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STABLE GASTRIC PENTADECAPEPTIDE BPC 157 HEALS CYSTEAMINE-COLITIS AND COLON-COLON-ANASTOMOSIS AND COUNTERACTS CUPRIZONE BRAIN INJURIES AND MOTOR DISABILITY

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Stable gastric pentadecapeptide BPC 157 was suggested to link inflammatory bowel disease and multiple sclerosis, and thereby, shown to equally counteract the models of both of those diseases. For colitis, cysteamine (400 mg/kg intrarectally (1 ml/rat)) and colon-colon anastomosis (sacrifice at day 3, 5, 7, and 14) were used. BPC 157 (10 μg/kg, 10 ng/kg) was applied either intraperitoneally once time daily (first application immediately after surgery, last at 24 hours before sacrifice) or per-orally in drinking water (0.16 μg/ml/12 ml/day till the sacrifice) while controls simultaneously received an equivolume of saline (5 ml/kg) intraperitoneally or drinking water only (12 ml/day). A multiple sclerosis suited toxic rat model, cuprizone (compared with standard, a several times higher regimen, 2.5% of diet regimen + 1 g/kg intragastrically/day) was combined with BPC 157 (in drinking water 0.16 μg or 0.16 ng/ml/12 ml/day/rat + 10 μg or 10 ng/kg intragastrically/day) till the sacrifice at day 4. In general, the controls could not heal cysteamine colitis and colon-colon anastomosis. BPC 157 induced an efficient healing of both at the same time. Likewise, cuprizone-controls clearly exhibited an exaggerated and accelerated damaging process; nerve damage appeared in various brain areas, with most prominent damage in corpus callosum, laterodorsal thalamus, nucleus reunions, anterior horn motor neurons. BPC 157-cuprizone rats had consistently less nerve damage in all damaged areas, especially in those areas that otherwise were most affected. Consistently, BPC 157 counteracted cerebellar ataxia and impaired forelimb function. Thereby, this experimental evidence advocates BPC 157 in both inflammatory bowel disease and multiple sclerosis therapy.

Key words: *colitis, anastomosis, cysteamine, intestinal bowel disease, cuprizone, stable gastric pentadecapeptide BPC 157, cerebellar ataxia*

INTRODUCTION

Several important findings recently appear in inflammatory bowel disease (IBD) and ulcerative colitis (1, 2) in particular, alterations of the brain-gut interactions ("brain-gut axis") (3) while stable gastric pentadecapeptide BPC 157 particular effects on nitric oxide (NO)-system (4), nonsteroidal anti-inflammatory drugs (NSAIDs)-toxicity, peripheral and central (5), gastrointestinal tract (6, 7) and wound-healing, and blood vessels (8), were recently reviewed. Here, BPC 157 was suggested to be a link between IBD and multiple sclerosis, that could be also used in practice per-orally (as an anti-ulcer peptide, stable in human gastric juice) (5-7, 9), and thereby, being equally effective against the models of both of those diseases. Thereby, BPC 157 was tested in rats subjected to cysteamine enema + colon-colon anastomosis colitis (9-11) and over-dose cuprizone application, a multiple sclerosis suited toxic experimental model (12).

Interesting from the viewpoint of inflammatory bowel therapy, endothelium has become one of the major areas of investigation in gut inflammation (13, 14). Likewise, the cell layers lining the intestines and blood-brain barrier define treating the inflammatory bowel disease as well as multiple sclerosis. Thereby, considering its effects (5-7, 9,15-17), we advocate the stable gastric pentadecapeptide BPC 157 in both inflammatory bowel disease and multiple sclerosis therapy. An additional argument is a direct demonstration of the healing of the severely injured muscles (15-17) and severely injured nerves (18) and brain injuries (19-23) not relied on immunomodulation.

At present, natalizumab, a humanized monoclonal antibody against the cell adhesion molecule α4-integrin, is advantageously used in the treatment of multiple sclerosis and Crohn's disease (24). On the other hand, with IBD-current biologic therapies limited to blocking tumor necrosis factor alpha some theoretical/practical inconsistencies appear. Mucosal endothelium is now well recognized to play an active role in the pathogenesis of both forms of inflammatory bowel disease, Crohn's disease and ulcerative colitis. Likewise, endothelial cells are regulating mucosal immune homeostasis, acting as "gatekeepers", controlling leukocyte accumulation in the interstitial compartment; the process mediated by leukocyte-endothelial adhesion molecules. However, with IBD-current

biologic therapies, all these mean some patients primary non-responders, experience a loss of response, intolerance or side effects, defining the urgent unmet need for novel treatments (13, 14). Likewise, also important for multiple sclerosis therapy, natalizumab also carries a specific risk of progressive multifocal leucoencephalopathy and reactivation of JC virus infection (24).

As an alternative, previously, there was an established BPC 157-NO system interaction (4, 8, 25-27) as well as a particular potential in angiogenesis (28-30) especially evident in the advanced healing process of the various tissues (25, 31-38) including those that may be almost avascular (*i.e.*, tendon, ligament) (37). Thereby, likely, fewer lesions in BPC 157-therapy, is possibly a particular effect of pentadecapeptide BPC 157 itself on the endothelium integrity (long-ago demonstrated (39)) since acting as a novel mediator of Robert's cytoprotection in stomach that essentially maintains endothelium integrity (5, 9). In support, the counteracting effect of BPC 157 on L-NAME was parallel with its endothelium protection and mucosal integrity maintenance (33); in rats with abdominal aorta anastomosis BPC 157 both prevents thrombotic clot formation and destroys already formed thrombosis (40) and also, after amputation, BPC 157 reduces bleeding time and thrombocytopenia after heparin, warfarin or aspirin administration (41). Likewise, when BPC 157 rescued doxorubicine-cardiac failure (33), supporting were the effects of BPC 157 medication on serum endothelin values: with a prevented increase along with prevented heart failure development and reversal of the increased values toward normal levels along with cardiac failure reversal (33).

This particular effect on endothelium integrity (termed also "wound healing therapy") (5-7, 9) may explain that initially for inflammatory bowel research, throughout a short term period and long term therapy, various intestinal lesions models were cured. Most improvement was achieved by BPC 157 regimens (42, 43) per-orally, parenterally and locally, even when complicated with relapse, poor healing of the fistulas, unhealed intestinal anastomosis (10, 11, 35, 42, 43) massive intestinal resection leading to short bowel (43) and functional incapability of the post-anastomotic remained intestine. Also, reversed was increased number of inflammatory cells (38, 44-46) and raised leukotriene B_4 (LTB_4), thromboxane B_2 (TXB_2), and myeloperoxidase (MPO) in the serum and inflamed tissues (47-49). And finally, BPC 157 increases macrophages activity (50). This marked potential effect (5-7, 9) is interesting since BPC 157 is originally an anti-ulcer peptide (GEPPPGKPADDAGLV, M.W. 1419), stable in human gastric juice, accordingly investigated in trials for inflammatory bowel disease, wound treatment, with no toxicity reported, where LD1 could be not achieved, effective alone without carrier (5-7, 9). Moreover, nonsteroidal anti-inflammatory drugs (NSAIDs), prototypic inhibitors of cyclooxygenase (COX) activity and PGE_2 production, can trigger or worsen the disease (51, 52). Contrary, BPC 157 consistently counteracts various lesions that may be produced by various NSAIDs (20-22, 53), and thereby suggested as an antidote for NSAIDs-toxicity (5). In these terms, the effects of BPC 157 on adjuvant arthritis a suited model of rheumatic arthritis, both prevention and rescue of the already established advanced failure (53), may be taken as a purposeful extension.

It particularly and consistently interacts with the NO-system, in various models and different species (4, 8, 25-27). Besides, it stimulated expression of early growth response 1 (egr-1) gene responsible for cytokine and growth factor generation and early extracellular matrix (collagen) formation (but also its repressor nerve growth factor 1-A binding protein-2 (nab2)) (44).

Therefore, considering significance of both NO-system and egr-1 gene-systems for inflammatory bowel disease (54) as well as for multiple sclerosis (55-57), these may be important also when confronted with severe complications of advanced and poorly controlled IBD as well as multiple sclerosis.

No therapy currently exists to repair demyelinated lesions in multiple sclerosis (58). However, besides arguments mentioned before, BPC 157 also provided direct demonstration of the healing of the severely injured muscles (15-17) and severely injured nerves (18, 59) and brain injuries (19-23) not relied on immunomodulation. Whether this approach may be suited to counteract these disabilities in multiple sclerosis patients, remains to be further determined. It should be, however, noted that there is a general lack of the studies that would demonstrate, for instance, the healing effect of disease-modifying drugs (DMDs) on the severely injured muscles and severely injured nerves. Unfortunately, several treatments, though successful in pre-clinical experimental autoimmune encephalitis trials, were either less effective in patients, worsened disease or caused unexpected, severe adverse events, as reviewed (60).

Therefore, the problem that the agents successful in pre-clinical experimental autoimmune encephalitis trials, were either less effective in patients, worsened disease or caused unexpected, severe adverse events (60), may be at least partly solved with the more suited cuprizone model that reflects a toxic experimental model (12). Cuprizone-induced demyelination in animals is accepted for studying multiple sclerosis-related lesions and is characterized by degeneration of oligodendrocytes more than by a direct attack on the myelin sheet (12).

As emphasized, currently, only injectable drugs are available for the treatment of multiple sclerosis (61). Therefore emerging oral therapies for multiple sclerosis are an especially attractive alternative to current DMDs (61). Thus, to highlight possible application of pentadecapeptide BPC 157 in both IBD and multiple sclerosis patients, this study shows BPC 157's potential to counteract the consequences of the cuprizone application, used in an extremely high regimen that highly exceeds those commonly applied (62). In cuprizone-rats, BPC 157 was given per-orally in the same regimen that may rescue rats with severe ulcerative colitis and colon-colon anastomosis.

MATERIALS AND METHODS

Animals

Male Wistar Albino rats (200–250 g) randomly assigned, 10 rats per each experimental group, were used in all experiments. They were fed with a standard rodent chow, *ad libitum*. All experiments were approved by the Local Ethics Committee.

Drugs and materials

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, cysteamine (Sigma,USA), and cuprisone (Sigma, USA) were accordingly used (5-7, 9, 62).

Colon-colon anastomosis complicated by the cysteamine-induced colitis

Under deep anesthesia the cysteamine (400 mg/kg) was applied through enema (1 ml/rat) at 8 cm proximal to the anus (as described before) (5-7, 9) and 10 minutes thereafter, the

colon-colon anastomosis was created 5 cm proximal to anus. Single-layer colon-colon anastomosis was performed with 7–0 polypropylene (Prolene; Ethicon, Hamburg, Germany) interrupted sutures; the abdominal incision was closed with 3–0 silk sutures. The sacrifice was at day 3, 5, 7, and 14.

Medication

BPC 157 (10 µg/kg, 10 ng/kg) was applied either intraperitoneally once time daily (first application immediately after surgery, last at 24 hours before sacrifice) or per-orally in drinking water (0.16 µg/ml/12 ml/day till the sacrifice) while controls simultaneously received an equivolume of saline (5 ml/kg) intraperitoneally or drinking water only (12 ml/day).

Anastomosis and cysteamine colitis healing assessment was performed at day 3, 5, 7, and 14 as described before (5-7, 9-11, 42, 43), the stool formation (scored 1 or 2, unformed or formed), weight loss (Δ, g, initial weight-post-surgery weight) and survival (number of survived and dead rats) were assessed initial as well. Briefly, as described (42), passage obstruction scored 0–3 (according to the loop diameters ratio close to anastomosis, loop diameters orally/loop diameters 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)); adhesion presentation 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; 3, exaggerated adhesions with whole anastomosis; 4, the mesenterial part of bowel also included; 5 neighboring loop also included; 6, many neighboring loops included; 7, neighboring loops, stomach, liver "packed"), cysteamine colitis the lesions areas were assessed by naive observers by a morphometrical analysis system (using a PC based program SFORM, VAMS, Zagreb, Croatia). Representative tissue sections were processed for further histologic analysis as described before (5-7, 9-11, 42, 43).

Biomechanics

Anastomosis dehiscence was biomechanically assessed in separate groups of animals, under deep anesthesia as described before (35, 42, 43). We studied the volume (ml) to the leak induction. Volume was infused through a syringeperfusion pump system (Argus 600, Argus Medical A6, Heimberg, Switzerland) (1 ml/10 s) in 10 cm colon segment with colitis and with anastomosis between 5 cm orally and 5 cm aborally, and corresponding 10 cm colon segment with colitis but without anastomosis.

Microscopy

The tissue specimens were immediately fixed in buffered formalin (pH 7.4), for 24 hours, dehydrated, and embedded in paraffin wax. The samples were stained with hematoxylin-eosin. Briefly, as described before (42), necrosis and granulation tissue formation were scored as follows: none (0); presentation less than 20% of the anastomosis area (1);

Table 1. Impaired forelimb function scoring (see 63, 64).

Parameter description	Score	Scoring description
Hindlimb clasping: Grasping the tail near its base and lifting the rat clear of all surrounding objects, observing the hindlimb position for 10 seconds	0	Hindlimbs are consistently splayed outward, away from the abdomen
	1	One hindlimb is retracted toward the abdomen for more than 50% of the time suspended
	2	Both hindlimbs are partially retracted toward the abdomen for more than 50% of the time suspended
	3	Hindlimbs are entirely retracted and touching the abdomen for more than 50% of the time suspended
The ledge test: Rat is placed on the cage`s ledge and it`s walking on the ledge and lowering in the cage is observed and scored	0	Rat walks along the ledge without losing its balance, and lowers itself back into the cage gracefully, using its paws.
	1	Rat loses its footing while walking along the ledge, but otherwise appears coordinated.
	2	Rat does not effectively use its hind legs, rear body reclines and rat lands on its head rather than its paws when descending into the cage. Body tremor can be observed.
	3	Rat falls off the ledge, or nearly so, while walking or attempting to lower itself, or shakes and refuses to move at all despite encouragement
Gait: Rat is observed and scored while walking on the flat surface	0	Rat moves normally, with its body weight supported on all limbs, with its abdomen not touching the ground, and with both hindlimbs participating evenly.
	1	Rat shows a tremor or appears to limp while walking, feet point away from the body during locomotion ("duck feet").
	2	Rat shows a severe tremor, severe limp, "duck feet", forelimb treading, dyskinesia is present less than 50% of the time.
	3	Rat has difficulty in moving forward and drags its abdomen along with the ground, akinesia or dyskinesia are present more than 50 % of the time.
Kyphosis: Rat's spine is observed and scored while rat is walking on the flat surface.	0	Rat is able to easily straighten its spine as it walks, and does not have persistent kyphosis.
	1	Rat exhibits mild kyphosis but is able to straighten its spine.
	2	Rat is unable to straighten its spine completely and maintains persistent but mild kyphosis.
	3	Rat maintains pronounced kyphosis as it walks or while it sits.

Table 2. The modified IBB scale for assessment of forelimb function impairment (see 66, 67).

Parameter	Score	Description
Pellet adjustment	0	Normal pellet adjustment, subtle movements without a loss of contact between the forepaws and the pellet during eating.
	1	Exaggerated pellet adjustment movements by the shoulder and/or elbow and/or wrist of the forelimbs that produce a complete loss of contact between forepaws and the cereal.
	2	Unability of pellet adjustement, no adjustment movement of one or both forelimbs
Predominant forepaw position	0	Partially flexed adaptable - The digits are partially flexed and conform to the shape of the pellet
	1	Extended non-adaptable - The digits are extended with an angle of >160° and do not conform to the shape of the held pellet.
Contact volar support	0	No support with the volar surface of the forepaw during eating (less than 5% of the time).
	1	Support of the pellet with the volar surface of the forepaw does occur during eating but not always (less than 95% of the time).
	2	No support with the volar surface of one or both forepaws during eating (less than 5% of the time).
Grasping method	0	Normal grasping method during more than 90% of the time of eating
	1	Normal grasping method during 60 to 90% of the time of eating
	2	Normal grasping method during 10 to 60% of the time of eating
	3	Normal grasping method less than 10 % of the time during eating
Ability of pellet grasping	0	Abilty to grasp pellet
	1	Inabilty to grasp pellet
Pellet raising	0	Ability to raise the pellet above the cylinder bottom
	1	Inability to raise the pellet above the cylinder bottom

presentation between 20% and 60% of the anastomosis area (2); presentation more than 60% of the anastomosis area (3), and edema, inflammatory cells, granulocytes, macrophages, fibroblasts were scored, scored on a four-point scale 0-3 (not; little; much; very much), and percentage of epithelization in anastomotic area and anastomotic area (mm) without new smooth muscle strands were assessed as before (42). Mallory-Gomory (reticulin, collagen), or immunohistochemically for desmin (Dako, Glostrup, Denmark) (newly formed muscle) and were examined in a blinded fashion. For the morphometrical analysis special software programs SFORM and ISSA (VAMSTEC, Zagreb, Croatia) were used. Five high power fields were randomly selected for the analysis.

Cuprizone-brain damage and motoric disability

Cuprizone 2.5% of diet regimen was combined with BPC 157 in drinking water 10 μg/kg or 10 ng/kg, 0.16 μg/ml/12 ml/day or 0.16 ng/ml/12 ml/day till the sacrifice after four days, with the additional applications once daily, last application at 24 hours before sacrifice: cuprizone 1 g/kg intragastrically while BPC 157 was given 10 μg/kg or 10 ng/kg intragastrically, controls received intragastrically an equivolume (5 ml/kg) of saline.

Behavioral assessment: cerebellar ataxia scoring

A protocol for the rapid and sensitive quantification of disease in rodent models of cerebellar ataxia (63) is described in *Table 1*.

Rats were assessed for fine motoric impairment with modified Irvine, Beatties and Bresnahan forelimb scale (IBB scale) that can detect recovery of both proximal and distal forelimb function including digit movements during a naturally occurring behavior that does not require extensive training or deprivation to enhance motivation (64). While IBB scale is more detailed and adjusted for fine motoric impairment due to cervical spinal cord injury, our modified and simpler scale reflects impairment due to cuprizone induced central nervous system injury. Rats were tested in 30 cm tall glass cylinder. The animals were first adapted to testing environment after a shortened pellet (1×0.3 cm), their usual diet, was administered in the cylinder. Rats were recorded and afterwards assessed (slow-down video) for forelimb function impairment. The overall score of forelimb function impairment was obtained from 6 parameters. The highest score of 10 points reflects the greatest impairment, while the lowest score of 0 points represents normal forelimb function. The modified IBB scale (64) is presented in *Table 2*.

Accordingly with previous studies (63, 64), animal responsiveness to forceps stimuli is tested with blunt-nosed thumb forceps with serrated tips for increased grip. Animals are tested in glass cylinder, where they adapt for 10 minutes prior to assessment. Experimenter lowers the forceps in cylinder in front of the rat, stimulates it`s whiskers and slightly touches it`s chest while recording and scoring rat`s reaction to stimuli. Healthy controls rear, raise their both forelimbs and grasp the forceps

Table 3. Animal responsiveness to forceps stimuli (see 63, 64).

Parameters assessed	Score
Rearing in a response to forceps stimuli	0
Raising both forepaws and touching forceps with volar surface	0
Raising only left forepaw and touching forceps with volar surface	1
Raising both forepaws in a direction of forceps but without touching them with volar surface	1
Raising only left forepaw in a direction of forceps but without touching them with volar surface	2
Losing body balance after rearing	3
Unresponsiveness at the beginning of testing	4
Complete unresponsiveness	5

Table 4. Mortality rate in rats that received cysteamine enema, and then colon-colon anastomosis, and thereby, poor anastomosis healing along with prominent ulcerative colitis. * P<0.05, *vs*. control.

Regimens	Medication	Number of survived/dead rats throughout days after cysteamine enema + colon-colon anastomosis			
		Day 3	Day 5	Day 7	Day 14
Once daily, intraperitoneally	Saline 5 ml/kg	10/5	7/8	6/9	4/11
	BPC 157 10 µg/kg	15/0*	15/0*	15/0*	15/0*
	BPC 157 10 ng/kg	15/0*	15/0*	12/3*	11/4*
Per-orally in drinking water (12ml/day till the sacrifice)	Drinking water	10/5	7/8	6/9	4/11
	BPC 157 (10 µg/kg, 0.16 µg/ml)	15/0*	15/0*	15/0*	13/2*

Table 5. Cerebellar ataxia scoring (0–3, Min/Med/Max). Impaired forelimb function scoring in rats that received cuprizone diet, and then cuprizone intragastrical applications, and thereby, cuprizone toxicity manifestations. BPC 157 (10 µg/kg, 10 ng/kg) was applied either per-orally in drinking water (0.16 µg/ml/12 ml/day; 0.16 ng/ml/12 ml/day till the sacrifice) and intragastrically after cuprizone instilations, while controls received simultaneously drinking water only (12 ml/day) as well as an equivolume of saline (5 ml/kg) intragastrically after cuprizone instillations. * P<0.05, at least, *vs*. control.

Noxious procedure for 4 days:	Therapy (/kg): (i) in drinking water (12 ml/day) till the sacrifice, nothing or BPC 157 (10 µg, 10 ng) (0.16 µg, 0.16 ng/ml/) +(ii) saline or BPC 157 (10 µg, 10 ng), intragastrically, after cuprizone instillations	Cerebellar ataxia scoring (0–3, Min/Med/Max). Impaired forelimb function scoring in rats that received cuprizone diet, and then cuprizone intragastrical applications			
		Hindlimb clasping: Grasping the tail near its base and lifting the rat clear of all surrounding objects, observing the hindlimb position for 10 seconds	The ledge test: Rat is placed on the cage`s ledge and it`s walking on the ledge and lowering in the cage is observed and scored	Gait: Rat is observed and scored while walking on the flat surface.	Kyphosis: Rat`spine is observed and scored while rat is walking on the flat surface
(i)cuprizone 2.5% diet + (ii) cuprizone 1 g/kg intragastrically once daily	Saline 5 ml	2/2/3	1/1/1	2/2/3	3/3/3
	BPC 157 10 µg	0/0/0*	0/0/0*	0/0/0*	0/0/0*
	BPC 157 10 ng	1/1/1*	0/0/1*	0/1/2*	0/1/1*

nose defensively. Animals affected with cuprizone toxicity react only with one or no forelimb and have difficulty with maintaining body balance while rearing. Scoring system is presented in *Table 3*.

Behavioral assessment: microscopy

Brains were fixed in 4% paraformaldehyde (PFA) for two days. The brain was cut into consecutive coronal sections. Brain slabs were dehydrated in graded ethanol and embedded in paraffin. Paraffin blocks were cut into 5 and 3- µm slices. Paraffin slices cut into 5-µm were deparaffinated in xylene, rehydrated in graded ethanol and stained with hematoxylin and eosin. Paraffin slices cut into 3-µm were deparaffinated in xylene, rehydrated untill 70% ethanol and stain in 0.1% cresyl violet solution for Nissl staining method. The intensity and distribution of brain lesions (balloonized or red neurons), were described and evaluated semi-quantitatively as follows (19-23): 0–5% balloonized or red neurons (1), 6–10% balloonized or red neurons (2), 11–15% balloonized or red neurons (3), 16–20% balloonized or red neurons (4), 21–30% balloonized or red neurons (5), >31% balloonized or red neurons (6). The degree of neuronal loss in hippocampal regions CA1 were described and evaluated semi-quantitatively as follows: 0–10% (1), 11–20% (2) and >21% (3). For motor neuron count, spinal cords from anesthetized rats were collected and post-fixed for 12 hours in 4% PFA. Paraffin-embedded spinal cords were serially sectioned at 5 µm steps, mounted on slides, and processed for Nissl staining. Images of ten contiguous sections were analyzed. Motor neurons were identified as cells positive for Nissl staining, with clear nucleus and nucleolus. Counting was performed in a blinded fashion. The intensity of red neurons were described and evaluated semi-quantitatively as follows: 0–25% (1), 26–50% (2) and >50% (3).

Statistical analysis

Statistical analysis was performed using parametric two-way mixed model ANOVA (one factor is repeated-measures) and Student Newman-Keuls test to compare the difference between groups. Fisher's exact probability test was used to assess the number of dead and surviving rats. A P value of 0.05 or less was considered statistically significant.

RESULTS

Here, to evidence a link between inflammatory bowel disease and multiple sclerosis that may be useful in practice, BPC 157 was used per-orally in rats subjected to cysteamine enema + colon-colon anastomosis colitis (9-11) and over-dose cuprizone application, a multiple sclerosis suited toxic experimental model (12). BPC 157 was used in the same regular regimens (10 µg or 10 ng/kg, per-orally/intragastrically), shown to be effective in all BPC 157 studies (5, 9). Since cuprizone regimen was several times higher than those regularly used in cuprizone studies (62) we used simple tests (*Tables 1-3*) to determine animal responsiveness and impairment. The anastomosis and the ulcerative colitis at the same time were assessed grossly, biomechanically, clinically and

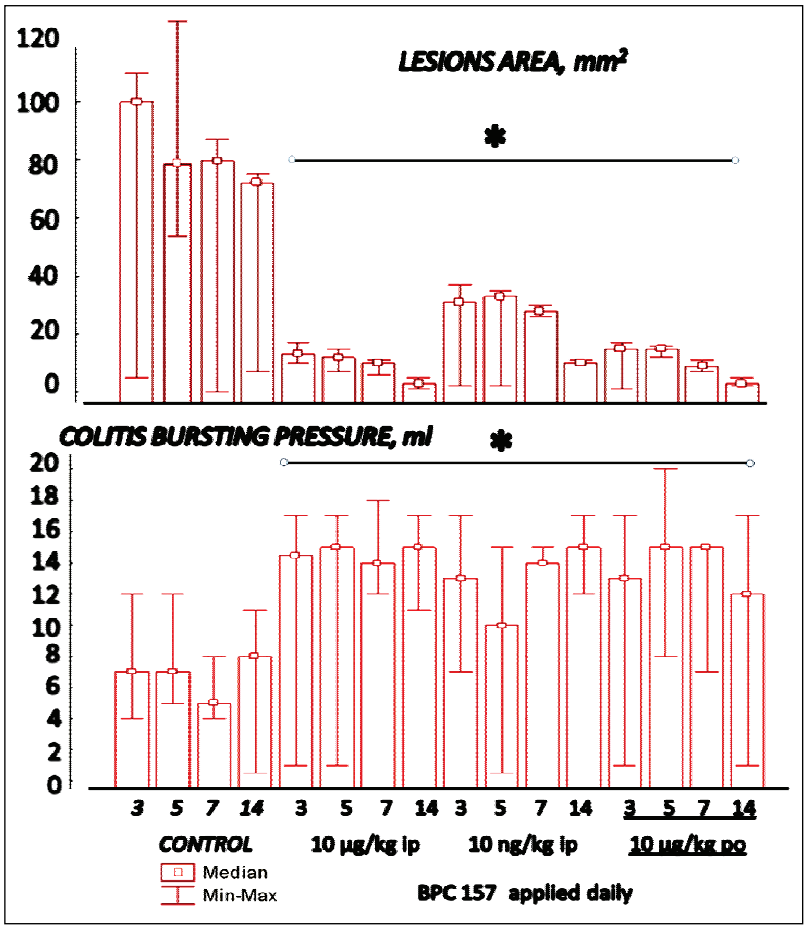

Fig. 1. Cysteamine enema, and then colon-colon anastomosis, and thereby, poor anastomosis healing along with prominent ulcerative colitis. Counteraction by various BPC 157 regimens. Presentation of colitis lesions, damaged area, mm^2, and colitis bursing pressure assessed as the volume (ml) of infunded water before leaking appearance in colitis-intestine. BPC 157 (10 µg/kg, 10 ng/kg) was applied either intraperitoneally, once time daily (first application immediately after surgery, last at 24 hours before sacrifice) or per-orally in drinking water (0.16 µg/ml/12 ml/day till the sacrifice) while controls simultaneously received an equivolume of saline (5 ml/kg) intraperitoneally or drinking water only (12 ml/rat/day). *P<0.05, at least, *vs.* control.

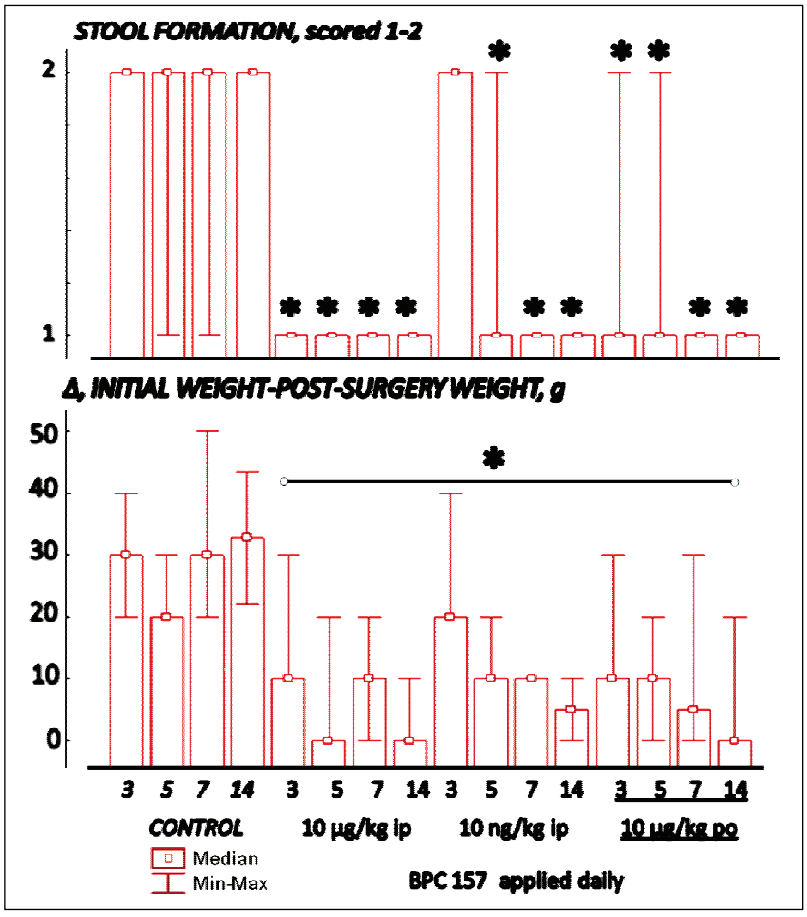

Fig. 2. Cysteamine enema, and then colon-colon anastomosis, and thereby, poor anastomosis healing along with prominent ulcerative colitis. Counteraction by various BPC 157 regimens. Presentation of non-formed and formed stool (scored 1–2), and weight loss (as Δ, g (initial weight-post-surgery weight)). BPC 157 (10 µg/kg, 10 ng/kg) was applied either intraperitoneally once time daily (first application immediately after surgery, last at 24 hours before sacrifice) or per-orally in drinking water (0.16 µg/ml/12 ml/day till the sacrifice) presented with mostly formed stool and pre-surgery weight achieved. Controls simultaneously received an equivolume of saline (5 ml/kg) intraperitoneally or drinking water only (12 ml/rat/day), and presented with non-formed stool and sustained weight loss. * P<0.05, at least, *vs.* control.

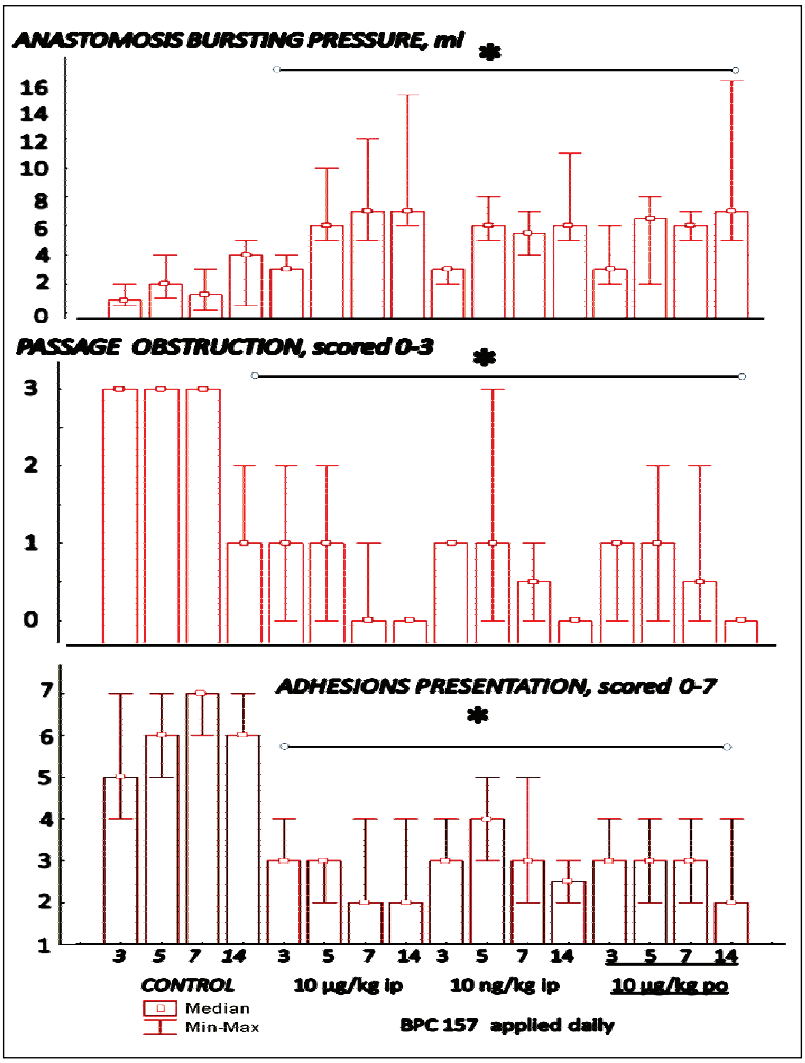

Fig. 3. Cysteamine enema, and then colon-colon anastomosis, and thereby, poor anastomosis healing along with prominent ulcerative colitis. Counteraction by various BPC 157 regimens. Presentation of anastomosis bursting pressure (assessed as the volume (ml) of infunded water before leaking appearance in anastomosis), passage obstruction (scored 0–3), and extent of adhesions formation (scored 0–7). BPC 157 (10 µg/kg, 10 ng/kg) was applied either intraperitoneally once time daily (first application immediately after surgery, last at 24 hours before sacrifice) or per-orally in drinking water (0.16 µg/ml/12 ml/day till the sacrifice) presented with small extent of adhesions, increased anastomosis bursting pressure and no passage obstruction. Controls simultaneously received an equivolume of saline (5 ml/kg) intraperitoneally or drinking water only (12 ml/rat/day), and presented with significant adhesions, small bursting pressure and significant passage bstruction. * P<0.05, at least, *vs*. control.

microscopically (*Table 4*, *Figs. 1-7*). As an illustration, the BPC 157 animals exhibited less colitis lesions and advanced anastomosis healing and functioned much better and mostly achieved pre-operative weigh. They exhibited the higher survival rate (*Table 4*), formed feces 3 days following surgery (*Fig. 2*) and the absence of passage impairment (*Fig. 3*). Likewise, cuprisone + BPC 157 rats functioned markedly better (*Tables 5-7*, *Fig. 8*) and exhibited less nerve damage in various brain areas (*Table 8*, *Fig. 9*).

Cysteamine colitis

In general, the controls could not heal cysteamine colitis and colon-colon anastomosis. Like in previous studies (10, 11), either of BPC 157 regimens exhibited all colitis lesions consistently attenuated, macroscopically (*Fig. 1*), clinically (*Figs. 2, 3*) and microscopically (*Figs. 4-6*). As described before, in rats treated with BPC 157 we substantiated a strong benefit (*i.e.*, less necrosis, increased epithelization, new strands of smooth muscle formed, and more granulation, less number of inflammatory cells) (*Figs. 4-7*). And thereby, their biomechanic assessment demonstrated markedly higher intestine strength before leaking (*Figs. 1, 3*) along with a general improvement (*i.e.*, formed stool, no passage obstruction, less adhesions presentation, and maintained weight) (*Figs. 2, 7*). Thereby, beneficial course was not hampered by the close anastomosis in rats that received BPC 157.

Colon-colon anastomosis

Specifically, the anastomosis in controls healed poorly (*Figs. 3-7*), grossly, clinically, microscopically, and in particular, biomechanically, and they could sustain without leaking only a very small volume (*Fig. 3*). Likewise, their adjacent colon relatively far from anastomosis, but with colitis, could sustain only a very small amount of the infunded water (*Fig. 1*).

Contrary, the colon-colon anastomosis and adjacent colon in animals treated with the pentadecapeptide BPC 157 sustained markedly higher infunded volume without leaking. From the 7th postoperative day, they presented values comparative to those of healthy animals (*Figs. 1, 3*).

Likewise, unlike controls presented with huge adhesions, and prominent passage impairment, BPC 157 rats exhibited markedly improved post-operative course, markedly less adhesions and no passage obstruction (*Figs. 3-7*).

Cuprizone-brain damage and motoric disability

The used cuprizone regimen, several times higher than those regularly used in cuprizone studies (62), rapidly induced marked functional disability along with severely affected various brain areas. For instance, as an illustration, control animals affected with cuprizone toxicity spare right forelimb, and thereby react only with one or no forelimb and have difficulty with maintaining body balance while rearing.

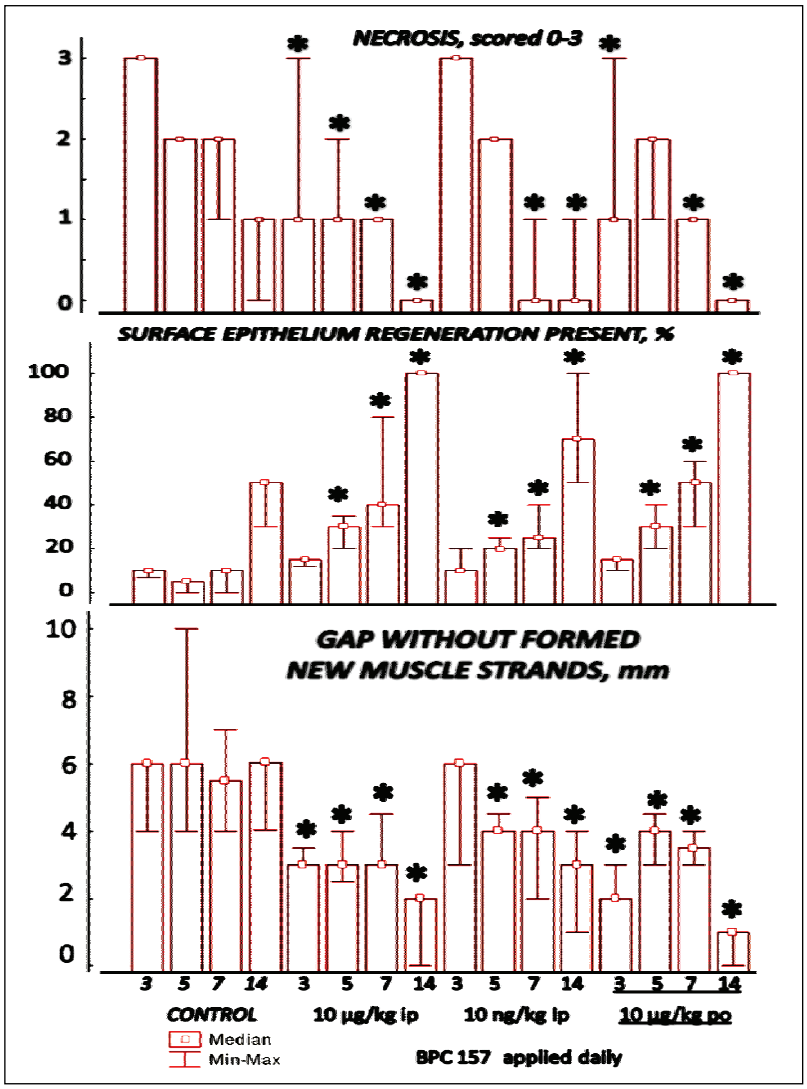

Fig. 4. Anastomotic gap presentation microscopically assessed in in rats that received cysteamine enema, and then colon-colon anastomosis, and thereby, poor anastomosis healing along with prominent ulcerative colitis if not rescued by various BPC 157 regimens. Necrosis (scored 0–3), percentage of gap with surface epithelium regeneration present, gap without new strands of smooth muscle. BPC 157 (10 µg/kg, 10 ng/kg) was applied either intraperitoneally once time daily (first application immediately after surgery, last at 24 hours before sacrifice) or per-orally in drinking water (0.16 µg/ml/12 ml/day till the sacrifice) while controls simultaneously received an equivolume of saline (5 ml/kg) intraperitoneally or drinking water only (12 ml/rat/day). * P<0.05, at least, *vs*. control.

Table 6. Modified Irvine, Beatties and Bresnahan forelimb scale (IBB scale, scored 0–2, 0–3, 0–1) (63, 64) for assessment of forelimb function impairment in rats that received cuprizone diet, and then cuprizone intragastrical applications, and thereby, cuprizone toxicity manifestations. BPC 157 (10 µg/kg, 10 ng/kg) was applied either per-orally in drinking water (0.16 µg/ml/12 ml/day; 0.16 ng/ml/12 ml/day till the sacrifice) and intragastrically after cuprizone instilations, while controls received simultaneously drinking water only (12 ml/day) as well as an equivolume of saline (5 ml/kg) intragastrically after cuprizone instillations. * P<0.05, at least, *vs*. control.

Noxious procedure for 4 days: (i) cuprizone 2.5% diet + (ii) cuprizone 1 g/kg, intragastrically, once daily	Therapy (/kg): (i) in drinking water (12 ml/day) till the sacrifice, nothing or BPC 157 (10 µg, 10 ng), (0.16 µg, 0.16 ng/ml/) + (ii) saline or BPC 157 (10 µg, 10 ng), intragastrically, after cuprizone instillations	Modified Irvine, Beatties and Bresnahan forelimb scale (IBB scale, scored 0–2, 0–3, 0–1)) for recovery of both proximal and distal forelimb function including digit movements during a naturally occurring behavior in rats that received cuprizone diet, and then cuprizone intragastrical applications.					
		Pellet adjustment	Predominant forepaw position	Contact volar support	Grasping method	Ability of pellet grasping	Pellet raising
	Saline 5 ml	2/2/3	1/1/1	2/2/3	2/2/2	1/1/1	1/1/1
	BPC 157 10 µg	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*	0/0/0*
	BPC 157 10 ng	1/1/1*	0/0/1*	0/1/1*	0/1/1*	0/0/0*	0/0/0*

Contrary, BPC 157 rats do not spare right forelimb, and they react simultaneously with both forelimbs, and grasp the forceps, and maintain body balance while rearing (*Tables 5-7*, *Fig. 8*).

Nerve damage appeared in various brain areas, with most prominent damage in corpus callosum, laterodorsal thalamus, nucleus reunions, anterior horn motor neurons. Thereby, considering BPC 157 therapy, it is important to indicate

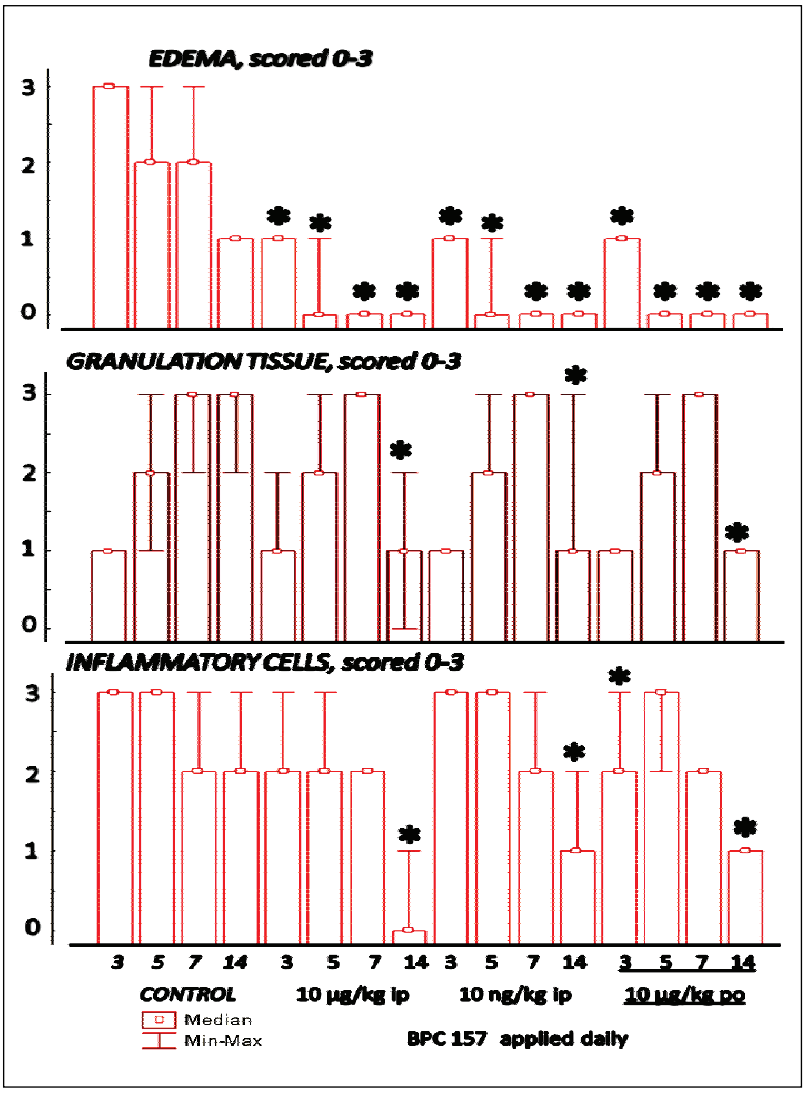

Fig. 5. Anastomotic gap presentation microscopically assessed in in rats that received cysteamine enema, and then colon-colon anastomosis, and thereby, poor anastomosis healing along with prominent ulcerative colitis if not rescued by various BPC 157 regimens. Edema, granulation tissue, inflammatory cells scored 0–3. BPC 157 (10 µg/kg, 10 ng/kg) was applied either intraperitoneally once time daily (first application immediately after surgery, last at 24 hours before sacrifice) or per-orally in drinking water (0.16 µg/ml/12 ml/day till the sacrifice) while controls simultaneously received an equivolume of saline (5 ml/kg) intraperitoneally or drinking water only (12 ml/rat/day). *P<0.05, at least, *vs*. control.

Table 7. Responsiveness to forceps stimuli in rats (64) (scored 0–5, min/med/max) that received cuprizone diet, and then cuprizone intragastrical applications, and thereby, cuprizone toxicity manifestations. BPC 157 (10 µg/kg, 10 ng/kg) was applied either per-orally in drinking water (0.16 µg/ml/12 ml/day; 0.16 ng/ml/12 ml/day till the sacrifice) and intragastrically after cuprizone instilations, while controls received simultaneously drinking water only (12 ml/day) as well as an equivolume of saline (5 ml/kg) intragastrically after cuprizone instillations. * P<0.05, at least, *vs*. control.

Noxious procedure for 4 days: (i) cuprizone 2.5% diet + (ii) cuprizone 1 g/kg, intragastrically, once daily	Therapy (/kg): (i) in drinking water (12 ml/day) till the sacrifice, nothing or BPC 157 (10 µg, 10 ng, 0.16 µg, 0.16 ng/ml/) + (ii) saline or BPC 157 (10 µg, 10 ng), intragastrically, after cuprizone instillations		
	Control (drinking water (12 ml/day) + saline 5 ml, intragastrically)	BPC 157 (10 µg, 0.16 µg/ml, in drinking water) + (10 µg, intragastrically)	BPC 157 (10 ng, 0.16 ng /ml, in drinking water) + (10 ng, intragastrically)
Responsiveness to forceps stimuli	4/4/5	0/0/0*	0/0/0*

consistently less nerve damage appeared in all damaged areas in all cuprizone + BPC 157 rats. However, the most important evidence is consistent beneficial effect of BPC 157 also in those areas that were most affected (*Table 8*, *Fig. 9*).

DISCUSSION

BPC 157 was suggested to be a link between inflammatory bowel disease and multiple sclerosis, that could be also used in

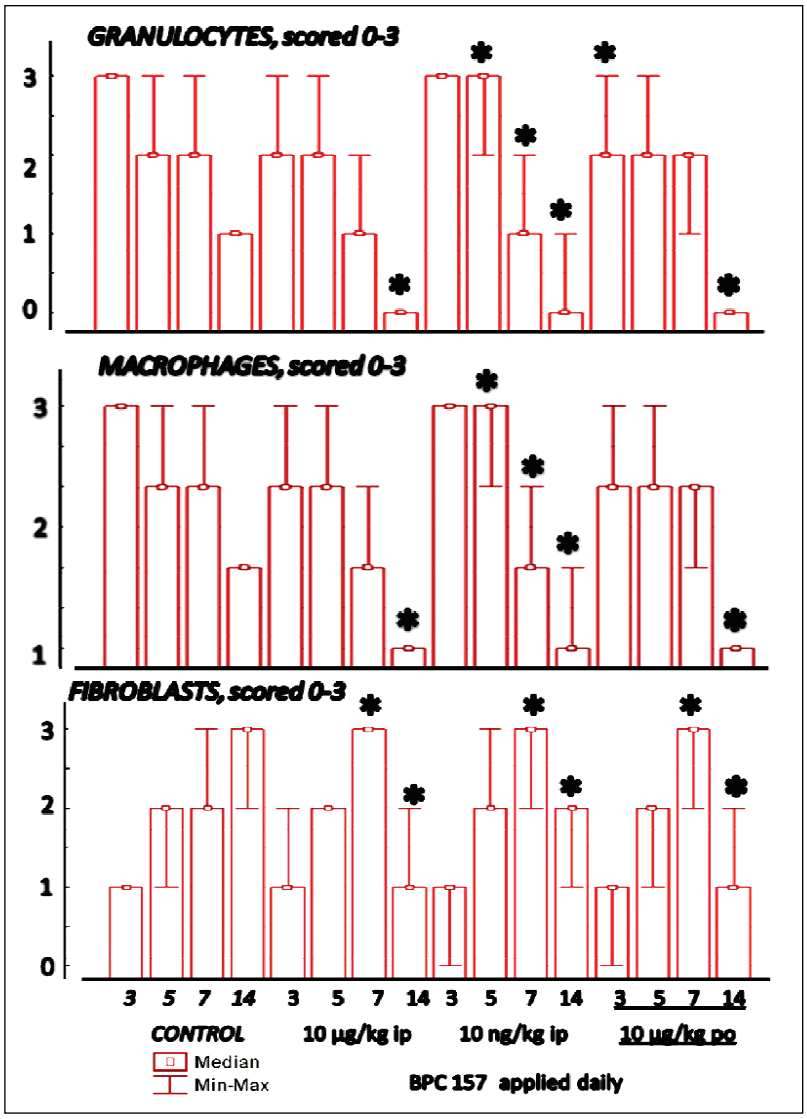

Fig. 6. Anastomotic gap presentation microscopically assessed in rats that received cysteamine enema, and then colon-colon anastomosis, and thereby, poor anastomosis healing along with prominent ulcerative colitis if not rescued by various BPC 157 regimens. Granulocytes, macrophages, fibroblasts scored 0–3. BPC 157 (10 µg/kg, 10 ng/kg) was applied either intraperitoneally once time daily (first application immediately after surgery, last at 24 hours before sacrifice) or per-orally in drinking water (0.16 µg/ml/12 ml/day till the sacrifice) while controls simultaneously received an equivolume of saline (5 ml/kg) intraperitoneally or drinking water only (12 ml/rat/day). *P<0.05, at least, *vs*. control.

Table 8. Brain and spinal cord lesions (scored 0–5, or 1–3, Min/Med/Max) in rats that received cuprizone diet, and then cuprizone intragastrical applications, and thereby, cuprizone toxicity manifestations. BPC 157 (10 µg/kg, 10 ng/kg) was applied either per-orally in drinking water (0.16 µg/ml/12 ml/day; 0.16 ng/ml/12 ml/day till the sacrifice) and intragastrically after cuprizone instilations, while controls received simultaneously drinking water only (12 ml/day) as well as an equivolume of saline (5 ml/kg) intragastrically after cuprizone instillations. *P<0.05, at least, *vs*. control.

Noxious procedure for 4 days: (i) cuprizone 2.5% diet + (ii) cuprizone 1 g/kg, intragastrically, once daily	Therapy (/kg): (i) in drinking water (12 ml/day) till the sacrifice, nothing or BPC 157 (10 µg, 10 ng,0.16 µg, 0.16 ng/ml/) + (ii) saline or BPC 157 (10 µg, 10 ng), intragastrically, after cuprizone instillations		
	Control (drinking water (12 ml/day) + saline 5 ml, intragastrically)	BPC 157 (10 µg, 0.16 µg /ml, in drinking water) + (10 µg, intragastrically)	BPC 157 (10 ng, 0.16 ng /ml, in drinking water) + (10 ng, intragastrically)
Cingulate cortex	2/2/3	1/1/1*	1/1/2*
Temporal neocortex	3/3/3	2/2/2*	2/3/3
Corpus calosum	5/5/5	2/2/2*	2/2/2*
Nucleus reuniens	5/5/5	2/2/2*	3/3/3*
Laterodorsal thalamic nuclei	5/5/5	3/3/3*	3/3/4*
Hippocampus, CA1	2/3/3	1/1/1*	1/1/2*
Motor neurons	6/6/6	1/1/1*	2/2/2*

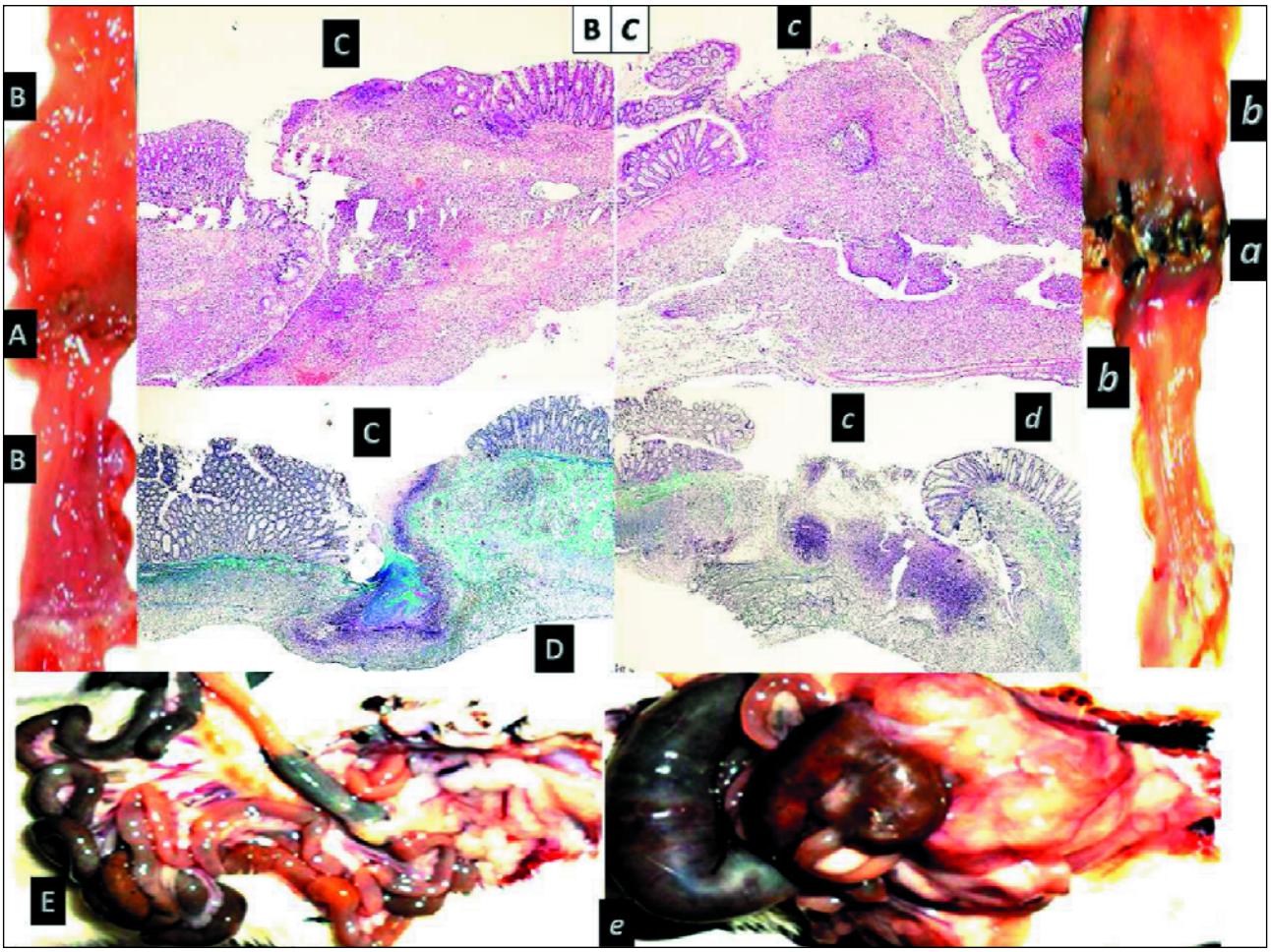

Fig. 7. Illustrative course after induction of ulcerative colitis (cysteamine enema) and colon-colon anastomosis in controls, not presented in cysteamine enema + colon-colon anastomosis + BPC 157 rats. Controls (C, right, small italics) (saline 5 ml/kg intraperitoneally or only drinking water 12 ml/day) presented with very poor anastomosis healing, *i.e.*, day 7, necrosis and dehiscience (*a*), along with prominent ulcerative colitis, present below and up anastomosis (*b*); and day 3 (HE (upper), Mallory (middle) staining, ×40): wide gap (*c*), great amount of inflammatory cells, collagen (green fibers, Mallory staining) only in mucosa and submucosa (*d*); day 7: subileus with adhesions (*e*). Animals that received BPC 157 (B, left, capitals) (10 µg/kg, 10 ng/kg) either intraperitoneally once a day (first application immediately after surgery, last at 24 hours before sacrifice) or per-orally in drinking water (0.16 µg/ml/12 ml/day till the sacrifice), demonstrated significantly improved healing, *i.e.*, day 7; advanced anastomosis healing (A), macoscopicallly preserved mucosa (B), day 3 (HE (upper), Mallory (middle) staining, ×40): smaller gap (C), less inflammatory cells, collagen in mucosa and submucosa and granulation tissue (green fibers, Mallory staining) (D); day 7: functionally (no ileus), presentation was similar to healthy animals (E).

practice per-orally (as an anti-ulcer peptide, stable in human gastric juice) (5-7, 9), and thereby, being equally effective against the models of both of those diseases. Thereby, this study focused on BPC 157 effectiveness as a comparative link in both diseases, as far it may be indicated by the two models, cuprizone and cysteamine enema and then colon-colon anastomosis that fairly mimic corresponding human conditions, multiple sclerosis (12) and inflammatory bowel disease, colitis and colon-colon anastomosis (9). Considering the endothelium integrity as a common denominator for both inflammatory bowel disease and multiple sclerosis, due to its particular effect of on endothelium integrity shown in advanced healing effect in the various tissues; muscle (15-17), nerve (18, 59) and brain injuries (19-23) not relied on immunomodulation, and especially in GI tract for inflammatory bowel research, *i.e.*, relapse (10, 11), poor healing of the fistulas (35), unhealed intestinal anastomosis (42, 43), massive intestinal resection leading to short bowel (43), and functional incapability of the post-anastomotic remained intestine (43), and, along with these, particularly for multiple sclerosis, direct demonstration of the healing of the severely injured muscles and severely injured nerves and brain injuries (15-23, 59) so far not mentioned with DMDs (61), these results obtained in two models, cysteamine enema and then colon-colon anastomosis and cuprizone may define a particular way not relied on immunomodulation.

Analyzing this study with same dosage regimen used, and the same per-oral protocol, this shows BPC 157's potential to counteract the consequences of the cuprizone application even an extremely high regimen that highly exceeds those commonly applied (62, 65). In general, this BPC 157's protocol accords with all protocols used before in studies evidencing BPC 157's healing of the severely injured muscles and severely injured nerves and brain injuries (15-23, 59).

Likewise, along with previous beneficial effect of BPC 157 in gastrointestinal tract, and inflammatory bowel disease complications that were accordingly solved (10, 11), poor healing of the fistulas (35), unhealed intestinal anastomosis (42, 43), massive intestinal resection leading to short bowel (43)) this

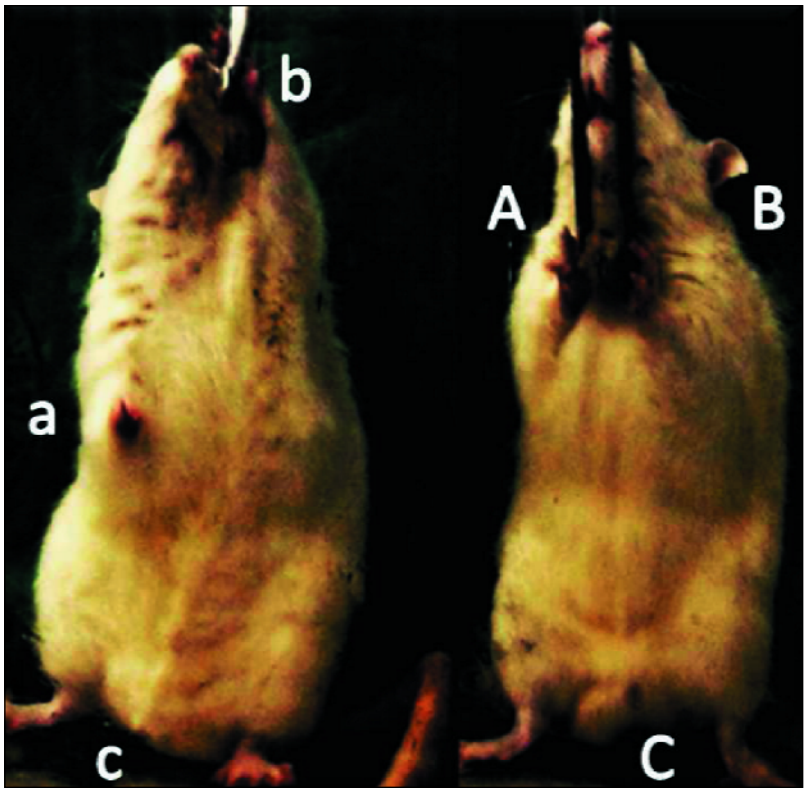

Fig. 8. Presentation of right forelimb sparing and disability in cuprisone (control, small letters, left), not presented in cuprisone + BPC 157 rats (capitals, right). Control, left, rat not using right forelimb (a), reacting with only left (b) forelimb (or no forelimb) upon challenge with forceps, having difficulty with maintaining body balance (c), common presentation in cuprizone control rats. BPC 157, right, normal functioning of right forelimb (A), simultaneous reaction with both forelimbs (B), and grasp the forceps (A, B), and maintaining body balance as healthy rats (C), common presentation in cuprizone + BPC 157 rats.

study shows BPC 157's high potential to heal colon-colon anastomosis along with cysteamine colitis, thus, potential to counteract the consequences of the anastomosis for cysteamine colitis, and *vice versa*, convalescing the increased healing failure of anastomosis healing due to concomitant presentation of severe colitis. Thus, providing the suitability of the both models, and damaging regimens used, these arguments (*i.e.*, chosen cuprizone administrations multiply beyond those cuprizone regimens commonly used and this should ascertain the value of the obtained counteracting effect; accordingly with inflammatory bowel disease, cysteamine damaging effect particularly includes both upper and lower GI tract lesions (5-7, 9) may evidence BPC 157 effectiveness as a comparative link in both diseases.

Specifically, the addition of cuprizone (bis-cyclohexanone oxalydihydrazone), to the diet process (66) produces a massive demyelination in different areas of the CNS and particularly in the corpus callosum (67-69). Demyelination is closely followed by recruitment of microglia and by phagocytosis of the disrupted myelin membrane. If the administration of cuprizone is terminated, an almost complete remyelination takes place in a matter of weeks (68, 70, 71). Here, using a several times higher cuprizone diet, followed by concomitant additional intragastrical administrations of huge cuprizone quantity, we clearly exaggerate and speed up the damaging process; and accordingly nerve damage appeared in various brain areas, with most prominent damage in corpus callosum, laterodorsal thalamus, nucleus reunions, anterior horn motor neurons, regional heterogeneities which suggest differences in the underlying pathophysiology as it had been previously claimed (72). Thereby, it is important to indicate that with consistently less nerve damage in all damaged areas in all BPC 157-cuprizone rats, the most important evidence is the consistent beneficial effect of BPC 157 also in those areas that were most affected, and thereby markedly improved gross presentation and functions in BPC 157 rats. Consistently, BPC 157 counteracted cerebellar ataxia (63), and impaired forelimb function (64), thus, it recovers both proximal and distal forelimb function including digit movements during a naturally occurring behavior that does not require extensive training or deprivation to enhance motivation (64). Even more intriguing is the presentation of right forelimb sparing and disability (reacting with only left or no forelimb upon challenge with forceps have difficulty with maintaining body balance while rearing), common in cuprizone rats, not presented in BPC 157-cuprizone rats (normal functioning of right forelimb, simultaneous reaction with both forelimbs, and grasp the forceps, and maintain body balance while rearing as healthy rats). Like in previous studies, BPC 157 consistently attenuates all encephalopathies that affect various brain areas, and that were induced by different agents, given in huge over-dose, such as paracetamol, diclofenac, ibuprofen or insulin. It also counteracts corresponding behavioral disturbances *i.e.*, prolonged sedation (diclofenac (22), ibuprofen (21)), seizures (insulin (23), paracetamol (20)). Likewise, a consistent beneficial effect of BPC 157 was evidenced in mice after traumatic brain injury (19). In accordance with the primary injury to the brain that initiates a secondary injury process of traumatic brain injury, the BPC 157 counteracting effects concerned the immediate consequences of severe head injury in mice, the preserved consciousness (with less unconsciousness and fatality). Also, BPC 157 attenuated and improved the postponed deleterious outcome (lower brain edema, significantly lower number and size of haemorrhagic traumatic lacerations, significantly lower intensity of subarachnoidal bleeding, significantly less intraventricular haemorrhage shown microscopically, declined fatality) (19).

Besides, considering the cuprizone effect on schizophrenia and thereby antipsyhotics in cuprizone model (73) BPC 157 also counteracts amphetamine toxicity, both acute and chronic (74, 75), like MPTP-toxicity and lethality (76), as well as catalepsy that may be produced by various neuroleptics (77). In addition, considering the serotonin system (*i.e.*, fluoxetine (78) sertraline

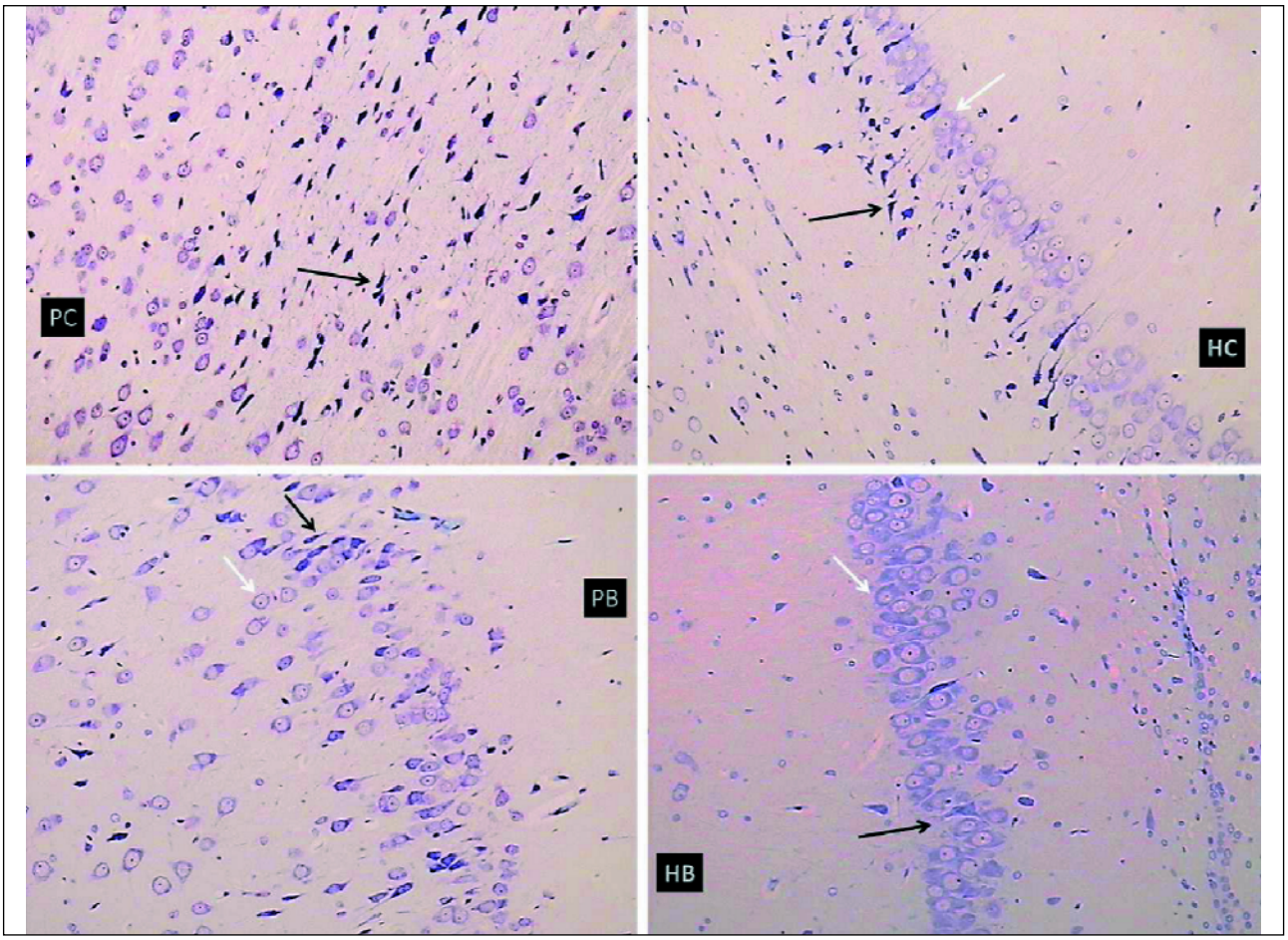

Fig. 9. Brain lesions in rats that received cuprizone diet, and then cuprizone intragastrical applications, and thereby, cuprizone toxicity manifestations. BPC 157 (10 µg/kg, 10 ng/kg) was applied either per-orally in drinking water (0.16 µg/ml/12 ml/day; 0.16 ng/ml/12 ml/day till the sacrifice) and intragastrically after cuprizone instilations, while controls received simultaneously drinking water only (12 ml/day) as well as an equivolume of saline (5 ml/kg) intragastrically after cuprizone instillations. Characterisistic presentation of parietal neocortex (PC, PB), left (control (PC), more neurons damage (black arrow indicates ballonized neuron); BPC 157, (PB), less neurons damage (black arrow indicates ballonized neuron; white arrow indicates normal neuron) and hippokampal region CA1, right (control (HC), more neurons damage (black arrow indicates ballonized neuron, white arrow indicates normal neuron); BPC 157 (HB), less neurons damage (black arrow indicates ballonized neuron; white arrow indicates normal neuron). Nissl ×20.

(79), promote remission in acute experimental autoimmune encephalomyelitis) BPC 157 affects with region specific influence the brain serotonin synthesis (α-[14C]methyl-l-tryptophan autoradiographic measurements) (80), reduces the duration of immobility to a greater extent than imipramine (81). Also, given peripherally, BPC 157 counteracts serotonin-syndrome, and has a particular increase in serotonin in substantia nigra, acutely and chronically (80).

Thus, it seems that these findings provide convincing evidence that BPC 157 may be also useful in cuprizone-rats. In addition, BPC 157 exhibits a particular effect on muscle healing in general terms as well. Of note, the extent of the demonstrated beneficial effect on recovery of muscle function and integrity is considerable, and involves the transection- (15, 37) crush- (16, 17) lesion of striated muscle, and healing impairment induced by systemic corticosteroid application (16), as well as function impairment induced by centrally acting agents (74, 75). Finally, BPC 157 markedly improves the healing of traumatic nerve (18) and traumatic brain injury (19) and ameliorates encephalopathies that occur in severely intoxicated animals (20-23) and therefore, exhibits particular neuroprotective ability. Besides, the BPC 157 healing involves also the smooth muscle functions, formations of new smooth muscle stands at the site of injury (5), prominent adaptation after resection injury (43) and rescuing of the failed sphincter function (82-85). Therefore, seen from the viewpoint of the inflammatory bowel disease-multiple sclerosis connections throughout BPC 157, the final proof should be the corresponding effectiveness in both models, multiple sclerosis as well as ulcerative colitis. Thus, the BPC 157's extension to inflammatory bowel disease (or from inflammatory bowel disease) seems to be well backed presented with favorable both animal data in various models of intestinal damages, and clinical data in inflammatory bowel patients (5-7, 9).

Summarizing, while current biological therapy which presumably seeks to target specific immune and biochemical abnormalities at the molecular and cellular level, the stable gastric pentadecapeptide BPC 157 evidence is more general, and from this point of view, even more suited for additional applications. For instance, reversed were increased number of inflammatory cells (38, 44-46) and the rise of leukotriene B_4 (LTB_4), thromboxane B_2 (TXB_2), and myeloperoxidase (MPO) in the serum and inflamed tissues (47-49). BPC 157 increased macrophages activity (44). BPC 157 counteracted deleterious effects of NO-system blockade (*i.e.*, BPC 157 reversed NO-

system blockade-complete failure of fistulas healing (35), and the same BPC 157-NO-system interaction was also seen in other models and in various species (4, 8, 25-27)). BPC 157 stimulated expression of egr-1 gene (but also its repressor nab2 (44)) and this should be implemented in BPC 157's wound healing capability (*i.e.*, gastrointestinal tract, muscle, nerve, brain lesions healing)) (5-7, 9), and cytokine and growth factor generation and early extracellular matrix (collagen) formation (44). Therefore, for BPC 157 in both inflammatory bowel disease and multiple sclerosis, we should consider significance of both NO-system and egr-1 gene-systems for both inflammatory bowel disease and multiple sclerosis (54-57). Multiple sclerosis is characterized by the progressive damage or loss of oligodendrocytes and this may be closely related to egr-1 gene (58). Thereby, more use of models of inflammatory bowel disease (and in particular, more effectiveness in cysteamine colitis rats with colon-colon anastomosis), better evidence for multiple sclerosis models (especially, cuprizone application) would be ascertained. Thus, various intestinal lesions models cured, even when complicated with relapse; poor healing of the fistulas; intestinal anastomosis; massive intestinal resection leading to short bowel, and functional incapability of the post-anastomotic remained intestine, all together indicate most improvement achieved by BPC 157 regimens, per-orally, parenterally and locally (10, 11, 35, 42, 43). Such advanced effectiveness (macro/microscopically, functionally, biomechanically) in healing may be perceived as an additional proof of BPC 157/inflammatory bowel disease/multiple sclerosis concept.

Especially, considering the anastomosis as a particular intestinal wound that needs all processes to be accommodated before the healing of the anastomosis could occur (5-7, 9, 42, 43), the suggested particular effect on endothelium integrity, termed also "wound healing therapy", clearly covers the cysteamine colitis healing in the same way (5-7, 9). This generalization may explain initial particular extensions from inflammatory bowel research, throughout a short term period and long term therapy, toward the disturbances, such as multiple sclerosis search as well (5-7, 9).

Clearly, huge stability, *i.e.*, stable in human gastric juice more than 24 hours, and use without carrier (otherwise, peptide + carrier(s) -complex bears considerable methodological/activity dilemmas), prominent antiulcer effect, and a safe application in inflammatory bowel disease patients with improved wound/intestinal anastomosis healing, adhesion prevention, collagen deposition, and anastomotic strength, along with prominent effect in cuprizone rats (*i.e.*, particularly intoxicated with much higher diet and additional intragastrical applications) (5-7, 9) as well as in other encephalopathies (20-23) and brain damage (19) and muscle (15-17) and nerve (18) disorders, all together support the special pentadecapeptide characteristics and practical importance. Thereby, we advocate the stable gastric pentadecapeptide BPC 157 in both inflammatory bowel disease and multiple sclerosis therapy.

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Conflict of interests: None declared.

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