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Osteogenic Effect of a Gastric Pentadecapeptide, BPC-157, on the Healing of Segmental Bone Defect in Rabbits: A Comparison with Bone Marrow and Autologous Cortical Bone Implantation

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Gastrectomy often results in increased likelihood of osteoporosis, metabolic aberration, and risk of fracture, and there is a need for a gastric peptide with osteogenic activity. A novel stomach pentadecapeptide, BPC-157, improves wound and fracture healing in rats in addition to having an angiogenic effect. Therefore, in the present study, using a segmental osteoperiosteal bone defect (0.8 cm, in the middle of the left radius) that remained incompletely healed in all control rabbits for 6 weeks (assessed in 2 week intervals), pentadecapeptide BPC-157 was further studied (either percutaneously given locally [10 μg/kg body weight] into the bone defect, or applied intramuscularly [intermittently, at postoperative days 7, 9, 14, and 16 at 10 μg/kg body weight] or continuously [once per day, postoperative days 7–21 at 10 μg or 10 ng/kg body weight]). For comparison, rabbits percutaneously received locally autologous bone marrow (2 mL, postoperative day 7). As standard treatment, immediately after its formation, the bone defect was filled with an autologous cortical graft. Saline-treated (2 mL intramuscularly [i.m.] and 2 mL locally into the bone defect), injured animals were used as controls. Pentadecapeptide BPC-157 significantly improved the healing of segmental bone defects. For instance, upon radiographic assessment, the callus surface, microphotodensitometry, quantitative histomorphometry (10 μg/kg body weight i.m. for 14 days), or quantitative histomorphometry (10 ng/kg body weight i.m. for 14 days) the effect of pentadecapeptide BPC-157 was shown to correspond to improvement after local application of bone marrow or autologous cortical graft. Moreover, a comparison of the number of animals with unhealed defects (all controls) or healed defects (complete bony continuity across the defect site) showed that besides pentadecapeptide intramuscular application for 14 days (i.e., local application of bone marrow or autologous cortical graft), also following other pentadecapeptide BPC-157 regimens (local application, or intermittent intramuscular administration), the number of animals with healed defect was increased. Hopefully, in the light of the suggested stomach significance for bone homeostasis, the possible relevance of this pentadecapeptide BPC-157 effect (local or intramuscular effectiveness, lack of unwanted effects) could be a basis for methods of choice in the future management of healing impairment in humans, and requires further investigation. (Bone 24:195–202; 1999)

Key Words: Gastric pentadecapeptide BPC-157; Intramuscular application; Local application; Osteogenic effect; Bone marrow; Autologous cortical bone; Implantation; Segmental bone defects; Rabbits; Stomach.

Introduction

Between the commonly used methods for fracture healing impairment, particularly defect pseudoarthrosis, such as stable osteosynthesis with intrafragmentary compression,[33] nonvascularized[12] and vascularized bone grafts[56] and the method of Ilizarov[57] relying on distraction osteogenesis, autologous bone grafting may be the simplest and best way of treating pseudoarthrosis and minor bone defects. However, its failure has been observed in 30% to 50% of patients.[4,14] Postoperatively, patients with iliac bone grafting would often have more pain from the donor site than from the primary operation. Although this pain usually resolves over a period of several weeks, it could also persist (e.g., in 10%,[33] 25%,[54] or even 61%[31] of patients) along with the other complications (i.e., fracture of the wing of the ilium, herniation of abdominal contents and meralgia paraesthetica).[21,39,67]

Thus, new therapeutical attempts are justified. Interestingly, gastrectomy would produce increased osteoporosis, metabolic aberration and risk of fractures.[29,30,70] Because gastrectomy-induced bone loss was not corrected by calcium addition,[30] and not considered being related to vitamin D deficiency,[29] the possible involvement of a hypothetical gastric hormone was suggested.[30] Consequently, the possibility that a peptide originated from stomach mucosa would promote fracture healing is not entirely unexpected since gastric epithelial cells are known to have a property of inducing osteogenesis if appropriately transplantated.[44]

In this, pentadecapeptide BPC-157 may also be interesting. Unlike other peptides beneficial capacity, regularly limited with

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a short half time,[58] it was recently confirmed that BPC-157 is very stable.[3,20,28,38,41,43,47–55,63–66] Along with its gastric juice origin,[28,43,47–55] incubated in human gastric juice or in water, this pentadecapeptide was not subjected to any degradation during 24 h unlike standard peptides (e.g., hEGF and hTGF) stable in water, but rapidly degraded in human gastric juice (e.g., already after 15 min).[64] Considering the existence of BPC-157 in gastric juice, and its unusual stability,[3,20,28,38,41,43,49–53,63–66] its salutary effect on different lesions gastrointestinal lesions, induced by various challengers,[28,43,49–53] it may be likely that this peptide has some biological actions, particularly in cytoprotection reactions modulation. It is suggested that BPC, formed constitutively in the gastric mucosa (body), and being in gastric juice would always protect the stomach against injury.[28,43,49–54] Along with these, the evidence was collected that this pentadecapeptide has a strong anti-inflammatory activity in both acute and chronic inflammation models[46,49] and reducing the release of inflammatory mediators (i.e., myeloperoxidase, leukotriene B_4, tromboxane B_2) in vitro and in vivo,[64–66] would markedly attenuate various experimental lesions in other organs (i.e., liver,[55] pancreas[52] and heart[3,49]). Besides, this pentadecapeptide exerts a complex interaction with adrenergic and dopaminergic system in protection.[27] It would be probably released from somatosensory neurons as shown in experiments with neurotoxin capsaicin in nociception assay[46] and nasal and gastrointestinal[51] lesion studies. Recently, a particular interaction with the NO system was shown as well.[53] With the effectiveness clearly noted in both prophylactic and therapeutic regimens, when given in the conditions of the already established injury stability,[3,20,28,38,41,43,49–54,63–66] importantly, this pentadecapeptide would accelerate the healing of the wound, and provides a marked angiogenic effect.[43,47] In addition, it would significantly increase the healing of the fracture in rats,[46] induced by Penttinen's method.[38]

Thus, it was a logical approach further to test this pentadecapeptide on a segmental bone defect in rabbits. Because it is well known that percutaneous injection of bone marrow into the bone defect or nonunion sites eases bone healing,[7,20,36] this was used for further comparison. The application of an autologous cortical graft was used as a reference procedure.

Materials and Methods

Drugs

Pentadecapeptide BPC-157 (Gly-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val; MW 1419) is a partial sequence of human gastric juice protein BPC, freely soluble in water at pH 7.0 and in saline, prepared as described elsewhere.[28,43,47–54] Peptide with 99% (HPLC) purity (1-des-Gly peptide as impurity), dissolved in saline, was used in all of the experiments.[7,43,47–54]

Animals

New Zealand white, adult male rabbits, 2340 ± 150 g body weight (b.w.), two per cage, were used. All were uniformly fed: each got 300 g feed for rabbit raising (Poljoprerada, Zagreb, Croatia) every second day in alteration with 300 g oats and unrestricted water. The experimental work was approved by the local committee for animal care.

Creation of Nonunion Model

The bone defect representing the experimental nonunion model[23] was created on left forelegs in rabbits under general anesthesia (Ketalar 70 mg/kg b.w. i.m., Caneire, Inverin, Ireland) and sterile conditions. Following the skin incision and separation of the mid third of the radial diaphysis from the adjacent structures, 8 mm (double diameter of the diaphysis of the radius) long osseous-periosteal cylinder of the radial diaphysis was dissected. To obtain the corresponding bone defect in all the rabbits, a surgical saw with two parallel 8 mm removed blades was used for the dissection. After having washed it out with saline, the wound was closed by layers using Dexon 4-0 and silk 4-0 sutures.

To assure the further procedure, immediately after the operation, the injured foreleg underwent control X-ray and the animals, having the entire bone defect surface within means ± SD of the 26.25 ± 0.62 mm^2, were included in further experiments (see subsequent text). The immobilization was done with a cast splint during two weeks to prevent fracture of the ulna. A long-acting antibiotic preparation, Benzapen 1 mL i.m. (150,000 units benzatine-benzine penicillin, 150,000 U procaine-benzyl penicillin, Pliva, Zagreb, Croatia) was given postoperatively.

Treatment Regimens

After initial screening (see earlier) animals were randomly divided into seven groups of 12 rabbits each (total number 84 rabbits). All animals were killed after a 6 week period following injury induction.

Saline-treated (2 mL i.m. and 2 ml locally into the bone defect, given at the time of medication), injured animals were used as controls (group 1).

Bone Marrow Application (Group 2)

In the second group (BM), 4 mL bone marrow was aspirated percutaneously from the posterior iliac spine under general anesthesia (Ketalar 70 mg/kg b.w. i.m.) and sterile conditions on postoperative day 7. Then, 2 mL bone marrow was percutaneously injected into each bone defect. There were using 1.2 mm needles for both bone marrow aspiration and injection.

Pentadecapeptide BPC 157 Administration (Groups 3–6)

On postoperative day 7 and 14, animals of the third group (BPC loc.) were injected BPC-157 10 μg/kg b.w. percutaneously into each bone defect. Alternatively, the rabbits were intramuscularly treated with BPC-157 10 μg/kg b.w. on postoperative days 7, 9, 14, and 16 (group 4, BPC i.m.), or BPC-157 (10 μg or 10 ng/kg b.w.) was intramuscularly administered once daily during 14 subsequent days (postoperative days 7–21) (group 5 (BPC14d-μg) and group 6 (BPC14d-ng)).

Autologous Bone Graft (Group 7)

Immediately after its creation, the bone defect was filled with an autologous bone graft obtained in radius dissection using 2–3 mm cortical bone fragments.

Radiographic Assessment

The first X-ray of the treated extremities was made directly after the operation and, then, aiming at following up of callus formation, every 2 weeks (after 2, 4, and 6 weeks).

To minimize the possible technical mistakes accordingly with Tiedeman and coworkers (1991),[60] identical roentgenographic parameters (55 kV, 100 mA, and 0.025 sec exposure time) were used, and roentgenograms were taken in an anteroposterior direction. X-rays were evaluated using two quantitative methods:

area of radiologically observable callus and microphotodensitometry of the bone defect region.

Measurements of the area of the callus and microphotodensitometry were carried out in personal computer (Pentium, 100 MHz, 32 MB RAM and 2 GB hard disk) using a special program SFORM (VAMS Software Co., Zagreb, Croatia). All roentgenograms were scanned with a professional scanner (Lynotype, USA) using resolution of 300 DPI and 256 gray scale.

For superficial measurement of radiologically observable callus, (i) in the initial roentgenograms, done immediately after surgery, the area of the defect was assessed and expressed in mm^2 (means ± SD, being 26.25 ± 0.62 mm^2) (microdensitometry value of the bone defect region immediately after the operation represents the density of soft tissues without bones); (ii) thereafter, in the roentgenograms provided throughout mentioned 2 week intervals, the area of the defect that was occupied by callus was measured (mm^2). All measurements were carried out by three independent observers unaware of the treatment, but no significant difference between their assessments was noted.

Microphotodensitometry of the bone defect region was carried out averaging the values noted in 10 fields of 1.20 × 0.89 mm. The obtained values were standardized according to the values worked out for the fifth field aluminum key (thickness 2.7 mm) present in each X-ray image (calculated as 1.000). Therefore, besides above mentioned, any influence of possibly various X-ray and/or film exposure factors on densitometry results was further minimized.

Besides, to provide an additional insight into the healing of the segmental bone defect, the number of animals with healed or nonhealed defect is compared, where healed defects are defined as those with complete bony continuity across the defect site, as described by Gerhart et al.[18]

Histomorphometry

The animals were sacrificed after six weeks. Nondecalcified bone preparations were fixed, embedded in metacrilate and cut in 2 μm sections on a Leitz microtome 1.518 for histomorphometry. The preparations were dyed with toluidine blue and Goldner stain.

Histomorphometric indicators were measured under 40× microscopic enlargement within the previously determined field. The microscope was connected to a personal computer (Pentium, 100 MHz, 32 MB RAM and 2 GB Hard disk). For analysis of the area of the bone, or connective or cartilaginous tissue, the SFORM program (VAMS) was used. All measurements (expressed in mm^2) were carried out by three independent observers unaware about the treatment, but no significant difference between their assessments was noted.

Statistical Analysis

The obtained results were statistically processed. Arithmetic means ± standard deviation were worked out for each particular group. Variance analysis (ANOVA), i.e. Tukey HSD test[71] was used to analyze the differences observable between the groups. Fisher's exact test was used for evaluation of the number of animals with healed defects.

Results

Radiographic Evaluation

Surface of X-ray-observable callus. The surface of callus formed within the bone defect after two weeks in the groups treated locally with bone marrow or pentadecapeptide BPC 157, or continuously treated with higher pentadecapeptide dose intramuscularly, was twice as large as regards the controls (**Table 1**), but statistical difference was not reached.

Table 1. Areas of radiologically observable callus (mean ± SD, mm^2)

	Areas of radiologically observable callus (mm^2)		
	Callus surface after:		
Groups[a]	2 weeks	4 weeks	6 weeks
1. Control	3.9 ± 3.7	10.4 ± 6.3	12.7 ± 6.2
2. BM	9.3 ± 7.1	23.3 ± 2.7[b]	23.4 ± 3.0[b]
3. BPC loc.	7.8 ± 7.4	17.2 ± 7.5	19.0 ± 8.2
4. BPC i.m.	6.5 ± 5.9	15.6 ± 6.2	18.9 ± 7.0
5. BPC14d-mg	7.9 ± 6.0	20.5 ± 5.6[b]	22.4 ± 4.4[b]
6. BPC14d-ng	6.8 ± 7.9	14.9 ± 8.5	20.2 ± 6.7
7. Bone graft			23.9 ± 2.7[b]

Group 1 (control): saline-treated (2 mL i.m. and 2 mL locally into the bone defect, given at time of medication), injured animals used as controls. Group 2 (BM): 4 mL bone marrow was aspirated percutaneously from the posterior iliac spine under general anesthesia and sterile conditions on postoperative day 7, then 2 mL bone marrow was percutaneously injected into each bone defect. Group 3 (BPC loc.): on postoperative days 7 and 14, 10 μg/kg b.w. of BPC-157 was percutaneously administered into each bone defect. Group 4 (BPC i.m.): 10 μg/kg b.w. i.m. BPC-157 given on postoperative days 7, 9, 14, and 16. Group 5 (BPC14d-μg): 10 μg/kg b.w. i.m. BPC-157 given once daily during 14 subsequent days (postoperative days 7–21). Group 6 (BPC 14d-ng): 10 ng/kg b.w. i.m. BPC-157 given once daily during 14 subsequent days (postoperative days 7–21). Group 7 (bone graft): immediately after creation, the bone defect was filled with an autologous bone graft obtained by radius dissection using 2–3 mm cortical bone fragments.
[a]Twelve rabbits in each experimental group.
[b]Significantly different from group 1 (control), $p < 0.001$.

Supportingly, it is evident that in all treated groups callus formation was the most prominent between weeks 2–4. Consequently, after four and six weeks, the advanced callus formation, significantly larger ($p < 0.001$) than in controls (i.e., after 6 weeks, 48.5% of the bone defect), was noted in animals treated with autologous bone marrow (i.e., 89.0% after 6 weeks) as well as BPC-157-treated rabbits, if this pentadecapeptide was given continuously (7–21 postoperative days) intramuscularly in the dose of the 10 μg/kg once daily (i.e., 85.3% after six weeks). Importantly, in the rabbits treated locally with autologous bone graft, a similar improvement was noted also after 6 weeks (i.e., 91.1% of bone defect surface presented as newly formed bone) (after 2 and 4 weeks the surface of X-ray-observable callus was not measured since it was impossible to distinguish newly formed bone from the bone graft) (Table 1).

Microphotodensitometry. Callus mineralization was followed up using microphotodensitometry of the bone defect region. The increase in bone callus density compared with the initial value (microdensitometry value of the bone defect region immediately after the operation represents the density of soft tissues without bones) is shown in **Table 2**.

Compared with the control values, after two and four weeks, the statistically significant increase ($p < 0.005$) of bone callus density was observed only in the group treated with autologous bone marrow as compared with the controls. After 6 weeks, values of callus mineralization were statistically significantly higher ($p < 0.001$) in the group continuously treated intramuscularly with the pentadecapeptide BPC-157 (10 μg/kg) as well.

Number of animals with healed defect. To give an additional insight into the healing process in the control and treated groups, and to demonstrate the course of individual healing in the

Table 2. Optical density measurements of defects[a]

Groups[b]	Optical density (*D*) measurements of the defects (% of optical values of 2.7-mm-thick fifth field aluminum key)			
	Postoperative optical density of defects			
	Optical density of soft tissues without bone	Optical density of the defects after 2 weeks	Optical density of the defects after 4 weeks	Optical density of the defects after 6 weeks
1. Control	0.690 ± 0.061	0.736 ± 0.079	0.819 ± 0.197	0.812 ± 0.113
2. BM	0.700 ± 0.037	0.920 ± 0.151[c]	1.277 ± 0.254[c]	1.350 ± 0.396[d]
3. BPC loc.	0.720 ± 0.046	0.806 ± 0.095	1.125 ± 0.248	1.085 ± 0.314
4. BPC i.m.	0.726 ± 0.055	0.855 ± 0.149	1.061 ± 0.278	1.069 ± 0.240
5. BPC14d-mg	0.678 ± 0.077	0.825 ± 0.111	1.120 ± 0.188	1.213 ± 0.226[d]
6. BPC14d-ng	0.702 ± 0.027	0.823 ± 0.146	1.032 ± 0.187	1.120 ± 0.132
7. Bone graft	1.105 ± 0.212	1.094 ± 0.138	1.423 ± 0.217	1.403 ± 0.270

For explanation of group characteristics refer to footnote to Table 1.
[a]Mean photodensitometry measurements and standard deviation are expressed as a percentage of the optical values of the 2.7-mm-thick fifth field aluminum key.
[b]Twelve rabbits in each experimental group.
[c]$p < 0.005$, [d]$p < 0.001$ vs. control.

corresponding groups, that is, whether the defect is healed or not, the healing was assessed as simply described by Gerhart and coworkers 1993. To ascertain the differences in the number of animals with the healed defect in the control and in the treated groups, defining the healed defects as those with complete bony continuity across the defect site, we clearly showed that no rabbit in control group had a completely healed defect. Compared with these control values, an apparently increased number of the rabbits with the healed bone was noted in all treated groups: bone marrow or autologous bone graft, or pentadecapeptide BPC-157. Namely, besides the groups treated locally with bone marrow or autologous bone graft, or rabbits that received intramuscularly the pentadecapeptide BPC-157 10 μg/kg or 10 ng/kg b.w. for 14 days, significantly more rabbits showed healed defects also in groups treated with other pentadecapeptide regimens: 10 μg/kg BPC 157 locally, or 10 μg/kg i.m., but intermittently (**Table 3**).

Histological Evaluation

The process of callus formation within the bone defect develops through enchondral ossification. After 6 weeks, in the bone defect region there is newly formed bone (woven and lamellar), cartilage and fibrous tissue in various ratios. In some animals (controls in particular, **Figure 1**) the bone defect region was filled with fibrous tissue, with newly formed bone and cartilaginous islets being found in the margins of the dissected radius and in the narrow zone along the ulna. In some animals (mostly from the bone marrow group, **Figure 2**, BPC 157 10 μg/kg i.m. continuously for two weeks, **Figure 3**, and autologous bone graft group) the bone defect was completely filled with newly formed bone (mostly lamellar) and smaller cartilaginous islets. In the remodeling process, the formation of the new medullar canal started.

Table 3. Healing of the bone defect assessed as the number of rabbits with healed bone defect (12 rabbits per group)[a]

Groups	Number of the rabbits with the healed bone defect (12 rabbits per each group)		
	First 2 weeks	Week 4	Week 6
1. Control	0	0	0
2. BM	0	10[b]	11[b]
3. BPC loc.	0	5[b]	6[b]
4. BPC i.m.	0	4	6[b]
5. BPC14d-mg	0	6[b]	9[b]
6. BPC14d-ng	0	3	6[b]
7. Bone graft	0	6[b]	9[b]

For explanation of group characteristics refer to footnote to Table 1.
[a]Healed defects are defined as those with complete bony continuity across the defect site, as described by Gerhart et al.[18]
[b]$p < 0.05$, at least vs. controls (group 1).

Measurements of newly formed bone, cartilaginous and fibrous tissue surfaces are given in **Table 4**. In the controls, the surface of newly formed bone callus occupies less than a half (48.9%) of the entire bone defect surface. As compared with the controls, the statistically significant difference ($p < 0.001$) was observed in bone callus of bone marrow-treated animals (88.4% of the bone defect surface), in animals treated with BPC-157 given in micrograms during 14 days (82.7%), in animals treated with BPC-157 given in nanograms during 14 days (75.6%) and in animals treated with a cortical autograft (84.3%).

There was no statistically significant difference among the groups in the size of cartilaginous callus. On the other hand, fibrous tissue within the bone defect was most prominent among the controls (49.5% of the entire bone defect surface), significantly higher ($p < 0.001$) than in groups treated with pentadecapeptide BPC 157 intramuscularly for 14 days, either with the 10 μg/kg (13.8%) or 10 ng/kg dose (22.1%), or bone marrow (14.3%), autologous bone graft (20.8%) locally.

Separate vital staining studies (oxytetracycline 50 mg/kg i.m. at postoperative day 14, calcein 30 mg/kg i.m., at postoperative day 28, alizarin 20 mg/kg i.m. at postoperative day 38) generally correlate with the above described findings. In treated groups (pentadecapeptide BPC-157, bone marrow, autologous bone graft) it was a common trend toward the earlier appearance of callus formation than in controls. However, due to possible influence of the fluorochromes themselves (although this possibility was minimized) (for review, see Sun et al.[44]) these results were not included in the present report (**Figure 4**).

Discussion

Clinical methods presently employed in the management of fracture healing impairment (bone grafts, vascularized bone grafts, stable osteosynthesis with interfragmentary compression,

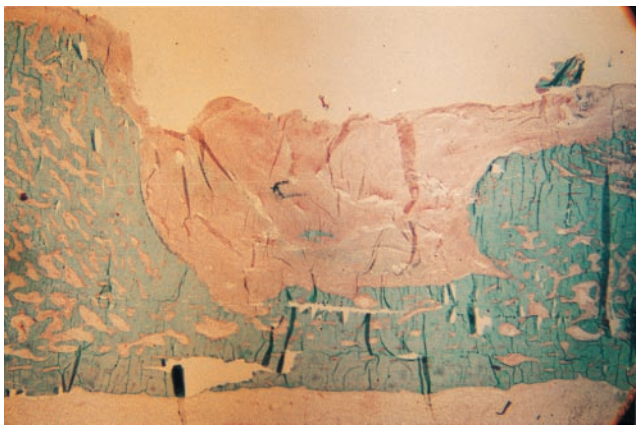

Figure 1. Histological appearance, 6 weeks postoperatively, of a radial defect in a control rabbit. The bone defect region is filled with fibrous tissue, the newly formed bone is in the margins of the dissected radius (Goldner stain; original magnification: ×10).

bone transport by the method of Ilizarov) require a sophisticated surgical technique, besides limitations due to high morbidity and many complications. Phemister's classic technique for autologous bone grafting involves two operative procedures, with potential morbidity at both the donor and the recipient site.[33] Great efforts are therefore made to find out less complicated and more effective methods. Thus, coupled to the gastric epithelial cells property of inducing osteogenesis[44] and suggested stomach significance for bone homeostasis[29,30,69] and hypothetical involvement of a gastric hormone,[30] the possibility that a peptide originated from stomach mucosa already shown to accelerate fracture healing in rats[46] may also promote healing of segmental bone defects, is not entirely unexpected.

In the present study a well known rabbit nonunion model[23] was used. Consistently with pseudoarthrosis development in patients, after six weeks in the majority of controls animals the poor formation of bone calluses was noted (e.g., the bone defect region was filled with fibrous tissue, with newly formed bone and cartilaginous islets being found in the margins of the dissected radius and in the narrow zone along the ulna). Likewise,

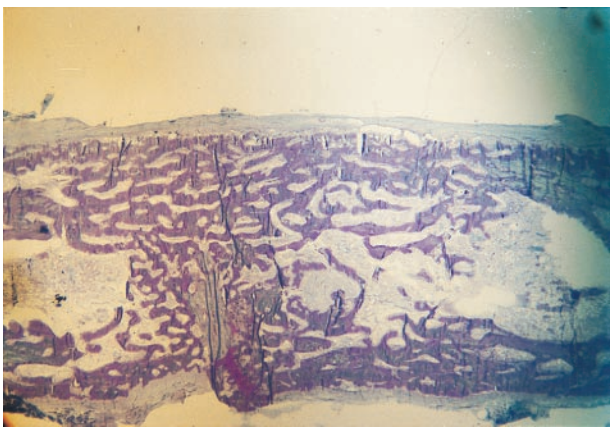

Figure 2. Histological appearance, 6 weeks postoperatively, of a radial defect that had been treated locally with autogenous bone marrow. The bone defect is filled with newly formed bone. There are small areas of fibrous tissue and cartilage. There is remodeling of trabecular bone and early formation of a new medullary cavity (toluidine blue stain; original magnification: ×10).

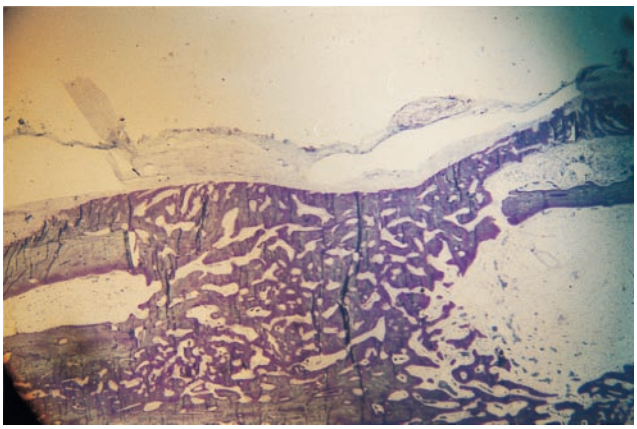

Figure 3. Histological appearance, 6 weeks postoperatively, of a radial defect that had been treated with pentadecapeptide BPC-157 at a dose of 10 μg/kg b.w. i.m. daily during a 14 day period. The bone defect is completely filled with trabecular bone (toluidine blue stain; original magnification: ×10).

the application of autologous bone graft as an osteogenic graft would produce a marked benefit like as a percutaneous application of autologous bone marrow clearly improved otherwise seriously impaired bone healing. These findings are consistent with the previous evidence[7,17] confirmed both experimentally and clinically. Thus, the reported evidence that a novel stomach pentadecapeptide, named BPC-157, would also improve markedly an otherwise delayed bone healing, merits particular attention.

Considering the mechanism, it should be noted that bone marrow is a complex tissue composed of red and white blood cells, their precursors and a connective-tissue network called "stroma."[22] Cells from the stroma morphologically appear as fibroblasts, reticulocytes, adipocytes and endothelial cells regulate the differentiation of haemopoietic cells through direct interaction via cell surface proteins and through the secretion of growth factors.[11,32,40] Some cells of the stroma, determined osteogenic progenitor cells (DOPC),[15] also have the potential to differentiate into bone and cartilage,[2] even without any inducing agent when bone marrow is transplanted to heterotopic sites. Taken together, it seems understandable that a percutaneous

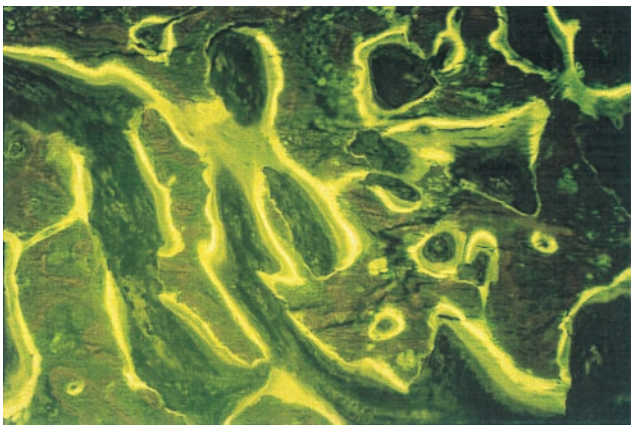

Figure 4. Vital staining studies (oxytetracycline 50 mg/kg i.m. at postoperative day 14, calcein 30 mg/kg i.m. at postoperative day 28, alizarin 20 mg/kg i.m. at postoperative day 38) generally correlate with previously described findings. Pentadecapeptide BPC-157 given at 10 μg/kg b.w. i.m. for 14 days (original magnification: ×260).

Table 4. Histological evaluation[a]

Groups[b]	Areas of bone and cartilaginous and fibrous callus (mm^2)		
	Bone	Cartilage	Fibrous tissue
1. Control	12.0 ± 6.4	0.7 ± 0.7	12.1 ± 6.3
2. BM	21.6 ± 3.5[c]	0.3 ± 0.5	3.5 ± 2.8[c]
3. BPC loc.	17.8 ± 4.5	0.3 ± 0.4	7.0 ± 3.6
4. BPC i.m.	18.2 ± 5.4	1.0 ± 1.3	6.3 ± 4.8
5. BPC14d-mg	20.3 ± 4.3[c]	0.8 ± 0.8	3.4 ± 3.9[c]
6. BPC14d-ng	18.6 ± 6.1[c]	1.0 ± 0.7	5.4 ± 5.4[c]
7. Bone graft	20.6 ± 4.6[c]	0.8 ± 1.7	5.1 ± 4.9[c]

For explanation of group characteristics refer to footnote to Table 1.
[a]Data include Areas of bone, cartilaginous and fibrous callus (means ± SD, mm^2). Surface of bone defect 25.25 ± 0.64 mm^2.
[b]Twelve rabbits per each experimental group.
[c]Significantly different from group 1 (control), $p < 0.001$.

injection of bone marrow into the bone defect or non-union sites eases bone healing.[6,20,36] Whether a similar mechanism could be suggested for pentadecapeptide BPC 157, remains to be seen.

Also applied locally into the bone defect, pentadecapeptide BPC-157, as could be assumed from the increased number of the rabbits with the healed defect, beneficially influences the healing of the bone defect that would otherwise remain unhealed in all control rabbits, at least throughout the tested experimental period. This is along with the increased number of the rabbits with healed defect also in other BPC-157 regimens. Considering local application, since the carrier is generally considered to perform several important functions, including the releasing osteoinductive protein at an effective dose during a period coincident with the accumulation of host target cells, possibly protecting osteoinductive protein from nonspecific proteolysis, and accommodating each step of the cellular response during bone formation,[8] the evidence that this pentadecapeptide was applied without special carrier, dissolved in saline, is probably important. It could suggest that this pentadecapeptide by itself is suitably stable (since non degraded in human gastric juice even for 24 h) providing its suitable presence at the defect site and the potential upregulation of the growth factor, as well as other local factors, triggering a sufficient stimulation of bone formation to close gaps. On the other hand, for systemic pentadecapeptide BPC-157 application, considering that besides the number with the healed defect, the other parameters (i.e., in radiographic assessment the callus surface, microphotodensitometry, quantitative histomorphometry) also revealed statistical difference, the effectiveness when it applied intramuscularly is probably more important. Namely, this salutary effect is increased with duration of the treatment (i.e., the continuous application for two weeks was noted to be clearly more efficacious than an intermittent medication (that would already increase the number of animals with the healed defect). In line with this may be the evidence that in the later intervals, if applied continuously, besides 10 μg/kg dose, even much lower amounts such as 10 ng/kg became effective also when assessed histomorphometrically. However, the precise mechanisms remain to be further explained. Considering that bone repair is a vascular and cellular process, like the healing of wound, and that this pentadecapeptide would beneficially increase the wound healing[43,49] as well, the process(es) common for wound and bone healing, could probably be beneficially influenced by its salutary activities. Recently, it was shown that this pentadecapeptide could protect endothelium,[51,55] modulates the release of nitric oxide (NO)[18,57] and it has angiogenic properties[43,53] and would promote new vessels formation, an activity regularly impaired markedly in nonunion healing.[44] Finally, since the cells of bone, especially those of the periosteum, endosteum and medullar cavity, are the source of healing tissue[44] they could be directly or indirectly targeted by this pentadecapeptide. The mentioned evidence that gastric epithelial cells are known to have a property of inducing osteogenesis,[44] along with possible stomach significance for bone homeostastis,[30,31] together with the evidence that this pentadecapeptide seems to be constitutively present in gastric mucosa being secreted into gastric juice[3,20,28,37,41,43,47–55,63–66] may provide an additional indication.

Finally, the noted evidence that this pentadecapeptide would markedly improve a nonunion healing, even when it was given one week after injury (and not immediately following surgery as standard autologous bone graft[12]) and applied intramuscularly, unlike other peptides (i.e., bone morphogenetic protein (BMP), mostly active only locally[9]), may be expected. As mentioned, this could be clearly explained based on its systemic activity[46] shown in gastrointestinal tract,[3,41,43,46–54,63–70] pancreas,[51] liver,[55] heart[3] and somatosensory neurons lesions,[51] where this pentadecapeptide was efficacious applied either intraperitoneally or intragastrically. Worth mentioning, besides a prophylactic activity, a therapeutic potential seen in the present study, could be also pointed out, since it was clearly effective when applied in the conditions of the already established severe organs damage (i.e., gastrointestinal lesions, acute pancreatitis, somatosensory neurons depletion).[46,49–51] In line with this beneficial activity is its mentioned unusual stability[3,20,28,38,41,43,48–54,63–66] since incubated in human gastric juice or in water, this pentadecapeptide was not subjected to any degradation at least for 24 h, unlike other peptides (e.g., hEGF and hTGF, were stable in water, but rapidly, i.e., after only 15 minutes, degraded in human gastric juice).[63] Along with this may be the finding that this pentadecapeptide, as an additional advantage, could be stable and easily applied in saline[3,20,28,38,41,43,47–55,63–66] without collagen medium (for review see Einhorn[13]).

In summary, intramuscular administration of pentadecapeptide BPC-157 produced results comparable with percutaneous injection of autologous bone marrow, or autologous bone grafting (whereas its effectiveness could be seen also after local administration). Naturally, in the light of the suggested stomach significance for bone homeostasis,[29,30,70] the full relevance of this pentadecapeptide BPC-157-positive effect remains to be further established. Its application is quite simple and surely accompanied with fewer complications, for instance, no evidence for extracortical new bone formation, bony hypertrophy or ectopic bone formation was noted (avoiding the risk of reluctant osteogenesis outside the desired area), unlike some studies with BMP.[8,32,42] Finally, this pentadecapeptide in toxicological studies is apparently nontoxic, even applied in very high doses,[46] and it could be a promising basis for further management of healing impairment in patients.

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