

## BPC 157's effect on healing

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**Summary** — The 15 amino acid agent BPC 157, showing a wide range of organoprotective action in different experimental models, was used in our experiments in order to establish its influence on different elements connected with the healing process. Elements thought to be of greatest importance in the process of healing are formation of granulation tissue, angiogenesis and production of collagen. In our work we tested the influence of BPC 157 on: granulation tissue and collagen formation, on angiogenesis as well as on tensile strength development, using three experimental rat models: 1) skin incisional wounds; 2) colon-colon anastomoses; and 3) angiogenesis model with synthetic sponge implantation. The specimens were histologically assessed for collagen, reticulin and blood vessels using scoring and morphometry. In all experiments significant differences between BPC 157-treated animals and controls were found, showing a strong, promoting involvement of BPC in the healing process. It is worth noting that these effects were achieved by different routes of application, including intragastric and local, making BPC 157 a potentially useful therapeutic agent.

**BPC / healing / skin wounds / colon-colon anastomoses / synthetic sponge / reticulin / collagen / histology / morphometry**

### Introduction

One of the processes essential for maintaining integrity of higher organisms is the process of reparation or healing. Beginning at the moment of tissue damage it represents a dynamic equilibrium between negative and positive events (necrosis and pus formation *versus* activation of macrophages and fibroblasts). Result of the healing process is formation of a, more or less abundant, scar enduring a certain load.

Several peptides are considered to play an important role in the inflammatory response and wound healing. Growth factors such as TGF- $\beta$ , PDGF, EGF, FGF as well as IL-1 and TNF (Rothe, 1989) have been investigated in a wide spectrum of *in vivo* and *in vitro* experiments in order to establish their effect on different elements included in the healing process. TGF- $\beta$ , PDGF, EGF and FGF have been shown to accelerate wound healing at least in some animal models (Mustoe *et al*, 1987; Brown *et al*, 1988; McGee *et al*, 1988).

The 15 amino acid agent BPC 157 (synthetic fragment of the gastric juice peptide BPC), showing a wide range of organoprotective action in different experimental models, was used in our experiments in order to establish its influence on various elements connected with the healing process. Previous experiments strongly suggested its involvement in tissue repair, *ie* experimental gastric ulcer healing (Sikiric *et al*, 1993, 1994).

Normal healing presumably includes three stages: i) inflammatory phase including vascular and cel-

lular elements; ii) fibroblast proliferation and collagen synthesis with early scar formation; and iii) remodelling of the scar. Different tissue elements are expressed in these processes. Elements thought to be of greatest importance in the healing process are production of collagen supported by formation of granulation tissue and angiogenesis (Eckersley and Dudley, 1988; Szabo *et al*, 1991). In accessing the dynamics of scar formation, quantitative analysis of collagen development as well as granulation tissue formation and angiogenesis, using *in vivo* models, seems most appropriate.

In our work we tested the influence of BPC 157 on granulation tissue and collagen formation, using three different experimental models: incisional skin wounds, colon-colon anastomoses, and synthetic sponge implants.

### Materials and methods

#### *Animals*

In all experiments albino rats of either sex, Wistar strain, 180–220 g bw, six per group were used.

#### *Chemicals*

BPC 157 was prepared as reported previously (Sikiric *et al* 1993) and applied immediately after wound induction (wounds, 10  $\mu$ g/kg bw ip; anastomoses, 10  $\mu$ g, 10 ng/kg bw ip, ig locally; angiogenesis, 50  $\mu$ g, 10  $\mu$ g, 10 ng/mL in the sponge). Controls received an equal volume of saline.

## Procedure

### Skin incisional wounds

Three cm skin wounds were placed in the middle line on back of the animals, one wound per animal. After killing (day 2, 4, 5, 7, 14) the wounds were excised, fixed in 10% neutral formaline, processed and embedded in paraffin (Bradbury *et al.*, 1990).

### Colon-colon anastomoses

Termino-terminal colon-colon anastomoses were performed after excision of 5 mm of bowel. The animals were killed after 2, 5, 7 and 10 days. After resection of anastomosis area with 2 cm surrounding colon (1 cm from each side) tissue was prepared for histology as described.

### Sponge implantation (angiogenesis)

Synthetic sponges ( $V = 0.25$  mL) were soaked with BPC or saline and placed subcutaneously as described (Szabo *et al.*, 1991). After killing (day 3 and 7) the sponges and the surrounding tissue were excised and processed for histology.

### Histological assessment

After sectioning the slides were, in addition to routine HE, stained for collagen (van Gieson stain), reticulin (silver impregnation after Gomory) and blood vessels (immunohistochemistry). Immunohistochemistry for smooth muscle actin was performed using ABC technique and primary anti-actin monoclonal antibody (DAKO, Glostrup) according to manufacturer's guidelines. Expression of each component was analysed morphometrically and expressed as percentage of investigated area or number in area (blood vessels). In the sponge implantation model the thickness of granulation tissue was also determined (table I). For morphometrical analysis we used PC-based software: Sform by Vams, Zagreb.

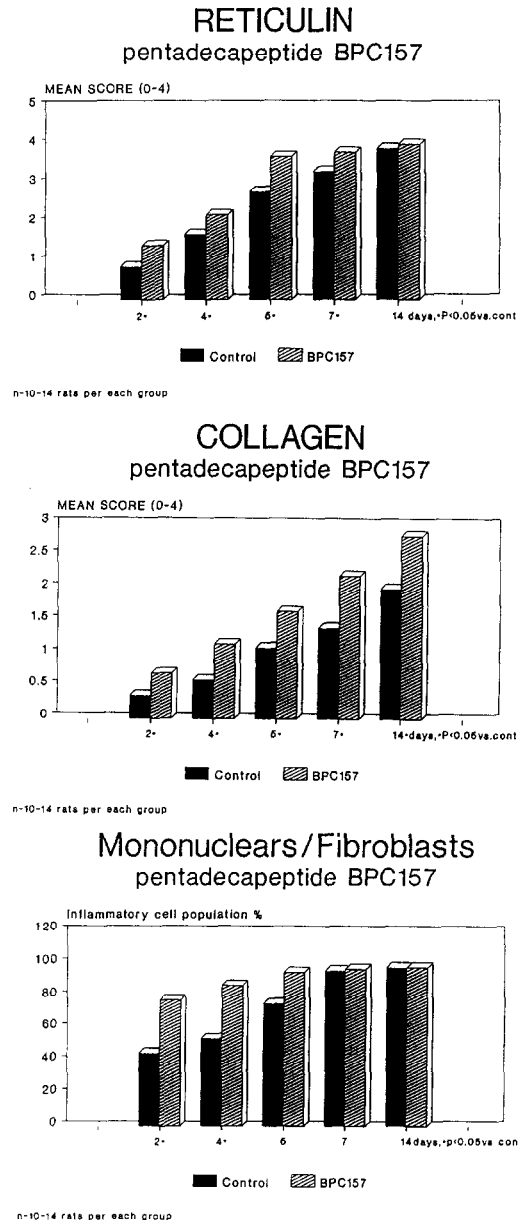
### Statistical analysis

Scores (in incisional wounds) were analysed by Kruskal-Wallis and Wilcoxon rank sum test. Granulation tissue, angiogenesis and inflammatory response were analysed by ANOVA and Scheffes test after establishing normal distribution by Kolmogorow-Smirnoff test. Percentage of collagen and reticulin was analysed by ANOVA and Scheffes test after arc-sin transformation.  $P$  values of  $\leq 0.05$  were considered statistically significant (Daniel, 1995a, b).

## Results

### Skin incisional wounds

In this model time-dependent evolution of reticulin and collagen was observed in all groups (fig 1). Reticulin and collagen formation in BPC 157-treated animals seem to be accelerated compared with controls. Treated animals showed almost fully developed reticulin already after 5–7 days. Control showed a comparable healing after 14 days. Col-



**Fig 1.** Healing elements: reticulin, collagen and mononuclear inflammatory cells with fibroblasts. Incisional skin wounds.

lagen developed slower in both groups, although the BPC-treated animals expressed a faster dynamic. Animals treated with BPC 157 were characterised by a prominent mononuclear inflammatory infiltrate

**Table I.** Histologically assessed parameters.

Model	Assessed parameter				
	Reticulin	Collagen	Inflammation	Granular tissue	Angiogenesis
Colon-colon	+	+	+		
Skin wounds	+	+	+		
Sponge				+	+

already after day 2 compared to 5–7 days in saline-treated controls.

#### Colon-colon anastomoses

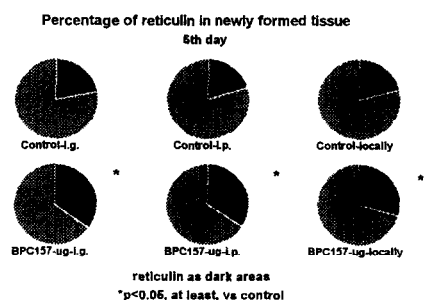
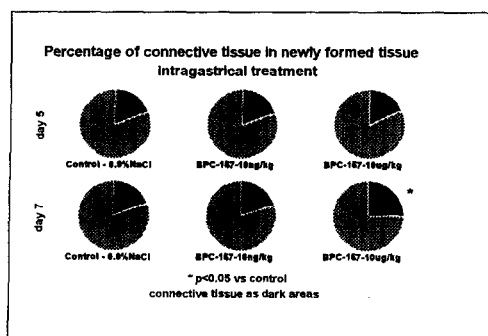
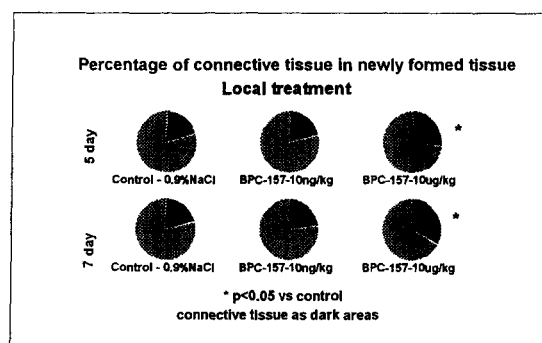
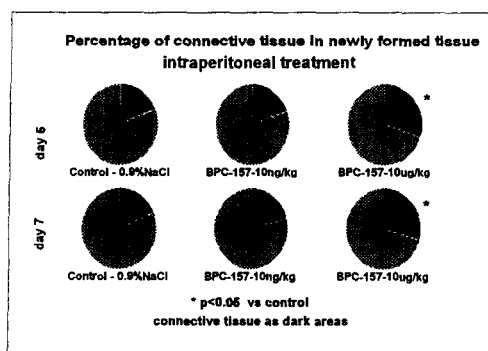
Differences between control animals and BPC 157-treated animals were also demonstrated in this set of experiments (fig 2A, B, C). The inflammatory cell population did not show notable difference until day 7 when a significant decrease was observed in treated animals. The dynamics of reticulin and collagen formation was also accelerated by BPC 157. Collagen formation showed slight differences according to route of application. Local and peritoneal application yielded a collagen increase in both microgram and nanogram dosages, while intragastrical application was effective only in micrograms.

Reticulin formation showed a difference between controls and BPC-treated animals only at day 5 and in microgram dosage, but regardless of route of application.

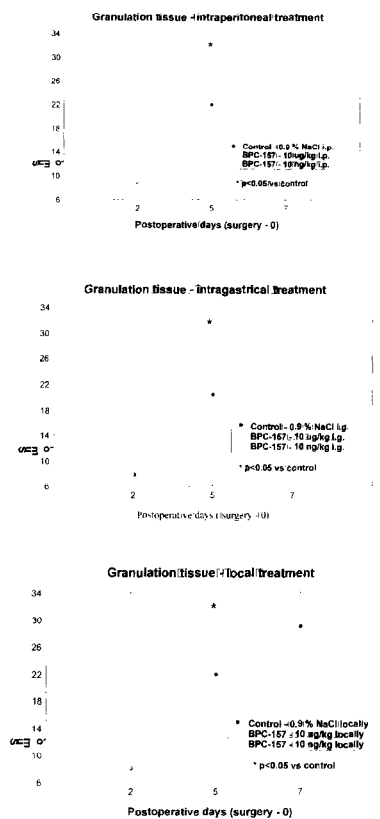
#### Sponge implantation

Newly formed vascular spaces were counted in this experiment. BPC 157 showed a dose- and time-related effect in increasing the number of new blood vessels (fig 3). This effect was more prominent at day 3 post implantation where animals treated with 10 micrograms of BPC 157 reached the level of control animals at day 7. Development of granulation tissue was also dose- and time-dependently increased by BPC treatment. At day 3, animals implanted with sponges soaked with 50  $\mu\text{g}/\text{mL}$  of

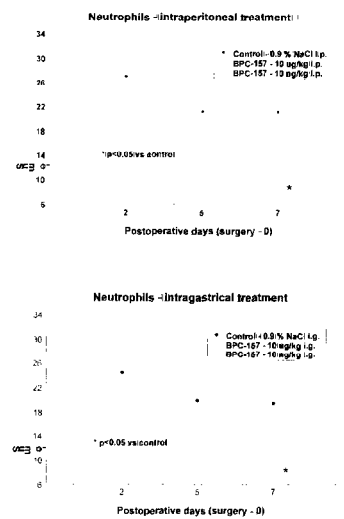
## 2A



2B



2C



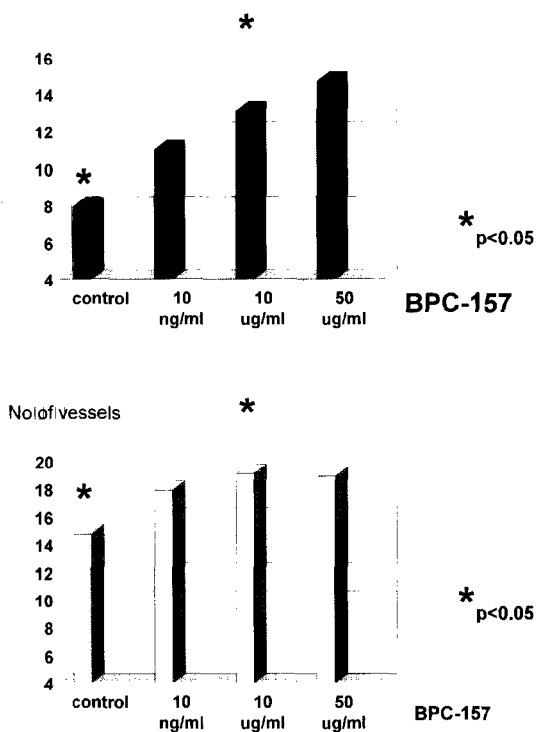
**Fig 2. A.** Percentage of connective tissue and reticulin in newly formed (granulation) tissue. Colon-colon anastomoses, days 5 and 7. **B.** Granulation tissue formation. Colon-colon anastomoses. **C.** Acute inflammatory reaction: neutrophils. Colon-colon anastomoses.

BPC 157 showed significantly better developed granulation tissue. This concentration yielded also a significant increase of granulation tissue formation after day 7.

## Discussion

In order to establish the potential effect of BPC 157 on wound healing and its elements we produced a series of experiments on three experimental models: incisional skin wounds, colon-colon anastomoses and synthetic sponge implantation model. Reticulin and collagen formation, granulation tissue formation and angiogenesis were evaluated as well as the type of inflammatory response. The rationale of skin and colon wounds is in different healing patterns and dynamics of these organs related to their collagen structures (Eckersley and Dudley, 1988; Eyre *et al*, 1988; Hendriks and Mastboom, 1990). Our results demonstrated a very clear, time- and dose-dependent positive effect of BPC 157 on parameters thought to be of great importance in the process of

wound healing, namely reticulin and collagen formation as well as granulation tissue development and angiogenesis (Lehto *et al*, 1985). Granulation tissue, consisting of fibroblasts producing collagen, collagen itself and newly formed blood vessels represent the basic structure in tissue repair. Its continuous maturation by becoming more dense with more collagen and fewer vessels ends up in an collagenous, avascular scar. Formation of new blood vessels in the early healing period is important for adequate support in oxygen and nutrients to the scene of tissue damage, while later production of reticulin and mature collagen gives the wound its final shape and strength. Reticulin, demonstrated by silver nitrate impregnation is mainly composed of collagen type III, representing newly formed connective tissue, while van Gieson trichrome demonstrates mature collagen (Bradbury *et al*, 1990). In our investigation we demonstrated that BPC 157-treated animals show, dose-dependent, earlier and more abundant reticulin and collagen formation as compared to controls. In skin wounds reticulin was demonstrable already after 2 days, more abundant



**Fig 3.** Number of newly formed vascular spaces after 3 and 7 days. Synthetic sponge implantation model.

as compared to controls after 5 days. After 7 days the amount of reticulin in all groups was approximately the same. Of great interest for further elucidation of the mechanism of BPC action seem to be the results of the sponge implantation assay. From different experiments it seems that many peptides showing a positive influence in wound healing express also angiogenetic properties (Plate *et al*, 1994). This is also true for other substances involved in tissue repair, such as Sucralfat (Szabo *et al*, 1991). Therefore it seems that an adequately vascularised granulation tissue is a prerequisite for optimal wound healing. The sponge implantation experiment showed that BPC 157-treated animals developed significantly more new vessels in granulation tissue as compared to controls. Thus a potential mechanism through which BPC positively influences the healing process is the stimulation of angiogenesis in areas of tissue damage. From previous experiments it was demonstrated that BPC 157 exerts beneficial effects in gastric ulcer prevention and healing (Sikiric *et al*, 1993a, c, 1994). We also demonstrated its capability of endothelial cell protection in ethanol gastric lesion. Therefore we

can expect that protection of endothelial cells during damaging events plays an important role in angiogenesis. So BPC seems to be a potent factor of vascular protection and a stimulator of angiogenesis in wound healing. Blood vessels are not only important in oxygenation and nutrition. By which mechanism the 'protected' vessels stimulate BPC-mediated healing remains to be seen. The obvious reduction of granulocytes and stimulation of mononuclear inflammatory cell infiltration in our experiments is in full accordance with previous observations (Sikiric *et al*, 1994). In this respect it is of interest that BPC 157 significantly reduces acute inflammatory mediators (such as LTB<sub>4</sub> and TxB<sub>2</sub>) in serum as well as tissue and decreases MPO level in inflamed tissue as recently demonstrated (Veljaca *et al*, 1994a, b, 1995).

From our experiments we can conclude that BPC 157 significantly accelerates reticulin and collagen formation as well as angiogenesis together with stimulation of macrophages and fibroblasts infiltration representing thus a potential tool in wound healing management.

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