3/3**	*3/3/4**
Once time daily	7	*2/3/3**	*2/2/2**	1/2/2	*2/3/3**	*3/3/3**	*3/3/4**
	10	*3/3/3**	*2/3/4**	2/2/3	*3/3/4**	*3/3/3**	*3/3/4**
	14	*3/4/4**	*4/4/4**	2/2/3	*3/4/4**	*3/3/3**	*3/3/4**
	21	*4/5/5**	*4/4/4**	2/2/3	*3/4/5**	*3/3/3**	*3/3/4**
Drinking water	1	1/1/1	1/1/1	1/1/1	1/1/1	2/2/2	1/1/2
12 ml/rat/day	3	1/1/1	1/1/1	1/1/1	1/1/1	2/2/2	1/1/2
Till the sacrifice	5	1/1/1	1/1/1	1/1/1	1/1/1	2/2/2	1/1/2
	7	1/1/1	1/1/1	1/2/2	1/1/1	2/2/2	2/2/2
	10	1/1/2	1/1/2	2/2/2	1/1/1	2/2/2	2/2/2
	14	1/2/2	1/1/2	2/2/2	1/1/1	2/2/2	2/2/2
	21	2/2/3	1/1/2	2/2/2	1/1/1	2/2/3	2/2/2
BPC 157	1	1/1/1	1/1/1	1/1/1	1/1/2	*3/3/3**	2/2/2
10 μg/kg	3	*1/1/2**	1/1/1	*1/2/2**	*2/2/2**	*3/3/3**	2/2/2
0.16 μg/ml	5	*2/2/3**	*1/2/2**	*1/2/2**	*2/3/3**	*3/3/3**	*3/3/4**
12 ml/rat/day	7	*2/3/3**	*2/2/2**	1/2/2	*2/3/3**	*3/3/3**	*3/3/4**
Till the sacrifice	10	*3/3/3**	*2/3/4**	2/2/3	*3/3/4**	*3/3/3**	*3/3/4**
	14	*3/4/4**	*4/4/4**	2/2/3	*3/4/4**	*3/3/3**	*3/3/4**
	21	*4/5/5**	*4/4/4**	2/2/3	*3/4/5**	*3/3/3**	*3/3/4**
BPC 157	1	1/1/1	1/1/1	1/1/1	1/1/2	*3/3/3**	2/2/2
10 ng/kg	3	*1/1/2**	1/1/1	*1/2/2**	*2/2/2**	*3/3/3**	2/2/2
0.16 ng/ml	5	*2/2/3**	*1/2/2**	*1/2/2**	*2/3/3**	*3/3/3**	*3/3/4**
12 ml/rat/day	7	*2/3/3**	*2/2/2**	1/2/2	*2/3/3**	*3/3/3**	*3/3/4**
Till the sacrifice	10	*3/3/3**	*2/3/4**	2/2/3	*3/3/4**	*3/3/3**	*3/3/4**
	14	*3/4/4**	*4/4/4**	2/2/3	*3/4/4**	*3/3/3**	*3/3/4**
	21	*4/5/5**	*4/4/4**	2/2/3	*3/4/5**	*3/3/3**	*3/3/4**

* P˙0.05, at least, vs. control.

and thereby acts directly, thus certain effect on external fistulas [8–11] and now on internal fistulas healing. Concluding, this further emphasizes rectovaginal fistulas healing as the real healing congruence of different tissues healing.

In this, the practical peptidergic activity is seen by the fact that the peroral (in drinking water) application corresponds to that of the parenteral administration (for review see i.e., [2–7]). An interesting point is that using 3H-labelled pentadecapeptide BPC 157, t/2 was 66 h and 69 h in male and female rats after single oral administration [2–7].

As a final emphasize that would explain such effectiveness, as an original anti-ulcer peptide stable in the human gastric juice, BPC 157 is claimed to be there a novel mediator of Robert's cytoprotection [6], more advanced cytoprotective effects for more tissues healing, and thereby, a particular wound-healing mediator as well [6, 41]. Finally, that pentadecapeptide BPC 157's beneficial effect (both the wound and fistulas healing [8–16]) involves (for review see i.e., [2–7]) reduction in the number of the inflammatory cells, leukotriene B4 (LTB4), thromboxane B2 (TXB2), myeloperoxidase (MPO) level in the serum and inflamed tissues [42–44] and increases the macrophage activity [45].

5. Conclusions

Recto-vaginal fistula is an especial devastating condition that would certainly benefit from novel treatment [1]. In theory, a simultaneous beneficial effect (healing of the rectal defect, healing of the vaginal defect, closing of the rectovaginal fistula) is mandatory of the healing of the internal fistulas. This signifies a new quality in the combined tissues healing (i.e., wounds other fistula's healing [8–16], along with a beneficial effect in ulcerative colitis [2–6]), which is not a simple repeat of separate defect's healing but taking into account all the healing particularities of the fistula healing. There, regardless still limited clinical trials [2–6], BPC 157 is the agent that promptly improves healing of both rectal and vaginal lesions and mediated fistula closing (macro-/microscopically, and functionally, with no fistula leakage upon application of the maximal volume water) in rats. Thereby, with LD1 not achieved, implemented in inflammatory bowel disease trials [2–6], BPC 157 should be the practical hallmark of the further wound healing therapy in fistulas. On the other hand, at this moment, extrapolation of these findings will certainly benefit further clinical studies.

Conflict of interest

The authors state that they have no conflicts of interest.

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