

Achilles Detachment in Rat and Stable Gastric Pentadecapeptide BPC 157: Promoted Tendon-to-Bone Healing and Opposed Corticosteroid Aggravation

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ABSTRACT: Stable gastric pentadecapeptide BPC 157 (BPC 157, as an antiulcer agent in clinical trials for inflammatory bowel disease; PLD-116, PL 14736, Pliva, no toxicity reported) alone (without carrier) ameliorates healing of tendon and bone, respectively, as well as other tissues. Thereby, we focus on Achilles tendon-to-bone healing: tendon to bone could not be healed spontaneously, but it was recovered by this peptide. After the rat's Achilles tendon was sharply transected from calcaneal bone, agents [BPC 157 (10 µg, 10 ng, 10 pg), 6α-methylprednisolone (1 mg), 0.9% NaCl (5 mL)] were given alone or in combination [/kg body weight (b.w.) intraperitoneally, once time daily, first 30-min after surgery, last 24 h before analysis]. Tested at days 1, 4, 7, 10, 14, and 21 after Achilles detachment, BPC 157 improves healing functionally [Achilles functional index (AFI) values substantially increased], biomechanically (load to failure, stiffness, and Young elasticity modulus significantly increased), macro/microscopically, immunohistochemistry (better organization of collagen fibers, and advanced vascular appearance, more collagen type I). 6α-Methylprednisolone consistently aggravates the healing, while BPC 157 substantially reduces 6α-methylprednisolone healing aggravation. Thus, direct tendon-to-bone healing using stable nontoxic peptide BPC 157 without a carrier might successfully exchange the present reconstructive surgical methods. © 2006 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. *J Orthop Res* 24:982–989, 2006

Keywords: gastric pentadecapeptide BPC 157 (BPC 157); tendon to bone healing

INTRODUCTION

Generally, because the spontaneous healing of a tendon is poor,^{1–4} the tendon-to-bone healing is hampered with particular restrictions.^{1,3,5} Contrary, the improved healing of transected Achilles tendon,^{1,6} a tendon with the direct insertion to bone, is appealing for a new therapy attempt for Achilles detachment injury. With the stable gastric pentadecapeptide BPC 157 applied following transection, the tendon healing can be consistently improved without any further surgical assistance;⁶ that is, cut tendon ends were not reattached either using a bone tunnel or using anchors. In extension, this can also augment the healing of an Achilles tendon in a rat detachment model to adequately reattach the tendon to bone

accordingly with the former tendon to bone tissue presentation.¹ As suggested,¹ these approaches have been done to overcome the limitations of methods of the indirect reconstruction.^{3,5,7–10} Generally, an artificial bone tunnel^{3,5,10} is supposed to secure the tendon graft, but graft laxity and failure^{5,9} are not avoided. Also, graft ossification occurred when various growth factors have been applied in the tunnel to strengthen the graft to bone contact regardless of complexity of carrier systems.^{5,7,8,10}

Therefore, because there are few reports on effective direct healing of the Achilles tendon at the tendon edge to bone, we explore the stable gastric pentadecapeptide BPC 157 (nondegraded in human gastric juice more than 24 h, unlike minutes for other growth factors;¹¹ as an antiulcer agent^{11–14} in clinical trials for inflammatory bowel disease (PLD-116, PL 14736, Pliva) no toxicity reported¹⁵) that does not need a carrier to ameliorate the transected tendon^{1,6} and bone healing,¹⁰ and various wounds,¹²

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and does not induce cartilage or ossicles formation in other tissues.^{1,6,16} Bearing in mind Achilles tendon-to-bone area as hypovascular, hyponeural, and hypocellular region with distinct morphological and biomechanical properties,^{4,17,18} the impaired both healing and medication accessibility should be considered. The therapeutic efficacy can credit that applied systemically, pentadecapeptide BPC 157 heals both transected tendon and bone defect,^{6,16} and counteracts corticosteroid-impaired healing.¹³ Furthermore, this tendon attaches to bone with an intervening zone of fibrocartilage (direct type) that requests synthesis of strong collagen fibers of type I, mainly responses seen recently in a burn wound model treated with this pentadecapeptide.¹³ Thus, by applying various doses of BPC 157 and using functional, biomechanical, histological, and immunohistochemistry analysis, we studied the healing of rat's Achilles tendon sharply transected from calcaneal bone. Corticosteroid, which repeatedly showed inhibition of the healing process,¹³ but was still used clinically to relieve pain and expedite a player's return to active status,¹⁹ was applied alone or combined with pentadecapeptide.

MATERIALS AND METHODS

Experimental Protocol

Wistar Albino male rats 280–300 g b.w. randomly assigned were used in the experiments, approved by local Ethic Committee, and the effect assessed by the examiners completely unaware about the given protocol. Under ketamin hydrochloride anesthesia using an aseptic technique, the right hind leg was shaved and a 3-cm longitudinal skin incision was made. The Achilles tendon and plantaris tendon were identified and sharply detached from the calcaneal bone edge. The skin was closed using loupe magnification 3× with 5-0 intracutaneous monofilament nylon running suture (Ethicon Inc., Johnson and Johnson, Somerville, NJ) (Fig. 1). Gastric pentadecapeptide BPC157 (GEPPPGKPADDAGLV, M.W. 1419, prepared by Diagen, Ljubljana, Slovenia, as a partial of sequence of human gastric juice protein BPC, freely soluble in water at pH 7.0 and in saline, peptide with 99% [high-pressure liquid chromatography (HPLC) purity (1-des-Gly peptide as impurity), as described before^{1,6,12–14,116,20–22}] (10 µg, 10 ng, 10 pg), 6α-methylprednisolone (Depomedrol, Pharmacia & Upjohn) (1 mg), alone or in combination (6α-methylprednisolone 1 mg and BPC 157 10 µg) were given intraperitoneally (i.p.)/kg b.w., as in a previously effective regimen^{6,13,16} (control received simultaneously an equivolume of 0.9% NaCl; 5 mL/kg), one time daily, first application at 30 min after surgery, last at 24 h before assessment (function, biomechanic, microscopy,

immunochemistry at 1, 2, 3, 4, 7, 10, 14, and 21 days after Achilles detachment).

Functional Evaluation

For functional evaluation, the Achilles functional index (AFI)^{6,23,24} was assessed. Briefly, the hind paws of the rats were moistened, and the rats were allowed to walk down a confined runway. Measurements [the distance to the opposite foot (TOF), print length (PL), the distance between the second and fourth or intermediary toes (IT)] were made from each walking track defining particular parameters [de Medinaceli's print length factor (PLF); toe-spread factor (TSF); intermediary toe-spread factor (ITF)] included in AFI-calculation [AFI = 74(PLF) + 161(TSF) + 48(ITF) - 5], as follows: PLF = (NPL-EPL)/EPL; TSF = (ETS-NTS)/NTS; ITF = (EIT-NIT)/NIT, where N is the normal uninjured side, E is the experimental, injured side, and TS is total spreading.

Biomechanical Evaluation

For biomechanical evaluation the hind limbs that were used for biomechanical analysis were frozen at -80°C until testing. At the same day the biomechanical testing was carried out, the limbs were thawed and all soft tissues with the exemption of gastrocnemius muscle were removed. Tendon diameter was measured at the calcaneal bone edge with precision calipers. The proximal part of the Achilles tendon was fixed with specially prepared clamps designed to distribute the load evenly over an elastically gripped distal part of the soleus muscle and proximal part of the Achilles tendon. The distal part was gripped in the same manner at the metatarsal foot region. Specially designed gripes allow maintaining tension in one plain, elastically holding both parts of tested specimen without tissue damage and prevent slippage during the testing. The tendon was kept moist with balanced salt solution during preparation. The mechanical testing was performed on a computerized testing machine (Uniaxial Tensile Testing Machine—UTM Messphysik BETA 50-5, Altenmarkt, Austria). Once the tendon was clamped firmly in the test fixtures, a preload of 0.5 N was applied and the displacement reading was nulled. A single 1-cm/s ramp to failure test was performed. Failure load, failure deformation, stiffness, and Young elasticity module were determined via analysis of continuously recorded computer data.

Histologic Evaluation

For histology, the tendon-to-bone samples were prepared using standard procedure for tissue fixation, demineralization, and paraffin embedding. Briefly, the tendon was freed from surrounding tissue and as much muscle and fat as possible were removed. The specimens were fixed in 10% neutral-buffered formalin, demineralized in formic acid, dehydrated in alcohol, and embedded in paraffin. Serial sections of the specimen were cut longitudinally through their entire length,

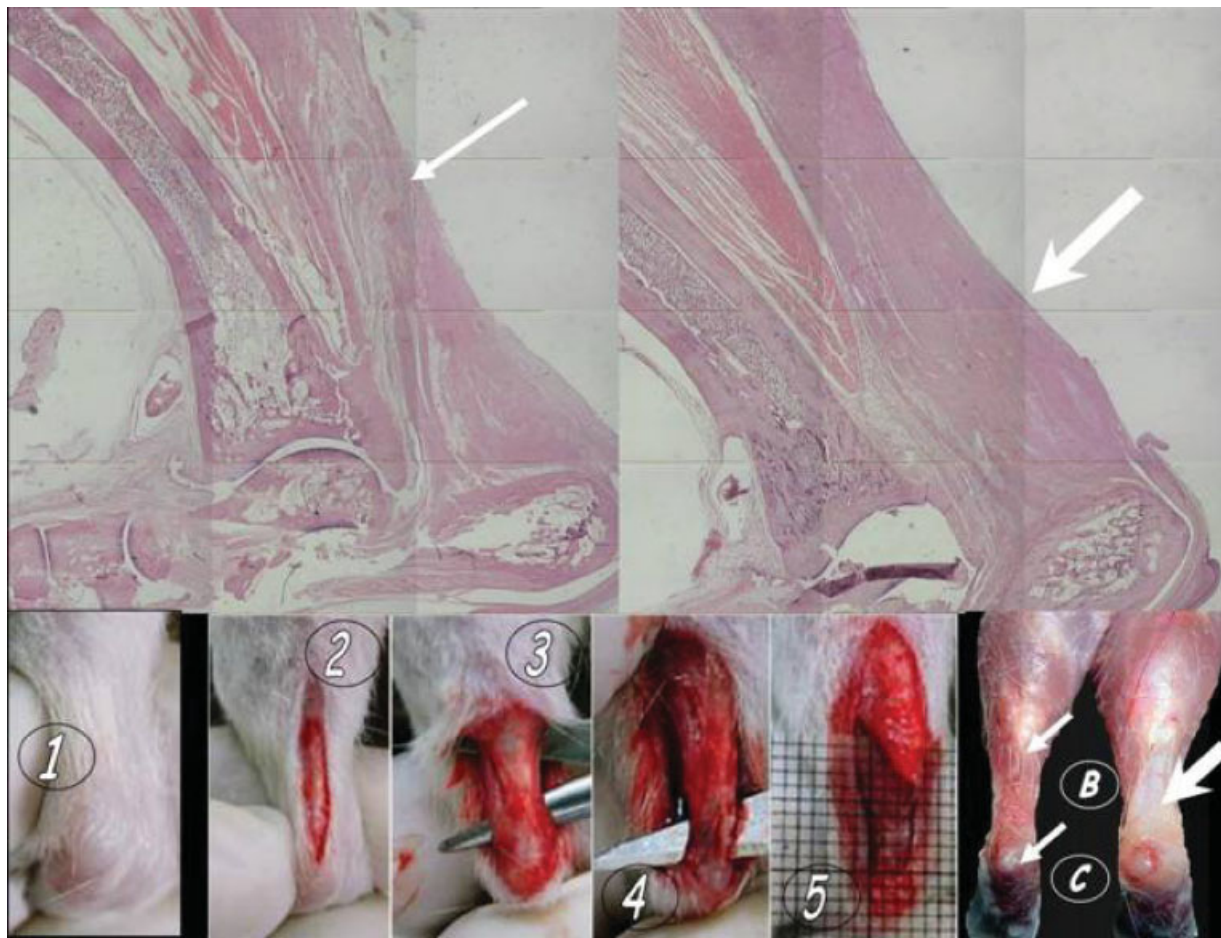


Figure 1. The presentation of surgery (down 1–5) and course Achilles detachment healing with and without therapy. HE, day 14, sagittal section, patchwork, objective $\times 2$ (upper part). In control (upper left) we present the gap between the tendon stump and calcaneus, partly filled with loose connective tissue and some fatty inclusions (arrow). In the treated animal (right picture) there is a well-formed connection throughout the gap area with only scarce fatty change. Gross presentation at day 10 in controls (C, left) with a significant gap between the tendon edge and bone with a clear stump (arrows), and in the BPC 157-treated rat (B, right) leg shows no defect between the tendon stump and calcaneal bone. The edge of the tendon stump cannot be recognized sharply with the connective tissue matrix (arrow).

giving insight to calcaneus, calcaneotendinal junction, tendon, and distal gastrocnemius muscle. The slides were stained using hematoxylin eosin. For each tendon three slides from the central part were selected and examined using a light microscope (Olympus-BX50, Olympus Optical Co Ltd, Tokyo, Japan). The assessment,²⁵ accordingly modified, includes (1) fiber structure and arrangement, (2) increased vascularity, (3) collagen stainability, (4) degeneration, using a scoring system (0–3), in relation to normal presentation with 0—no change, 1—mild, 2—severe, and 3—profound.

Immunohistochemistry

For the immunohistochemistry studies, after sacrifice, specimen was shortly immersed in liquid nitrogen, placed in an Eppendorf tube, and stored in liquid

nitrogen. The specimen was cut on a cryostat (Leica TP1020) and fixed in cold acetone for 10 min. The specimen were mounted on a silanized slide and stained using primary antibodies against collagen I and III and LSAB procedure method (DAKO, Glostrup, Denmark), according to manufacturer's specifications. We tested the area of tendon to bone junction (distal part) of the specimen using semiquantitative scale from 0–3, where 0 represents no color and 3 represents the strongest color intensity.

Statistical Analysis

For functional evaluation (AFI index) and biomechanical values (load to failure, stiffness, and Young elasticity modulus) analysis of variance were used. Data were presented as median and range. Changes in AFI were

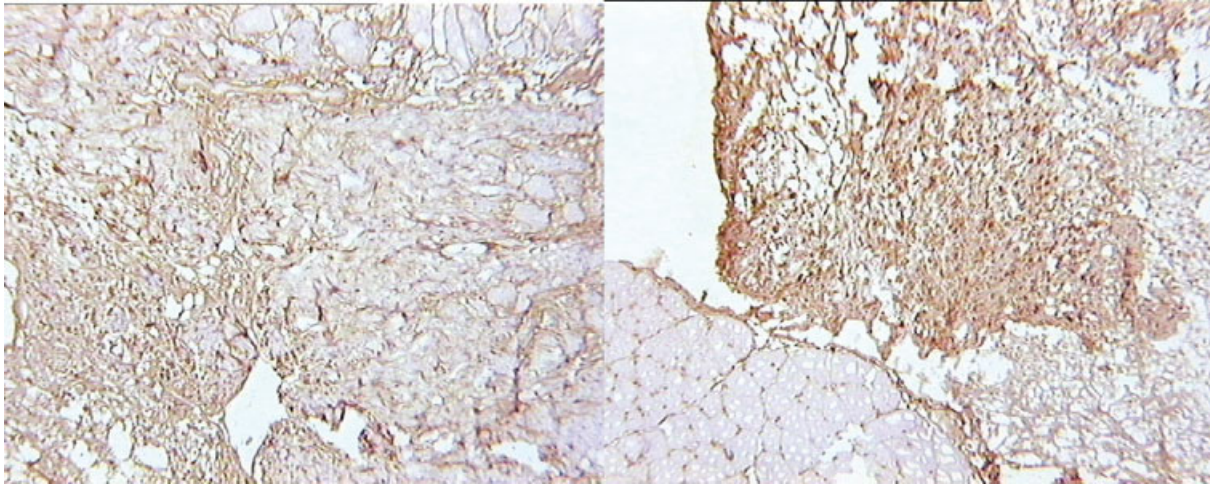


Figure 2. Day 4, tendon margin and granulation tissue, immunohistochemistry, collagen type III, objective $\times 6.3$. Loosely arranged positive fibers with no particular organization (left, control), a much stronger positivity, as a result of more fibers with stronger reactivity and a better longitudinal orientation (right, BPC 157).

expressed using the AUC method (area under curve, 1 cm y-axis represents 10 AFI units, 1 cm x-axis represents 1 day). The significant were p -values of $p < 0.01$. All results were confirmed with nonparametric Kruskal-Wallis tests with $p < 0.01$. Histological parameters and immunohistochemistry were analyzed using frequencies of each point scored for each testing after lesion for particular group. Thus, we used 2×2 frequency tables for a Fischer exact test to ascertain the connection between the particular group (control or experimental) and the pathologic score. As the pathologic scores were not normally distributed and the expected cell sizes are small (less than 5), this Fischer test is used as an alternative to the Pearson chi-square test to determine whether the sum-score difference between the two groups was statistically significant. The SPSS (release 11.0) statistical package (SPSS, Chicago, IL) was used for analysis. A probability level of $p < 0.05$ was considered significant.

RESULTS

Important for the tendon otherwise not repaired to the bone in controls, the positive outcome of Achilles detachment in BPC 157-treated rats has been evidenced since the beginning: improved AFI values (Fig. 1) are along with improved healing: biomechanical (Table 1), macroscopic (Fig. 1), microscopic (i.e., a positive shift in healing from the fourth postoperative day, abundant fibers with parallel orientation and low blood vessels, but prominent raise in the earliest critical period, and improved collagen patterns and increased appearance, with no hyaline degeneration; Fig. 1, Table 2), immunohistochemistry (the highest

stainability present consistently more collagen with predominance of the type I along with advanced healing period; Fig. 2, Table 2). Because they were noted with both $10 \mu\text{g}$ and 10 ng/kg daily regimens, these effects obviously are not accidental. On the contrary, 6α -methylprednisolone markedly aggravates the already detrimental healing course of Achilles detachment (i.e., failed function, biomechanical diffuse disruption of tendon at the lower part, loss of the fine fiber structure, increased waviness and separation of the fibers with the delayed vascularity, low collagen with predominant type III, and severe hyaline degeneration). This 6α -methylprednisolone aggravation is fully opposed when BPC 157 is given simultaneously with 6α -methylprednisolone.

DISCUSSION

We have evaluated without further surgical interventions (bone tunnel, or any attempt to actually surgically reattach the tendon to bone) whether gastric pentadecapeptide BPC 157—as a stable peptide with systemic treatment (without carrier) (like previously transected tendon⁶ or bone healing¹⁶)—could rescue Achilles tendon-to-bone healing from inadequate healing, worsened detachment, and degeneration and corticosteroid impairment, and found that it was possible, within the period and dose regimen shown in healing of the fully transected tendon,⁶ and also by providing this particular rat Achilles detachment model we use

Table 1. Functional and Biomechanical Evaluation of Achilles Tendon Detachment Course

		Presentation of Load to Failure (N), Young Elasticity Module $\times 10^5$ N/mm ² , and Stiffness (N/mm) in Rats at the Assessment day (12 Rats per each Group for Every Time Interval)					
Parameter Assessed	Days	Control	BPC 157 10 μ g/kg	BPC 157 10 ng/kg	BPC 157 10 pg/kg	MP	MP +BPC157 10 μ g/kg
		Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
AFI	1	-128 \pm 9	-100 \pm 7	-110 \pm 9	-120 \pm 11	-145 \pm 15	-120 \pm 14
	2	-120 \pm 8	-96 \pm 6	-109 \pm 8	-117 \pm 10	-142 \pm 13	-117 \pm 12
	3	-120 \pm 7	-94 \pm 7	-107 \pm 8	-117 \pm 8	-140 \pm 12	-116 \pm 10
	4	-118 \pm 9	-90 \pm 5	-105 \pm 8	-115 \pm 8	-139 \pm 12	-112 \pm 9
	7	-115 \pm 8	-74 \pm 5	-99 \pm 5	-113 \pm 7	-134 \pm 10	-109 \pm 9
	10	-110 \pm 8	-62 \pm 8	-95 \pm 6	-105 \pm 5	-128 \pm 12	-105 \pm 7
	14	-106 \pm 9	-36 \pm 6	-68 \pm 7	-102 \pm 6	-118 \pm 10	-99 \pm 6
	21	-104 \pm 6	-33 \pm 4	-64 \pm 3	-96 \pm 5	-118 \pm 9	-88 \pm 6
Load to failure (N)	Biomechanic	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
	4	3.5 \pm 0.3	6 \pm 0.5	5.2 \pm 0.5	3.6 \pm 0.5	2.7 \pm 0.8	3.3 \pm 0.5
	7	7 \pm 0.6	16 \pm 1.5	13 \pm 0.9	8 \pm 0.7	6 \pm 1.1	8 \pm 0.8
	10	17 \pm 1.5	27 \pm 2	22 \pm 1.8	19 \pm 1.6	12 \pm 1.6	20 \pm 1.6
	14	24 \pm 2	34 \pm 2.5	29 \pm 2	25 \pm 2.2	18 \pm 1.9	25 \pm 2.2
Young elasticity module $\times 10^5$ N/mm ²	4	0.43 \pm 0.05	0.66 \pm .05	0.59 \pm .05	0.44 \pm 0.05	0.29 \pm 0.02	0.42 \pm 0.04
	7	0.85 \pm 0.07	1.61 \pm 0.1	1.41 \pm 0.1	0.92 \pm 0.1	0.55 \pm 0.05	0.77 \pm 0.08
	10	2.09 \pm 0.2	2.85 \pm 0.2	2.48 \pm 0.2	2.21 \pm 0.2	1.50 \pm 0.1	1.80 \pm 0.1
	14	3.15 \pm 0.4	3.78 \pm 0.3	3.44 \pm 0.3	3.22 \pm 0.3	2.20 \pm 0.3	2.90 \pm 0.2
	21	3.71 \pm 0.4	4.37 \pm 0.4	4.30 \pm 0.4	3.93 \pm 0.3	2.20 \pm 0.3	2.95 \pm 0.2
Stiffness (N/mm)	4	5.1 \pm 0.5	7.5 \pm 0.6	7.0 \pm 0.6	5.4 \pm 0.5	3.8 \pm 0.3	4.8 \pm 0.4
	7	5.8 \pm 0.5	9.6 \pm 0.4	7.9 \pm 0.5	6.6 \pm 0.4	4.3 \pm 0.3	6.1 \pm 0.5
	10	5.4 \pm 0.6	8.5 \pm 0.3	7.1 \pm 0.5	6.5 \pm 0.4	4.4 \pm 0.4	5.7 \pm 0.5
	14	4.3 \pm 0.4	7.4 \pm 0.3	6.1 \pm 0.4	4.6 \pm 0.3	2.9 \pm 0.4	4.9 \pm 0.3
	21	2.4 \pm 0.4	5.2 \pm 0.3	4.0 \pm 0.4	3.2 \pm 0.3	2.4 \pm 0.3	3.4 \pm 0.4

Pentadecapeptide BPC 157 and 6 α -methylprednisolone (MP), applied intraperitoneally, alone or in combination. $p <$ vs. control, $p <$ vs. 6 α -methylprednisolone, $p <$ vs. control and vs. 6 α -methylprednisolone. Kruskal-Wallis test, $p <$ 0.01

to reproduce the events that occur in patients.¹ Contrary, the artificial bone tunnel and tendon graft allow dubious significance (no natural occurring site where tendon is surrounded by bone), where intraarticular and extraarticular position,³ graft laxity,⁵ and unpleasant ossification (i.e., with various factors and different carriers in the bone tunnel)^{6,7,9,26} are readily produced. No natural site exists where the tendon is surrounded by bone.³ To emphasize, pentadecapeptide-improved mechanical load is continuously providing a decisive element during the direct tendon-to-bone healing, particularly connective tissue healing. Clear impact of BPC 157 therapy (i.e., in transected tendon, it directly affects tendocytes⁶) on the functional recovery is important, considering that the tendon cannot otherwise be repaired to the bone, and healing cannot be advanced spontaneously. Noted from the

first postoperative day, the improved function and AFI can be a clear predictor of the positive outcome of healing (for review see, i.e., ref. 6), and structural integrity has been restored on the fourth day. Generally, after Achilles tendon detachment, BPC 157-treated rats show more blood vessels and dilated capillary beds at injury site in the early healing period (day 4). BPC 157 has angiogenic potential,^{6,21,22} directly protects endothelium,²² counteracts endothelin overproduction,²⁰ and modulates NO synthesis, and NO system function,¹⁴ and is substantially involved in tendon and bone injury and healing²⁷⁻²⁹ [i.e., BPC 157 opposes both L-arginine-NO synthesis overexpression, and NO synthesis inhibitor N^G-nitro-L-arginine methylester (L-NAME) harmful effect also seen in tendon and bone injuries].¹⁴ In this hypovascular region, these can improve clearing of cellular debris and toxic

Table 2. Histology and Immunohistochemistry Scoring (0–3) Presentation of Achilles Detachment Course Pentadecapeptide BPC 157 and 6 α -Methylprednisolone (MP), Applied Intraperitoneally, Alone or in Combination

Presentation of Fiber Structure, Vascularity, Collagen Staining, and Degeneration, as the Number of Rats Presenting with the Given Score (0–3) at the Assessment Day (12 Rats per Each Group per Each Time Interval) and Collagen III Immunohistochemistry (Median and Range)

Parameter Scored (0–3)	Control			BPC 157 10 μ g/kg			BPC 157 10 ng/kg			BPC157 10 pg/kg			MP			MP +BPC157 10 μ g/kg				
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Fiber structure	1	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	4	0	0	12	0	0	10	2	0	0	10	2	0	0	2	10	0	0	1	11
	7	0	2	10	0	10	2	0	7	5	0	0	0	0	6	6	0	1	5	6
	10	0	3	9	0	11	1	0	0	9	3	0	0	0	8	4	0	2	6	4
	14	0	4	8	0	11	1	0	0	9	3	0	0	0	8	4	0	3	8	1
	21	0	4	8	0	11	1	0	0	9	3	0	0	0	8	4	0	4	8	0
Vascularity	1	0	1	8	3	0	1	4	7	0	1	5	6	0	1	9	2	0	4	8
	4	0	1	10	1	3	7	2	0	2	6	4	0	0	2	9	1	0	6	6
	7	0	1	10	1	5	7	0	0	4	7	1	0	0	2	9	1	0	7	5
	10	0	3	9	0	8	4	0	0	7	5	0	0	0	4	8	0	0	10	2
	14	0	5	7	0	8	4	0	0	6	6	0	0	0	5	7	0	0	8	4
	21	0	5	7	0	8	4	0	0	7	5	0	0	0	6	6	0	0	8	4
Collagen staining	1	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	4	0	0	12	0	0	11	1	0	0	10	2	0	0	1	11	0	0	4	8
	7	0	0	11	1	5	7	0	4	7	1	0	0	0	2	10	0	2	6	4
	10	0	1	9	1	11	1	0	0	9	3	0	0	0	3	9	0	2	8	2
	14	0	3	9	0	11	1	0	0	9	3	0	0	0	5	7	0	4	8	0
	21	0	3	9	0	11	1	0	0	9	3	0	0	0	5	7	0	5	7	0
Degeneration	1	0	1	11	0	11	1	0	0	9	3	0	0	0	3	9	0	0	4	8
	4	0	1	11	0	11	1	0	0	9	3	0	0	0	3	9	0	0	4	8
	7	0	2	10	0	11	1	0	0	9	3	0	0	0	5	7	0	0	6	6
	10	0	2	10	0	11	1	0	0	9	3	0	0	0	7	5	0	0	8	4
	14	0	4	8	0	11	1	0	0	9	3	0	0	0	7	5	0	0	10	2
	21	0	4	8	0	11	1	0	0	9	3	0	0	0	7	5	0	0	10	2
Collagen type I Immunohistochemistry	4	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	7	0	0	12	0	0	11	1	0	0	10	2	0	0	1	11	0	0	4	8
	10	0	1	9	1	11	1	0	0	9	3	0	0	0	3	9	0	2	8	2
	14	0	3	9	0	11	1	0	0	9	3	0	0	0	5	7	0	4	8	0
	21	0	3	9	0	11	1	0	0	9	3	0	0	0	5	7	0	5	7	0
	4	0	1	11	0	11	1	0	0	9	3	0	0	0	3	9	0	0	4	8
7	0	2	10	0	11	1	0	0	9	3	0	0	0	5	7	0	0	6	6	
10	0	2	10	0	11	1	0	0	9	3	0	0	0	7	5	0	0	8	4	
14	0	4	8	0	11	1	0	0	9	3	0	0	0	7	5	0	0	10	2	
21	0	4	8	0	11	1	0	0	9	3	0	0	0	7	5	0	0	10	2	
4	0.62 \pm .05	/	/	/	0.50, 0.3	1.68 \pm .06	0.50, 0.3	1.50 \pm .04	0.33, 0.5	1.50 \pm .04	0.75 \pm .07	0.25 \pm .08	0.12 \pm .06	0.25 \pm .08	0.50 \pm .08	0.50 \pm .08	0.50 \pm .08	0.50 \pm .08	0.50 \pm .08	0.50 \pm .08
7	0.87 \pm .07	/	/	/	2.10 \pm .05	2.10 \pm .05	2.00 \pm .05	2.00 \pm .05	2.00 \pm .05	2.00 \pm .05	0.95 \pm .08	0.25 \pm .09	1.00 \pm .08	0.25 \pm .09	1.00 \pm .08	1.00 \pm .08	1.00 \pm .08	1.00 \pm .08	1.00 \pm .08	
14	1 \pm .05	/	/	/	2.43 \pm .06	2.43 \pm .06	2.25 \pm .06	2.25 \pm .06	2.25 \pm .06	2.25 \pm .06	1.10 \pm .08	0.50 \pm .04	1.25 \pm .04	0.50 \pm .04	1.25 \pm .04	1.25 \pm .04	1.25 \pm .04	1.25 \pm .04	1.25 \pm .04	
21	1 \pm .07	/	/	/	2.62 \pm .04	2.62 \pm .04	2.50 \pm .03	2.50 \pm .03	2.50 \pm .03	2.50 \pm .03	1.10 \pm .09	0.68 \pm .06	1.25 \pm .04	0.68 \pm .06	1.25 \pm .04	1.25 \pm .04	1.25 \pm .04	1.25 \pm .04	1.25 \pm .04	
4	/	/	/	/	0.5 \pm .06	0.5 \pm .06	0.33 \pm .06	0.33 \pm .06	0.33 \pm .06	0.33 \pm .06	/	/	0.12 \pm .08	/	0.12 \pm .08	0.12 \pm .08	0.12 \pm .08	0.12 \pm .08	0.12 \pm .08	
7	0.65 \pm .06	/	/	/	2.43 \pm .02	2.43 \pm .02	2.25 \pm .06	2.25 \pm .06	2.25 \pm .06	2.25 \pm .06	0.66 \pm .04	0.50 \pm .06	1.50 \pm .06	0.50 \pm .06	1.50 \pm .06	1.50 \pm .06	1.50 \pm .06	1.50 \pm .06	1.50 \pm .06	
10	0.80 \pm .04	/	/	/	2.62 \pm .02	2.62 \pm .02	2.50 \pm .04	2.50 \pm .04	2.50 \pm .04	2.50 \pm .04	0.90 \pm .05	0.25 \pm .08	1.00 \pm .06	0.25 \pm .08	1.00 \pm .06	1.00 \pm .06	1.00 \pm .06	1.00 \pm .06	1.00 \pm .06	
14	1 \pm .05	/	/	/	1.75 \pm .04	1.75 \pm .04	2.00 \pm .03	2.00 \pm .03	2.00 \pm .03	2.00 \pm .03	1.00 \pm .06	0.50 \pm .08	1.25 \pm .04	0.50 \pm .08	1.25 \pm .04	1.25 \pm .04	1.25 \pm .04	1.25 \pm .04	1.25 \pm .04	
21	1 \pm .04	/	/	/	0.65 \pm .08	0.65 \pm .08	1.00 \pm .08	1.00 \pm .08	1.00 \pm .08	1.00 \pm .08	0.68 \pm .08	0.68 \pm .08	1.25 \pm .04	0.68 \pm .08	1.25 \pm .04	1.25 \pm .04	1.25 \pm .04	1.25 \pm .04	1.25 \pm .04	

p < vs. control, *p* < vs. 6 α -methylprednisolone, *p* < vs. control and vs. 6 α -methylprednisolone. Fischer test *p* < 0.05, Kruskal-Wallis test, *p* < 0.01; /—no detected.

cytokines from the injury site before reparation of the extracellular matrix takes place.^{30,31} One of the most specific issues of Achilles tendon-to-bone healing is hyponeural and hypocellular environment that makes reparative process extremely difficult. Moreover, blood vessels are even absent from the fibrocartilage zones.^{4,17,18} The anatomy of the insertion is very complex and difficult to reproduce once disrupted. Using this model with detached tendon and no anchor or bone tunnel fixation we can evaluate the sole effect of the pharmacological agent on healing. The effect of BPC 157 showed significantly stronger tendon-to-bone insertion over the control used without bone tunnel fixation or anchors. Accordingly, pentadecapeptide BPC 157 significantly reduces levels of inflammatory markers such as myeloperoxidase (MPO), leukotriene-B4 (LTB4), and tromboxane-B2 (TxB2).¹⁵ Ruptured tendons present reduced quantities of type I collagen as well as significant proportion of type III collagen, essential for decreased biomechanical strength and uneven distribution of fibers in extra cellular matrix.³²⁻³⁴ With BPC 157, type III collagen is promptly cleared and substituted with collagen type I fibers (i.e., improved load to failure and elasticity correlate with the advanced collagen type I distribution), a finding already seen in thermally injured animals that received BPC 157 therapy.¹³ Furthermore, corticosteroid complications (here, the weakest biomechanical results, low collagen concentration, and uneven distribution, mainly type III with many areas of degeneration) are apparently counteracted by pentadecapeptide coadministration, as it has been recently noted in burned mice.¹³

Most important for a drug effect, the improved tendon-to-bone healing is the critical, well-balanced event. This suggests that the pentadecapeptide BPC 157 effectiveness in the improvement of the healing course after Achilles detachment is obviously a specific and valuable one. Otherwise, the tendon-to-bone recovery should be hampered with the initial tendon healing process as similar to fracture healing,²⁶ cartilage or ossicles formation in other tissues, that is, in Achilles tendon tissue with conventional osteogenic peptides [i.e., bone morphogenetic proteins (BMPs), osteogenic protein 1 (OP-1), cartilage-derived morphogenetic proteins 1, 2, 3 (CDMP-1,2,3)] or given intramuscularly, cartilage-, and bone-like tissue formation (i.e., recombinant adenovirus mediated CDMP-3 gene transfer into tendon).^{8,35,36} Furthermore, the pentadecapeptide BPC 157 efficacy can be a common link between the evidenced improvement of the tendon-to-bone healing and the improved healing

of both transected tendon⁶ and pseudoarthrosis.¹⁶ These fitted its consistent healing of various wounds,^{6,12-14,16,21,22} particular stability (nondegradation in gastric juice¹¹), and no need for carrier, easy applicability with no toxicity noted, corticosteroid-healing impairment antagonization, and potential clinical effectiveness.^{1,6,12-16,20-22,37} Thus, the use of stable gastric pentadecapeptide BPC 157 in soft tissue healing (no cartilage or ossicles formation in other tissues^{6,16}) can have possible advantages over currently suggested agents. It would be interesting to evaluate whether the BPC 157 effectiveness would be helpful even if the tendon, transected or detached, would be reattached either using a bone tunnel fixation or anchors.

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