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Peptide therapy with pentadecapeptide BPC 157 in traumatic nerve injury

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ABSTRACT

We focused on the healing of rat transected sciatic nerve and improvement made by stable gastric pentadecapeptide BPC 157 (10 μg, 10 ng/kg) applied shortly after injury (i) intraperitoneally/intragastrically/locally, at the site of anastomosis, or after (ii) non-anastomozed nerve tubing (7 mm nerve segment resected) directly into the tube. Improvement was shown clinically (autotomy), microscopically/morphometrically and functionally (EMG, one or two months post-injury, walking recovery (sciatic functional index (SFI)) at weekly intervals). BPC 157-rats exhibited faster axonal regeneration: histomorphometrically (improved presentation of neural fascicles, homogeneous regeneration pattern, increased density and size of regenerative fibers, existence of epineural and perineural regeneration, uniform target orientation of regenerative fibers, and higher proportion of neural vs. connective tissue, all fascicles in each nerve showed increased diameter of myelinated fibers, thickness of myelin sheet, number of myelinated fibers per area and myelinated fibers as a percentage of the nerve transected area and the increased blood vessels presentation), electrophysiologically (increased motor action potentials), functionally (improved SFI), the autotomy absent. Thus, BPC 157 markedly improved rat sciatic nerve healing.

1. Introduction

We focused on the improvement of the healing after traumatic nerve injury [1–12], transected rat sciatic nerve injury healing and a peptide therapy, using a small, orally active, anti-ulcer peptide [13–22] — stable gastric pentadecapeptide BPC 157 (MW 1419) effective in trials for inflammatory bowel disease therapy [19–22].

Knowing also BPC 157's wound healing capability, increased collagen and new blood vessels formation, decreased myeloperoxidase (MPO) activity and inflammatory cell influx, including healing of transected muscle and tendon [13,14,23–32], we thought that it could also influence the healing of transected nerve injuries, although generalization is not always applicable, since for example, tacrolimus (FK506) does not have a consistent effect on wound healing [33]. Important fact is that BPC 157 has undoubtedly a positive effect on muscle healing [27–30], providing the importance of the suggested regeneration of the damaged intramuscular nerve branches [34].

BPC 157 shares some characteristics with neuroimmunophilin ligands, of which tacrolimus (FK506) has emerged as a particularly promising therapeutic agent important in a nerve injury [1,2]. Neuroimmunophilin ligands cross the blood–brain barrier and are orally effective in a variety of animal models of ischemia, traumatic nerve injury and human neurodegenerative disorders [1]. BPC 157 given peripherally also crosses the blood–brain barrier influencing region-specific serotonin synthesis in the rat brain [35,36]. Unlike neuroimmunophilin ligands [3], it also exhibits a neurotropic effect attenuating 1-methyl-4-phenyl 1,2,3,6, tetrahydropyridine (MPTP) brain lesion and mortality [15].

Therefore, after sciatic nerve transection, BPC 157 was applied intraperitoneally, intragastrically or locally, at the site of anastomosis immediately after nerve anastomosis creation. In addition, after the nerve segment was dissected, BPC 157 was applied directly into the tube to completely fulfill the gap between the nerve stumps.

2. Materials and methods

All experimental procedures were approved by the local Ethics Committee and assessed by the observers blinded about the treatment given. We used male Wistar Albino rats, 200 g body weight, randomly assigned, at least 10 rats per experimental group per period. The surgical procedures were performed under a dissection microscope (Opton, Oberkochen, Germany) in deeply anaesthetized rats (pentobarbital, 65 mg/kg i.p.). The right sciatic nerve was exposed and completely transected with microscissors, at 5 mm distal from foramen infrapiriforme, and the proximal and distal stumps anastomozed with three 10-0 monofilament epineurial sutures at each end (Ethilon, Ethicon Inc, USA). Alternatively, the

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7 mm long nerve segment was dissected, and using two 10-0 monofilament epineurial sutures at each end, the severed nerve ends were sutured to 1 mm inside a 9-mm silicone tube (Heyer Schulte, USA; internal diameter 1.20 mm), forming a 7 mm nerve gap with a volume of 17.7 μl. After the operation, all muscle incisions and skin incisions were sutured (4-0 Vicryl (polyglactin 910, Ethicon, USA), Dermalon 2-0 (Davis & Geck, GB)).

2.1. Therapy

Medication includes pentadecapeptide BPC 157 (a partial of sequence of human gastric juice protein BPC, freely soluble in water at pH 7.0 and in saline); peptide with 99% (HPLC) purity (1-des-Gly peptide as impurity, manufactured by Diagen, Ljubljana, Slovenia, GEPPPGKPADDAGLV, M.W. 1419) [14–19,23–25,27–30,35–40]. BPC 157 dissolved in saline (10 μg/kg, 10 ng/kg) or saline (5.0 ml/kg) were applied intraperitoneally, intragastrically or locally, at the site of anastomosis (1 ml/bath) immediately after nerve anastomosis creation. After the tubing operation, to completely fulfill the gap with the tested solutions, an insulin needle with a volume of 17.7 μl of the pentadecapeptide BPC 157 (58 μg/ml, 58 ng/ml) or an equivolume of the saline was applied directly into the distal part of the tube, and the corresponding volume of the air was evacuated proximally with the other needle. Assessment procedure was carried out one or two

Table 1
Morphometrical and EMG analyses of sciatic nerve recovery after transection and nerve anastomosis, and therapy with stable gastric pentadecapeptide BPC 157 application. Mann–Whitney *U*-test ***p*<*0.05*** at least vs. control.

Application immediately after nerve anastomosis creation	Medication	Time after nerve injury	Conduction velocity m/s	The diameter of the axons (μm)	Diameter of myelinated fibers (μm)	Thickness of myelin sheet envelope(μm)	Number of myelinated fibers × 10^2 /mm^2	% of myelinated fibers area as % of neural tissue on nerve cross section	Number of blood vessels/ visual field
			Min/Med/Max	Min/Med/Max	Min/Med/Max	Min/Med/Max	Min/Med/Max	Min/Med/Max	Min/Med/Max
Intra-peritoneal	Saline 1 ml/rat 5 ml/kg	1 month	5.7/24.5/38.1	1.0/2.1/5.5	1.0/2.0/5.5	0.1/0.2/0.6	50/90/130	3.1/5.0/10.0	1.5/2.2/2.7
	BPC 157 2 μg/rat 10 μg/kg	1 month	***29.9/41.4/44.4****	***1.8/4.2/8.3****	***1.5/4.6/8.7****	***0.2/0.4/1.1****	***200/250/270****	***25.1/30.2/41.1****	***2.0/2.9/3.8****
	BPC 157 2 ng/rat 10 ng/kg	1 month	***26.6/38.3/45.6****	***2.0/3.5/8.2****	***2.0/4.4/8.5****	***0.1/0.4/1.1****	***200/240/260****	***20.1/29.3/37.3****	***1.7/2.7/3.1****
	Saline 1 ml/rat 5 ml/kg	2 months	15.4/29.3/36.6	0.5/2.7/8.1	0.5/3.0/6.2	0.1/0.3/1.2	100/150/170	6.0/8.2/15.1	3.0/3.6/4.3
	BPC 157 2 μg/rat 10 μg/kg	2 months	***25.0/53.3/66.6****	***1.0/4.8/11.1****	***1.5/4.9/9.2****	***0.3/0.7/1.5****	***250/310/350****	***44.2/59.0/61.3****	***6.6/7.3/7.6****
	BPC 157 2 ng/rat 10 ng/kg	2 months	***30.5/39.3/46.6****	***1.0/4.9/10.7****	***1.5/4.8/8.5****	***0.2/0.7/1.3****	***240/290/340****	***39.9/51.6/57.4****	***5.9/6.6/6.9****
Locally, at the site of anastomosis (1 ml/bath)	Saline 1 ml/rat 5 ml/kg	1 month	7.2/27.6/33.5	1.0/2.2/8.5	1.1/2.0/5.1	0.2/0.3/0.6	50/90/140	3.3/5.7/9.7	1.6/2.2/2.8
	BPC 157 2 μg/rat 10 μg/kg	1 month	***29.3/38.3/48.1****	***2.1/3.5/8.4****	***2.5/3.5/8.4****	***0.3/0.5/1.1****	***190/230/240****	***12.1/28.2/41.3****	***2.2/3.0/3.7****
	BPC 157 2 ng/rat 10 ng/kg	1 month	***20.7/36.9/42.4****	***2.0/3.5/8.1****	***2.4/3.4/8.0****	***0.2/0.5/1.1****	***160/250/250****	***9.8/25.9/39.9****	***1.9/2.5/2.9****
	Saline 1 ml/rat 5 ml/kg	2 months	17.5/30.3/33.3	1.0/2.5/8.0	1.0/2.5/6.8	0.1/0.3/0.9	70/160/180	5.2/9.4/17.1	3.2/3.9/4.7
	BPC 157 2 μg/rat 10 μg/kg	2 months	***25/33.4/55.5****	***2.1/5.4/10.3****	***2.5/5.5/10.1****	***0.3/0.7/1.4****	***260/330/360****	***45.0/59.4/62.5****	***6.5/7.5/7.9****
	BPC 157 2 ng/rat 10 ng/kg	2 months	***22.4/37.8/43.9****	***2.1/4.9/9.9****	***2.0/4.5/9.9****	***0.4/0.8/1.2****	***210/300/330****	***42.7/57.8/60.6****	***6.1/6.7/7.0****
Intra-gastrical	Saline 1 ml/rat 5 ml/kg	1 month	1.9/26.6/40.1	1.1/2.3/5.5	1.0/2.1/5.0	0.1/0.2/0.6	40/100/130	4.0/6.1/11.0	1.4/2.0/2.4
	BPC 157 2 μg/rat 0 μg/kg	1 month	***32.2/44.0/65.2****	***1.1/3.5/9.5****	***1.6/4.9/9.9****	***0.2/0.4/1.2****	***210/260/300****	***22.3/30.7/43.4****	***1.9/2.7/3.7****
	BPC 157 2 ng/rat 10 ng/kg	1 month	***28.3/40.4/48.2****	***1.1/3.6/10.0****	***1.7/3.9/9.1****	***0.2/0.4/1.2****	***230/250/310****	***19.6/29.0/38.7****	***1.5/2.4/2.9****
	Saline 1 ml/rat 5 ml/kg	2 months	23.3/26.6/33.3	1.1/2.0/8.1	1.0/2.2/6.7	0.1/0.2/0.7	60/150/160	5.3/10.1/20.2	3.1/3.8/4.5
	BPC 157 2 μg/rat 10 μg/kg	2 months	***20.0/51.9/70.1****	***2.1/5.4/10.1****	***2.4/4.9/9.9****	***0.2/0.6/1.3****	***250/340/350****	***40.3/53.6/60.6****	***6.4/7.4/7.7****
	BPC 157 2 ng/rat 10 ng/kg	2 months	***30.3/41.3/49.8****	***2.1/4.9/9.9****	***2.3/4.8/9.7****	***0.2/0.6/1.1****	***250/300/320****	***32.5/50.9/59.6****	***5.8/6.8/6.9****