Impact of pentadecapeptide BPC 157 on muscle healing impaired by systemic corticosteroid application

Pevec, Damira; Novinščak, Tomislac; Brčić, Luka; Šipoš, Kristijan; Jukić, Ivana; Starešinić, Mario; Miše, Stjepan; Brčić, Iva; Kolenc, Danijela; Kliček, Robert; ...

© Med Sci Monit, 2010; 16(3): BR81-88
PMID: 20190676

Received: 2008.01.03
Accepted: 2008.11.03
Published: 2010.03.01

Impact of pentadecapeptide BPC 157 on muscle healing impaired by systemic corticosteroid application

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Danira Pevec[1ABCDEF], Tomislav Novinscak[1ABCDEF], Luka Brcic[2ABCDEF], Kristijan Sipos[1ABCDEF], Ivana Jukic[1ABCDEF], Mario Staresinic[1ABCDEF], Sandro Mise[1ABCDEF], Iva Brcic[2ABCDEF], Danijela Kolenc[2ABCDEF], Robert Klicek[1ABCDEF], Tihomir Banic[1ABCDEF], Marko Sever[1ABCDEF], Ana Kocijan[1ABCDEF], Lidija Berkopic[1ABCDEF], Bozo Radic[1ABCDEF], Gojko Buljat[1ABCDEF], Tomislav Anic[1ABCDEF], Ivan Zoricic[1ABCDEF], Ivan Bojanic[1ABCDEF], Sven Seiwerth[2ABCDEF], Predrag Sikiric[1ABCDEF]

[1] Department of Pharmacology, University of Zagreb Medical School, Zagreb, Croatia
[2] Institute of Pathology, University of Zagreb Medical School, Zagreb, Croatia

Source of support: Departmental sources

Summary

Background: The effect of systemic and local peptide treatment effective in muscle contusion and then on counteraction of corticosteroid-induced impairment was tested. The pentadecapeptide BPC 157, given without a carrier, improved the healing of transected quadriceps muscle. It also improved muscle healing in rats with muscle crush injury when applied systemically or locally. Importantly, it counteracted corticosteroid-impairment in tendon to bone healing. Thus BPC 157 is proposed as an effective treatment that can improve muscle healing in spite of corticosteroid treatment.

Material/Methods: After the gastrocnemius muscle complex had been injured, rats received BPC 157 (intraperitoneally or locally as a cream) and/or 6α-methylprednisolone (intraperitoneally) only once (immediately after injury, sacrifice at 2 h) or once daily (final dose 24 hours before sacrifice and/or assessment procedure at days 1, 2, 4, 7, and 14). Muscle healing was evaluated functionally, macroscopically, and histologically.

Results: Without therapy, crushed gastrocnemius muscle complex controls showed limited improvement. 6α-methylprednisolone markedly aggravated healing. In contrast, BPC 157 induced faster muscle healing and full function restoration and improved muscle healing despite systemic corticosteroid treatment when given intraperitoneally or locally and demonstrated functionally, macroscopically, and histologically at all investigated intervals.

Conclusions: BPC 157 completely reversed systemic corticosteroid-impaired muscle healing.

key words: **BPC 157 • corticosteroid • peptide therapy • muscle healing**

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/878454

Word count: 2236
Tables: 4
Figures: 3
References: 42

Author's address: Predrag Sikiric, Department of Pharmacology, University of Zagreb Medical School, Salata 11, 10000 Zagreb, Croatia, e-mail: sikiric@mef.hr

BACKGROUND

Corticosteroids have been repeatedly shown to inhibit the healing process and may be a particular problem for muscle contusions healing. This type of injury is very frequent, being the leading cause of morbidity from sports-related injuries, and corticosteroids are commonly used to relieve pain and expedite a player's return to active status [1–4]. Furthermore, this problem is complicated in practice by a lack of suitable pharmacological agents, including standard peptides that can improve muscle healing in spite of corticosteroid treatment.

We recently demonstrated that the pentadecapeptide BPC 157 accelerated post-injury muscle healing with full function restoration [5,6]. It also recovered transected Achilles tendon [7–9] and transected quadriceps muscle [6], and thereby the tendon-muscle unit. Importantly, it counteracted negative aspects of corticosteroids [7,8,10] and corticosteroid impairment in tendon to bone [7,8] and thermal injury [10,11] healing. BPC 157 also seems to be of high physiological importance [12] as an anti-ulcer agent [10-14] effective in trials for inflammatory bowel disease (code name PL 14736, Pliva, Croatia), without toxicity reported so far [15–17]. Unlike standard peptides, it does not need a carrier and is resistant to highly degrading media [5–23]. BPC 157 actively stimulated early collagen organization even under very unfavorable conditions [5-11] and also stimulated the expression of early growth response 1 (*egr1*) gene and its repressor, nerve growth factor 1-A binding protein-2 (*nab2*) [21].

Thus, assuming that this stable peptide is advantageous in the therapy of crushed gastrocnemius muscle complex (GMC) [5] and is effective when given either locally or systemically, without a carrier, also in debilitated healing in corticosteroid-treated animals [7,8,10], we attempted to show its effect on the healing of crushed muscle in rats additionally impaired with 6α-methylprednisolone given systemically.

MATERIAL AND METHODS

Rats

We used male Wistar Albino rats, 280–320 g body weight, with at least 10 rats randomly assigned to each experimental groups.

Injury induction

We used the previously described experimental procedure for crush injury [5]. The hind limbs were shaved a day before trauma and the animals were anesthetized before injury induction. A force of 0.727 Ns/cm^2 was delivered to the maximum diameter of the GMC, 2 cm proximal to the insertion of the Achilles tendon. The experimental procedure was approved by the Ethics Committee of the University of Zagreb Medical School.

Medication

Medication (agents applied alone or in combination) was given only once, i.e. immediately after injury (sacrifice after 2 h), and/or once daily, receiving the final dose 24 hours before sacrifice and/or the assessment procedure at days 1, 2, 4, 7, and 14. The medication included 6α-methylprednisolone (Depo-Medrol, Pfizer, USA) 1.0 mg/kg intraperitoneally; pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, M.W. 1419, manufactured by Diagen, Ljubljana, Slovenia [5–11,13,18,24–30]) 10 μg/kg and 10 ng/kg intraperitoneally, given without a carrier or peptidase inhibitor, dissolved in saline or saline alone (5.0 ml/kg); or BPC 157 locally as 1.0 μg or 0.01 μg dissolved in distilled water per gram of commercial neutral cream (Belobaza, Belupo, Croatia), or commercial neutral cream applied as a thin cream layer at the injury site.

Functional evaluation

Functional evaluation, as previously described [5], was carried out by an observer unaware of the given treatment. It was performed at days 1, 2, 4, 7, and 14 following injury and included walking analysis and muscle function. DeMedinaceli's sciatic functional index (SFI) was used for the walking analysis, this being:

$$SFI = -38.3\times(EPL-NPL)/NPL+109.5\times(ETS-NTS)/NTS+13.3\times(EIT-NIT)/NIT-8.8$$

where EPL and NPL are print length on the experimental and normal sides, ETS and NTS toe spread between the first and fifth digits on the experimental and normal sides, and EIT and NIT the distance between the middle of the second and fourth toes on the experimental and normal sides. Muscle function was evaluated using Koka's extensor postural thrust (EPT), i.e. a modified motor function index (MFI) assessed as the weight on a digital weight scale (precision: 0.001 g) born by the experimental or normal hind limb at the moment when the rat, held upright with the hind limb extended and placed upon the scale, begins to bear weight on the scale, as: EPT=(NEPT–EEPT)/NEPT, where NEPT and EEPT are the EPT values on the normal and experimental sides, respectively.

Macroscopy

Macroscopy at 2 h and on days 1, 2, 4, 7, and 14 following injury included severity of GMC injury and the maximum hind limb GMC circumference at the maximum impact site, i.e. approximately 2 cm above the insertion of the Achilles tendon. The severity of GMC injury was presented as a score ranging from 0 to 9, a sum of scored hematoma + swelling (edema) + erythema (0–3 for each parameter, with 0 – no injury presented, 1 – minimal, 2 – medium, and 3 – severe injury).

Pathohistological analysis

For histological analysis, the muscle was fixed immediately after skinning the injured leg in buffered formalin (pH 7.4) for 24 h, dehydrated, and embedded in paraffin wax. Muscle samples were cut, stained with hematoxylin and eosin and immunohistochemically for desmin (Dako, Glostrup, Denmark), and examined in a blinded fashion. For morphometrical analysis, hot-spot assessment and the special programs SFORM and ISSA (VAMSTEC, Zagreb, Croatia) were used. Five high-power fields were randomly selected for analysis from the area of maximal tissue damage, which was detected on semi-serial muscle sections. Inflammatory cells

Table 1. Macroscopic evaluation after intraperitoneal application of BPC 157.

Assessed parameter Med (Min/Max)	Time	Control		BPC 157 10 ug/kg		BPC 157 10 ng/kg	
		Saline	6α-MP	Saline	6α-MP	Saline	6α-MP
Injury severity*	2h	8.5 (6.0/9.0)	6.75† (4.1/8.75)	5.2§ (2.0/7.0)	4.7‡ (2.0/8.2)	4.5§ (3.0/5.0)	4.5§ (3.0/7.5)
	1d	7.5 (6.0/8.0)	4.5† (3.9/7.5)	2.5§ (1.0/6.0)	2.35§ (0.5/5.5)	3.0§ (1.0/4.0)	2.3§ (0.5/5.0)
	2d	5.5 (2.0/8.0)	3.75† (2.0/4.45)	2.0† (1.0/5.0)	1.9‡ (0.5/5.5)	2.5† (2.0/4.0)	2.0† (1.0/5.5)
	4d	2.0 (1.0/4.0)	1.5 (0.5/4.5)	0.5§ (0.0/2.0)	0.4§ (0.0/3.0)	1.5§ (0.0/1.0)	1.0§ (0.0/3.0)
	7d	1.0 (0.0/3.0)	0.0 (0.0/3.0)	0.0 (0.0/0.0)	0.57 (0.0/1.0)	0.0 (0.0/0.0)	0.0 (0.0/0.5)
	14d	0.0 (0.0/0.0)	0.0 (0.0/0.0)	0.0 (0.0/0.0)	0.0 (0.0/0.0)	0.0 (0.0/0.0)	0.0 (0.0/0.5)
GMC circumference# (mm)	2h	4.5 (3.0/6.0)	4.0 (2.5/5.7)	3.5§ (2.0/4.0)	2.5§ (1.5/4.0)	3.0§ (1.0/4.0)	2.5§ (1.5/3.5)
	1d	4.5 (4.0/8.0)	3.6† (2.4/5.7)	3.5§ (0.0/4.0)	2.3 (0.5/3.5)	2.0‡ (1.0/5.0)	2.0 (1.0/3.5)
	2d	4.0 (2.0/9.0)	3.3† (1.8/7.2)	1.5§ (0.0/3.0)	0.7 (0.0/3.0)	2.0 (1.0/5.0)	0.8 (0.0/3.5)
	4d	2.0 (0.0/6.0)	0.0 (0.0/6.4)	0.5‡ (–1.0/3.0)	0.0‡ (–1.0/2.0)	0.0‡ (0.0/4.0)	0.0‡ (–1.0/3.0)
	7d	0.0 (–1.0/5.0)	–2.0‡ (–3.0/4.7)	0.0 (–3.0/3.0)	0.0 (–3.0/2.0)	0.0 (0.0/1.0)	0.0 (–1.0/3.5)
	14d	0.5 (0.0/2.0)	–3.0‡ (–4.0/2.7)	0.0† (–1.0/0.0)	0.0 (–1.0/0.5)	0.0 (0.0/1.0)	0.0 (–2.0/2.5)

6α-MP – 6α-methylprednisolone 1 mg/kg intraperitoneally; Med – median; Min – minimal value; Max – maximal value; * injury severity score: hematoma (0–3) +edema (0–3) +erythema (0–3); GMC – gastrocnemius muscle complex; h – hours; d – days; † $p<0.05$, ‡ $p<0.01$, § $p<0.001$ vs. control; # difference between the maximum hind limb gastrocnemius muscle complex (GMC) circumference after and before injury.

and myofibers with centralized nuclei were counted in the selected areas and fiber diameters (μm) and desmin-positive areas (expressed as the percentage of analyzed area) were also measured.

Statistical analysis

The Kruskall-Wallis and Mann-Whitney tests were used for statistical analysis. The data are presented as the minimum, median, and maximum. All values of $p<0.05$ were considered statistically significant.

Results

Without therapy after GMC crush, the controls commonly showed limited improvement. In regenerating muscle, stronger desmin immunoreactivity and more myoblasts with centralized nuclei were found after post-injury day 7 and also presented at day 14 with excessive scar formation along with failed function (Tables 1–4, Figures 1–3).

The BPC 157 protocols shifted the morphological and immunohistochemical parameters toward earlier muscle regeneration, along with a rapid function recovery. At 24 h post-injury, there was less edema and some cells in the location of satellite cells of myocytes adjacent to damage area became hyperplastic and expressed significant desmin immunoreactivity. At post-injury day 4, more desmin immunoreactivity in myoblastic cells and more regenerating myofibers with centralized nuclei were present, along with a substantial number of blood vessels in the damaged area with strong desmin immunoreactivity of the proliferating cells in their walls. In the end, together with less scar formation, full function was completely recovered and contracture was not present. After an initial increase, fewer inflammatory cells were present throughout the experiment.

These findings contrasted with those of the crushed rats that continuously received 6α-methylprednisolone alone. Aggravation finally presented on post-injury day 14, characterized by marked muscle atrophy, disorganized muscle fibers, and disruption of the normal tissue architecture. Inflammatory response by post-injury day 7 was delayed, with more inflammatory cells and macrophages, few fibroblasts and myotubes, and necrotic tissue still present. Soon after an initial marked decrease in tissue cellularity, with

Table 2. Macroscopic evaluation after topical application of BPC 157.

Assessed parameter Med (Min/Max)	Time	Control		BPC 157 1 ug (per g of cream)		BPC 157 10 ng (per g of cream)	
		Cream	6α-MP	Cream	6α-MP	Cream	6α-MP
Injury severity*	2h	8.0 (6.0/9.0)	6.5‡ (4.0/9.0)	3.5§ (1.5/6.0)	3.5§ (1.0/5.5)	3.5§ (1.0/6.0)	3.5§ (1.5/5.0)
	1d	6.5 (4.0/7.0)	4.0‡ (2.5/6.5)	1.5§ (1.0/5.0)	1.5§ (0.5/3.5)	2.5§ (2.0/4.0)	2.5§ (1.0/4.0)
	2d	5.5 (1.0/7.0)	3.0† (2.0/6.0)	2.0† (0.0/4.0)	2.0† (0.5/2.5)	1.5‡ (1.0/4.0)	1.5‡ (0.5/2.0)
	4d	1.5 (1.0/3.0)	1.5 (0.5/3.0)	0.5† (0.0/2.0)	0.5† (0.0/2.5)	1.5† (0.0/3.0)	0.5† (0.0/2.0)
	7d	0.0 (0.0/2.0)	1.0 (0.0/1.0)	0.0 (0.0/0.0)	0.0 (0.0/0.0)	0.0 (0.0/0.0)	0.0 (0.0/0.0)
	14d	0.0 (0.0/0.0)	0.0 (0.0/0.0)	0.0 (0.0/0.0)	0.0 (0.0/0.0)	0.0 (0.0/0.0)	0.0 (0.0/0.0)
GMC circumference# (mm)	2h	5.0 (3.0/7.0)	4.6 (3.1/6.3)	2.0§ (0.0/5.0)	1.7§ (1.0/3.4)	2.0§ (1.0/5.0)	1.6§ (0.8/3.3)
	1d	5.0 (3.0/6.0)	4.3 (2.4/7.3)	1.0§ (0.0/3.0)	0.5 (0.0/2.8)	3.5§ (1.0/5.0)	2.0 (0.5/4.7)
	2d	3.0 (1.0/5.0)	2.4 (1.7/4.5)	0.5§ (0.0/2.0)	0.2 (–1.6/2.4)	2.5 (0.0/4.0)	2.0 (1.0/3.1)
	4d	0.5‡ (0.0/3.0)	0.5 (0.0/4.5)	0.0 (–2.0/3.0)	0.0 (–1.5/1.5)	2.0 (0.0/4.0)	1.0 (–0.5/2.3)
	7d	1.0 (–1.0/2.0)	–2.0‡ (–3.0/5)	0.0 (–1.0/1.0)	0.0 (–1.4/1.6)	0.0 (0.0/1.0)	0.0 (–1.7/2.2)
	14d	0.0 (–1.0/1.0)	–3.0‡ (–4.0/3.0)	0.0 (–1.0/0.0)	0.0 (–2.1/1.1)	0.0 (0.0/1.0)	0.0 (–1.5/2.0)

6α-MP – 6α-methylprednisolone 1 mg/kg intraperitoneally; Med – median; Min – minimal value; Max – maximal value; * injury severity score: hematoma (0–3) +edema (0–3) +erythema (0–3); GMC – gastrocnemius muscle complex; h – hours; d – days; † $p<0.05$, ‡ $p<0.01$, § $p<0.001$ vs. control; # difference between the maximum hind limb gastrocnemius muscle complex (GMC) circumference after and before injury.

fewer inflammatory cells, deterioration occurred. However, this early recovery was not evident in functional tests as they became visibly worse than in the controls, which was consistent with the more pronounced contracture, worse muscle capability, and impaired walk.

All these pitfalls were fully counteracted in the BPC 157 rats on both systemic and local regimens. With grossly less visible lesion, the rats walked better, muscle function rapidly recovered, presenting a highly advanced healing course and with no post-injury contraction at the end, which was generally consistent with the microscopic and immunohistochemical findings (Tables 1–4, Figures 1–3).

Discussion

After crush, GMC defects permit only very poor spontaneous healing and crushed GMC controls exhibited only limited improvement [5]. Of note, this failure could be further aggravated; systemic application of corticosteroid induced an additional impairment. The resulting aggravation of muscle healing in the corticosteroid-treated rats with crushed GMC was consistent with the commonly described downhill corticosteroid course: early transient recovery, a sparing effect on the local muscle tissue with inhibition of the inflammatory response, followed by unwanted atrophy and failed collagen synthesis by inhibition of the inflammatory response in the long term [1–4]. BPC 157, in contrast, consistently improved muscle healing [5,6] when given intraperitoneally as well as locally (as a cream). The noted amelioration has to be a strong effect; the positive effect of BPC 157 on muscle crush injury [5] can clearly improve muscle healing in spite of corticosteroid treatment.

It should be noted, however, that the possible mechanism of BPC 157 on the recovery of crushed rat muscle treated with 6α-methylprednisolone remaines elusive, considering that glucocorticoids act on target tissue to produce a variety of effects, but their final significance has not been completely elucidated [1–4]. Even so, the noted evidence that BPC 157 reversed corticosteroid impairment in rats with crushed GMC is consistent with the described systemic counteractions to corticosteroid-impaired healing (tendon injuries [7,8], and burns [10]), particularly since these BPC 157 dose regimens and modes of application have been successfully used [7,8,10]. Together,

Table 3. Functional evaluation after intraperitoneal application of BPC 157.

Assessed parameter Med (Min/Max)	Time	Control		BPC 157 10 ug/kg		BPC 157 10 ng/kg	
		Saline	6α-MP	Saline	6α-MP	Saline	6α-MP
SFI	2h	– (–/–)	– (–/–)	– (–/–)	– (–/–)	– (–/–)	– (–/–)
	1d	–33.6 (–42.2/–29.4)	–29.4 (–45.25/–17.3)	–23.9† (–41.6/–9.8)	–21.7† (–40.3/–15.4)	–22.8† (–46.7/–14.5)	–22.4† (–47.3/–16.2)
	2d	–32.3 (–42.3/–21.0)	–27.3 (–40.6/–17.3)	–21.4† (–26.9/–1.0)	–18.8‡ (–34.8/–5.7)	–22.6‡ (–35.2/–2.8)	–20.7‡ (–34.2/–3.4)
	4d	–24.0 (–34.7/–12.6)	–19.7 (–37.4/–10.23)	–10.7‡ (–25.9/2.9)	–9.3‡ (–12.3/–4.7)	–14.8‡ (–25.8/–6.7)	–8.5‡ (–16.7/–4.2)
	7d	–18.3 (–22.4/–7.4)	–18.5 (–24.3/–5.7)	–4.5§ (–15.2/7.7)	–9.3§ (–15.2/–3.5)	–9.0† (–14.7/1.3)	–8.4§ (–12.3/1.1)
	14d	–11.0 (–24.5/0.4)	–20.4† (–24.11/–5.3)	–2.6§ (–8.3/7.2)	–2.3§ (–12.5/–3.7)	–2.0‡ (–12.4/4.7)	–2.4‡ (–14.9/1.5)
EPT	2h	– (–/–)	– (–/–)	– (–/–)	– (–/–)	– (–/–)	– (–/–)
	1d	0.5 (0.2/0.5)	0.46 (0.2/0.7)	0.3§ (0.1/0.3)	0.28§ (0.0/0.4)	0.3§ (0.1/0.3)	0.1§ (0.1/0.3)
	2d	0.2 (0.15/0.5)	0.17 (0.1/0.3)	0.1§ (0.0/0.3)	0.075 (0.0/0.3)	0.0§ (0.0/0.2)	0.045 (0.0/0.2)
	4d	0.2 (0.2/0.3)	0.15 (0.0/0.4)	0.1§ (0.1/0.2)	0.6§ (0.0/0.2)	0.1§ (0.1/0.1)	0.3§ (0.0/0.1)
	7d	0.2 (0.1/0.5)	0.38‡ (0.1/0.5)	0.1§ (–0.1/0.3)	0.1§ (0.0/0.3)	0.0§ (0.0/0.0)	0.05§ (0.0/0.15)
	14d	0.2 (0.0/0.2)	0.32‡ (0.1/0.5)	0.1§ (0.1/0.2)	0.15§ (0.0/0.3)	0.0§ (–0.1/0.1)	0.15§ (0.0/0.2)

6α-MP – 6α-methylprednisolone 1 mg/kg intraperitoneally; SFI – Sciatc Functional Index; EPT – Extensor Postural Thrust, motor function index; h – hours; d – days; Med – median; Min – minimal value; Max – maximal value; † $p<0.05$, ‡ $p<0.01$, § $p<0.001$ vs. control.

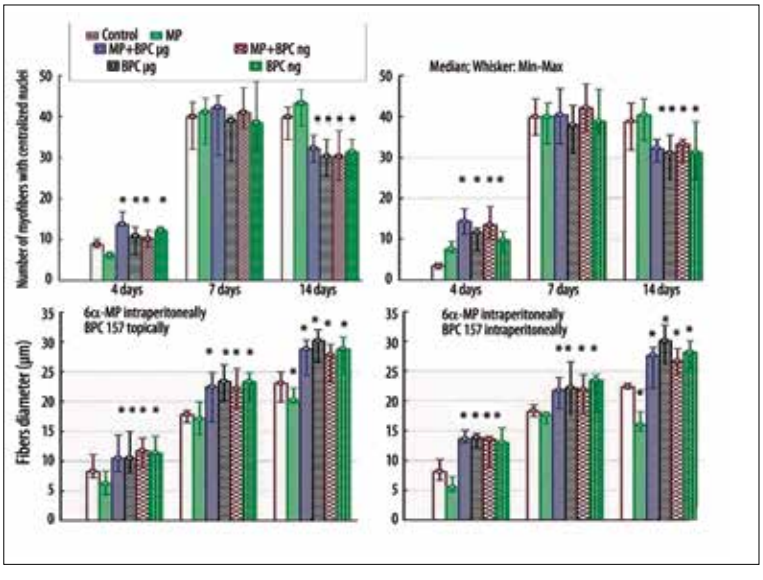

Figure 1. Presentation of myonuclei and myofibers diameters. The number of myoblasts with centralized nuclei in animals treated with BPC 157 alone or in combination with 6α-methylprednisolone is higher after 4 days compared with control animals and animals treated with 6α-methylprednisolone, while the results after 14 days are completely opposite, which suggests impaired muscle healing in the latter groups. The positive effect of BPC 157 and 6α-methylprednisolone is also evident in the constantly larger diameter of myofibers. *$p<0.01$, at least, vs. control.

these data suggest that BPC 157 can have a particular relationship with the corticosteroid system. Accordingly, in burned animals BPC 157 not only counteracted corticosteroid wound impairment, but also inhibited corticosteroid

Table 4. Functional evaluation after topical application of BPC 157.

Assessed parameter Med (Min/Max)	Time	Control		BPC 157 1 ug (per g of cream)		BPC 157 10 ng (per g of cream)	
		Cream	6α-MP	Cream	6α-MP	Cream	6α-MP
SFI	2h	– (–/–)	– (–/–)	– (–/–)	– (–/–)	– (–/–)	– (–/–)
	1d	–39.0 (–48.5/–30.7)	–35.2 (–51.3/–22.4)	–27.2† (–42.6/–14.6)	–26.5 (–39.4/–18.2)	–31.6‡ (–43.6/–13.0)	–28.6 (–40.4/–14.3)
	2d	–34.3 (–40.0/–23.8)	–30.7 (–44.2/–23.3)	–18.0† (–33.3/–10.5)	–15.7 (–33.6/–12.5)	–25.0‡ (–41.9/–6.8)	–21.3 (–38.4/–11.6)
	4d	–22.9 (–32.5/–10.2)	–17.3 (–31.7/–12.5)	–14.5 (–27.5/–4.7)	–13.6 (–24.0/–7.6)	–14.9 (–36.8/–11.5)	–11.8 (–24.1/–6.1)
	7d	–19.7 (–35.1/–6.5)	–15.2 (–25.5/–8.4)	–12.2† (–15.8/1.7)	–10.2 (–26.7/–4.0)	–14.3 (–26.4/–6.7)	–10.2 (–18.3/–4.3)
	14d	–15.6 (–24.8/–4.5)	–15.3 (–20.7/–7.2)	–7.3† (–11.7/15.3)	–5.5 (–12.4/3.2)	–4.4† (–10.7/2.6)	–2.6 (–9.3/3.1)
EPT	2h	– (–/–)	– (–/–)	– (–/–)	– (–/–)	– (–/–)	– (–/–)
	1d	0.4 (0.2/0.5)	0.37 (0.3/0.45)	0.3§ (0.1/0.3)	0.25§ (0.15/0.3)	0.3§ (0.1/0.3)	0.3§ (0.1/0.5)
	2d	0.2 (0.2/0.5)	0.15 (0.1/0.25)	0.1§ (0.0/0.2)	0.1§ (0.05/0.2)	0.1§ (0.1/0.2)	0.1§ (0.0/0.2)
	4d	0.3 (0.1/0.5)	0.24 (0.05/0.2)	0.1§ (0.1/0.2)	0.07§ (0.05/0.15)	0.1§ (0.1/0.1)	0.05§ (0.0/0.1)
	7d	0.2 (0.2/0.4)	0.35‡ (0.1/0.5)	0.1§ (0.1/0.2)	0.1§ (0.0/0.2)	0.1§ (0.0/0.2)	0.05§ (0.0/0.15)
	14d	0.2 (0.0/0.3)	0.35‡ (0.2/0.5)	0.0§ (–0.1/0.0)	0.0§ (–0.1/0.0)	0.0§ (–0.1/0.0)	0.0§ (–0.1/0.0)

6α-MP – 6α-methylprednisolone 1 mg/kg intraperitoneally; SFI – Sciatc Functional Index; EPT – Extensor Postural Thrust, motor function index; h – hours; d – days; Med – median; Min – minimal value; Max – maximal value; † $p<0.05$, ‡ $p<0.01$, § $p<0.001$ vs. control.

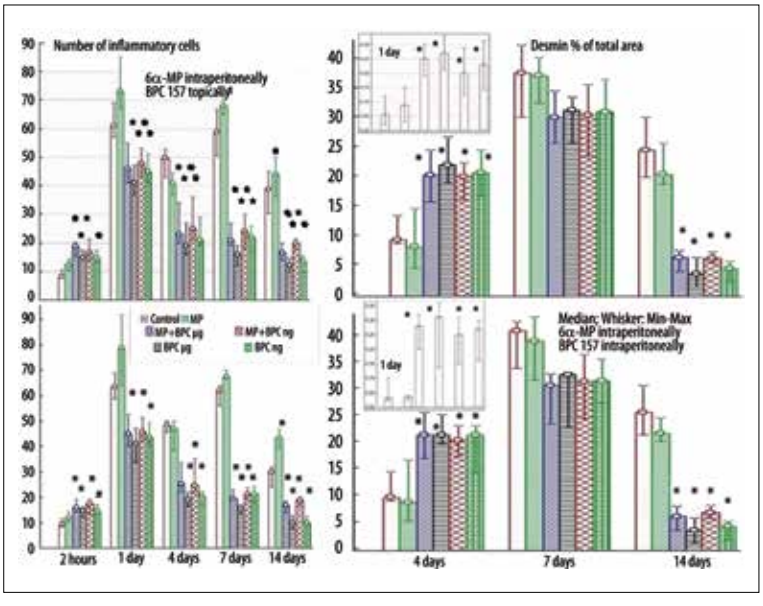

Figure 2. Inflammatory cells and desmin immunoreactivity. Comparing animals treated with BPC 157 alone or in combination with 6α-methylprednisolone with controls or 6α-methylprednisolone animals, a more robust inflammatory response in the earlier interval with a decrease in the later intervals is obvious, as well as initially increased desmin immunoreactivity. All this parameters favor improved healing. *$p<0.01$, at least, vs. control.

immunosuppression [8,9]. In rats with the Achilles tendon sharply detached from the bone edge, the stable peptide BPC 157 can be a novel therapeutic approach to improve early healing of ischemic or injured tissue. Namely, BPC

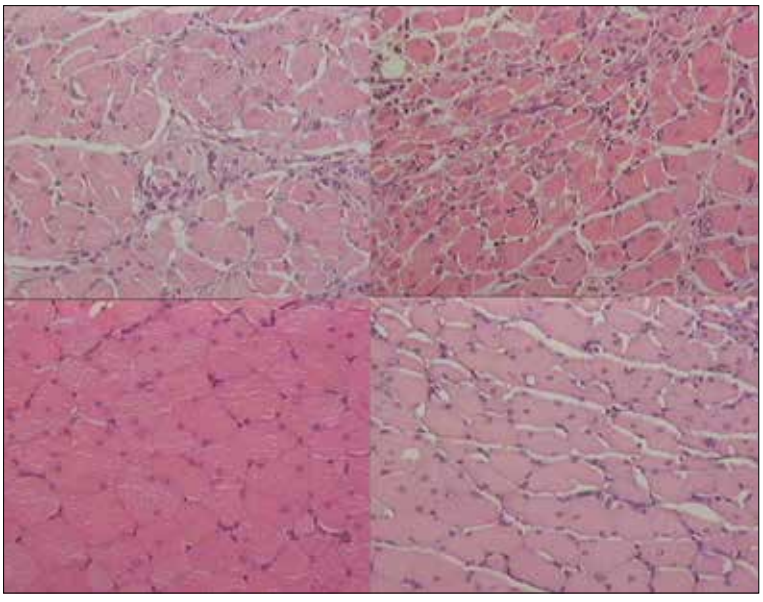

Figure 3. Histological presentation. Hematoxylin and eosin stained slides of muscle fibers 14 days after injury, demonstrating larger myofibers in BPC 157 and 6α-methylprednisolone+BPC 157 animals (lower left and right, respectively) with smaller numbers of centralized myonuclei compared with the control and 6α-methylprednisolone-treated animals (upper left and right, respectively). (Objective ×25).

157 decreased MPO activity and inflammatory cell influx with increased new blood vessel formation, unlike the decrease seen with 6α-methylprednisolone [7]. Thus BPC 157 application rescued both the regular healing drawbacks and the more complex impairment that appeared after prolonged systemic corticosteroid application. Commonly, after BPC 157 was applied, reversal of the impaired healing and better collagen organization occurred [5–11], pointing to its particular counteracting effect on corticosteroid treatment. Considering that peptides generally have a short half-life but prolonged effects (BPC 157 is no exception; for review see, e.g. [17,24]), it may be that also BPC 157 can successfully trigger a particular chain of secondary events increasing its beneficial effect. In support of this it was demonstrated in wound healing [21] that BPC 157 was more active than PDGF-BB in stimulating early collagen organization and expression of *egr1* gene and its repressor *nab2*. This can be important since egr-1 plays an important role in vascular recovery [31] and muscle function (continuous contractile activity led to a particular increase in egr-1 mRNA) [32]. Also, egr-1 has an important role for glucocorticoids and the activated glucocorticoid receptor [33]. Finally, BPC 157 also counteracted ACTH secretion, but was ineffective after adrenalectomy [27]. Therefore, BPC 157 can induce faster healing, usefully interacting with corticosteroid functions and modulating corticosteroid impairment [7,8,10,27].

Another possibility might be that, as previously described [13], pentadecapeptide BPC 157 interacted with the somatosensory neurons and their function, and this led to nerve healing and regeneration, which is very important for muscle healing [34]. Unlike standard growth factors, BPC 157 considerably affected/modulated the NO system in different species [25,26,28,30]. NO has an essential role in collagen tissue organization [35,36], and BPC 157/NO interaction [25,26,28,30] (vs. corticosteroids) corresponded to a more pronounced and better collagen organization. Importantly, systemic treatment with glucocorticoids significantly altered the expression levels of inducible nitric oxide synthase (iNOS) and GTP-cyclohydrolase I (GTP-CH I), the reaction-limiting enzyme in the biosynthesis of the iNOS cofactor (6R) 5,6,7,8-tetrahydropbiopterin (6-BH4), during the repair process. Expression of iNOS and GTP-CH I was suppressed by glucocorticoids in normal, and to a much greater extent in wounded skin [35]. In addition, as mentioned, unlike corticosteroids [37], BPC 157 has a strong angiogenic effect [5–11,24,29] and directly protects endothelium [24] and counteracts endothelin over-expression [30], which might be important considering endothelin's role in fibrosis development [38].

The final point can be related to the evidence that these muscle crush injuries may respond to therapy affecting the accompanying inflammatory process. Importantly, as mentioned, BPC 157's beneficial effect on wound healing paralleled the reduction in the inflammatory cells and MPO [5–11,18–21] and it also reduced levels of LTB4 and TXB2 in both the serum and inflamed tissues [39]. These can be perceived as a purposeful peptide healing delivery to injured muscle, important in resolving the corticosteroid anti-inflammatory controversy. Regularly, BPC 157 did not impede corticosteroid's initial recovery, but it did inhibit the latter's healing breakdown, characterized by failed healing in corticosteroid-treated rats with crush injury [7,8,10].

Finally, whatever the final mechanism(s), with evident appropriate healing and effective function recovery [5–11,18–21], because it is always given alone [5–30,39] BPC 157 overrides the unstable standard peptides regularly applied with carrier(s) and, thereby, the considerable methodological/activity dilemmas of peptide+carrier(s) complexes [40,41]. Carrier addition, particularly in wound healing [40–42], modified the original effects of growth factors (whose own activity is practically very weak) and could not solve problems in systemic/local peptide treatment for muscle healing [1–4,20], i.e. (in)efficiency, uncertain delivery and, consequently, their practical utility for corticosteroid disturbances. Thus BPC 157's effects can be much more accepted to be the peptide's own effects, and the proposed mechanism(s) more likely reliable.

CONCLUSIONS

The pentadecapeptide BPC 157 is capable to ameliorate wound healing and muscle contusion injury even in corticosteroid-treated rats. BPC 157 completely reversed systemic corticosteroid impairment in muscle healing, modulated corticosteroid's effects, and represents a potentially important peptide therapy.

REFERENCES:

1. Beiner JM, Jokl P: Muscle contusion injuries: current treatment options. J Am Acad Orthop Surg, 2001; 9: 227–37
2. Beiner JM, Jokl P, Cholewicki J, Panjabi MM: The effect of anabolic steroids and corticosteroids on healing of muscle contusion injury. Am J Sports Med, 1999; 27: 2–9
3. Järvinen TAH, Järvinen TLN, Minna Kääriäinen M et al: Muscle injuries: biology and treatment. Am J Sports Med, 2005; 33: 745–62
4. Paolini JA, Orchard JW: The use of therapeutic medications for soft-tissue injuries in sports medicine. MJA, 2005; 183: 384–88
5. Novinscak T, Brcic L, Staresinic M et al: Gastric pentadecapeptide BPC 157 as an effective therapy for muscle crush injury in the rat. Surg Today, 2008; 38: 716–25
6. Staresinic M, Petrovic I, Novinscak T et al: Effective therapy of transected quadriceps muscle in rat: gastric pentadecapeptide BPC 157. J Orthopaed Res, 2006; 24: 1109–17
7. Krivic A, Majerovic M, Jelic I et al: Modulation of early functional recovery of Achilles tendon to bone unit after transection by BPC 157 and methylprednisolone. Inflamm Res, 2008; 57: 205–10
8. Krivic A, Anic T, Seiwerth S et al: Achilles detachment in rat and stable gastric pentadecapeptide BPC 157: promoted tendon to bone healing and opposed corticosteroid aggravation. J Orthop Res, 2006; 24: 982–89
9. Staresinic M, Sebecic B, Jadrijevic S et al: Gastric pentadecapeptide BPC 157 accelerates healing of transected rat Achilles tendon and *in vitro* stimulates tendocytes growth. J Orth Res, 2003; 21: 976–83
10. Sikiric P, Seiwerth S, Mise S et al: Corticosteroid-impairment of healing and gastric pentadecapeptide BPC-157 creams in burned mice. Burns, 2003; 29: 323–34
11. Mikus D, Sikiric P, Seiwerth S et al: Pentadecapeptide BPC 157 cream improves burn-wound healing and attenuates burn-gastric lesions in mice. Burns, 2001; 127: 817–27
12. Wood JD: The first Nobel prize for integrated systems physiology: Ivan Petrovich Pavlov, 1904. Physiology, 2004; 19: 326–30
13. Sikiric P, Seiwerth S, Grabarevic Z et al: Beneficial effect of a novel pentadecapeptide BPC 157 on gastric lesions induced by restraint stress, ethanol, indomethacin, and capsaicin neurotoxicity. Dig Dis Sci, 1996; 41: 1604–14
14. Xue XC, Wu YJ, Gao MT et al: Protective effects of pentadecapeptide BPC 157 on gastric ulcer in rats. World J Gastroenterol, 2004; 10: 1032–36
15. Ruenzi M, Stolte M, Veljaca M et al: Ulcerative Colitis Study Group. A multicenter, randomized, double blind, placebo-controlled phase II study of PL 14736 enema in the treatment of mild-to-moderate ulcerative colitis. Gastroenterology, 2005; 128: A584
16. Veljaca M, Krnic Z, Brajsa K et al: The development of PL 14736 for treatment of inflammatory bowel disease. IUPHAR-GI Section Symposium, Honolulu, Hawaii. 2002; 13–15 July: O-32
17. Veljaca M, Pavic-Sladojev D, Mildner B et al: Safety, tolerability and pharmacokinetics of PL 14736, a novel agent for treatment of ulcerative colitis, in healthy male volunteers. Gut, 2003; 51: A309
18. Seiwerth S, Sikiric P, Grabarevic Z et al: BPC 157's effect on healing. J Physiol (Paris). 1997; 91: 173–78
19. Seveljevic Jaran D, Cuzic S, Dominis Kramaric M et al: Accelerated healing of excisional skin wound by PL 14736 in alloxan-hyperglycemic rats. Skin Pharmacol Physiol, 2006; 19: 266–74
20. Xue XC, Wu YJ, Gao MT: Study of the protective effects of pentadecapeptide BPC 157 on wounds in small type pigs. Chin New Drugs J, 2004; 12: 602–4
21. Ivetic Tkalcevic I, Cuzic S, Brajsa K et al: Enhancement by PL 14736 of granulation and collagen organization in healing wounds and the potential role of egr-1 expression. Eur J Pharmacol, 2007; 570: 212–21
22. Veljaca M, Chan K, Guglietta A: Digestion of h-EGF, h-TGF alpha, and BPC-15 in human gastric juice. Gastroenterology, 1995; 108: 761
23. Gjurasin M, Miklic P, Buljat G et al: Selective healing effect of the gastric pentadecapeptide BPC 157 on injured part of the nerve in subtotal sciatic nerve transection. Dig Dis Sci, 2003; 48: 1878
24. Sikiric P, Seiwerth S, Grabarevic Z et al: The beneficial effect of BPC 157, a 15 amino acid peptide BPC fragment, on gastric and duodenal lesions induced by restraint stress, cysteamine and 96% ethanol in rats. A comparative study with H2 receptor antagonists, dopamine promotors and gut peptides. Life Sci, 1994; 54(5): PL63–68
25. Grabarevic Z, Tisljar M, Artukovic B et al: The influence of BPC 157 on nitric oxide agonist and antagonist induced lesions in broiler chicks. J Physiol (Paris), 1997; 91: 139–49
26. Turkovic B, Sikiric P, Seiwerth S et al: Stable gastric pentadecapeptide BPC 157 studied for inflammatory bowel disease (PLD-116, PL14736, Pliva) induces nitric oxide synthesis. Gastroenterology, 2004; 126: 287
27. Sikiric P, Petek M, Rucman R et al: A new gastric juice peptide BPC – an overview of stomach/stress/organoprotection hypothesis and BPC beneficial effects. J Physiol (Paris), 1993; 87: 313–27
28. Sikirić P, Seiwerth S, Grabarević Z et al: The influence of a novel pentadecapeptide, BPC 157, on N(G)-nitro-L-arginine methylester and L-arginine effects on stomach mucosa integrity and blood pressure. Eur J Pharmacol, 1997; 332: 23–33
29. Sikiric P, Separovic J, Anic T et al: The effect of pentadecapeptide BPC 157, H2 blockers and sucralfate on new vessels and new granulation tissue formation. J Physiol (Paris), 1999; 93: 479–85
30. Lovric-Bencic M, Sikiric P, Separovic J et al: Doxorubicine congestive heart failure-increased big-endothelin 1 plasma concentration. Reversal by amlodipine, losartan and gastric pentadecapeptide BPC 157 in rat and mouse. J Pharmacol Sci, 2004; 95: 19–26
31. Lee YS, Jang HS, Kim JM et al: Adenoviral-mediated delivery of early growth response factor-1 gene increases tissue perfusion in a murine model of hindlimb ischemia. Mol Ther, 2005; 12: 328–36
32. Irrcher I, Hood DA: Regulation of Egr-1, SRF, and Sp1 mRNA expression in contracting skeletal muscle cells. J Appl Physiol, 2004; 97: 2207–13
33. Wong DL: Why is the adrenal adrenergic? Endocr Pathol, 2003; 14: 25–36
34. Lehto MUK, Jarvinen J: Muscle injuries, their healing process and treatment. Ann Chir Gynaecol, 1991; 80: 102–8
35. Frank S, Madlener M, Pfeilschifter J, Werner S: Induction of inducible nitric oxide synthase and its corresponding tetrahydrobiopterincofactor synthesizing enzyme GTP-cyclohydrolase I during cutaneous wound repair. J Invest Dermatol, 1998; 111: 1058–6436. Whittle BJR, Boughton-Smith NK, Moncada S: Biosynthesis and role of the endothelium-derived vasodilator, nitric oxide in gastric mucosa. Ann NY Acad Sci, 1992; 664: 126–39
37. Hashimoto I, Nakanishi H, Shono Y et al: Angiostatic effects of corticosteroid on wound healing of the rabbit ear. J Med Invest, 2002; 49: 61–66
38. Rockey DC, Chung JJ: Endothelin antagonism in experimental hepatic fibrosis. Implications for endothelin in the pathogenesis of wound healing. J Clin Invest, 1996; 98: 1381–88
39. Veljaca M, Lesch CA, Pllana R et al: BPC-15 reduces trinitrobenzene sulfonic acid-induced colonic damage in rats. J Pharmacol Exp Ther, 1994; 272: 417–22
40. Kurtz CA, Loebing TG, Anderson DD et al: Insulin-like growth factor I accelerates functional recovery from Achilles tendon injury in a rat model. Am J Sports Med, 1999; 27: 363–69
41. Urist MR: The first three decades of bone morphogenetic protein. Osteologie, 1996; 4: 207–33
42. Thomaidis V, Kazakos K, Lyras DN et al: Comparative study of 5 different membranes for guided bone regeneration of rabbit mandibular defects beyond critical size. Med Sci Monit, 2008; 14(4): BR67–73