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ANTIINFLAMMATORY EFFECT OF BPC 157 ON EXPERIMENTAL PERIODONTITIS IN RATS

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The pentadecapeptide BPC 157 has been shown to have anti-inflammatory and wound healing effects on multiple target tissues and organs. The purpose of the present study was to investigate the effect of BPC 157 on inflammation and bone resorption in experimental periodontitis in rats. First the acute effect of BPC was tested on gingival blood flow by laser doppler flowmetry. Then periodontitis was produced by a silk ligature placed around the lower left first molar. Rats were treated with BPC 157 (once daily for 12 days) or vehicle. At day 13, the gingivomucosal tissues encircling the molars were removed on both sides. Inflammation was assessed by Evans blue plasma extravasation technique and by histology. Alveolar bone loss was analyzed by microCT. BPC 157 had no effect on gingivomucosal blood flow. Twelve day ligature caused a significantly increased Evans blue extravasation in the gingivomucosal tissue, histological signs of inflammation, and alveolar bone destruction. BPC 157 treatment significantly reduced both plasma extravasation, histological alterations and alveolar bone resorption. In conclusion, systemic application of BPC 157 does not alter blood circulation in healthy gingiva. Chronic application of the peptide has potent antiinflammatory effects on periodontal tissues in ligature induced periodontitis in rats. Taken together, this proof of concept study suggests that BPC 157 may represent a new peptide candidate in the treatment of periodontal disease.

Key words: *pentadecapeptide BPC 157, periodontitis, rat, inflammation, blood flow, gingiva, laser doppler flowmetry, Evans-blue extravasation, bone resorption, micro computed tomography*

INTRODUCTION

The gastrointestinal epithelium represents an important interface between the host and the external environment, serving both as a surface for absorption as a defence against ingested pathogens. In the oral cavity, a unique feature to be handled by the host defence is that continuously replaced bacteria may obtain a firm anchorage on the nonshedding tooth surface and will thereby remain in close contact with the soft tissues surrounding the tooth for a long time and evoke inflammation (1, 2). This chronic inflammatory disease of the soft and hard supporting tissues of the teeth is periodontitis, which is one of the most frequent human diseases (3, 4). While periodontitis supports the protection against local microbial attack, this inflammatory reaction can also damage the surrounding cells and connective tissue structures, including alveolar bone causing tooth loss (5, 6).

It has been well established that inflammatory diseases of the periodontium are most frequently of bacterial origin. The toxins, enzymes and metabolites of bacteria (predominantly Gram-negative anaerobic) present in the dental plaque play a key role in the initiation of the inflammatory process, but the exact pathomechanism is far from being understood in detail (5-8).

In the present study, a well established rat model of periodontitis was utilized, which involves a ligature around the cervix of the mandibular first molar tooth (9, 10). A similar model has previously been used in several species (11-14). In this model, ligation acts as (i) a mechanical trauma on the dentogingival area, thereby reducing tissue integrity and allowing for intense host-plaque interaction and (ii) a plaque-formation-promoting factor, thus increasing the number of bacteria. Initiation of periodontal disease by bacteria is well-documented, and the end result, destruction of the alveolar bone and other connective tissues is readily observed. However, the molecular events that promote these alterations are incompletely understood (6).

BPC 157 is a pentadecapeptide first described in 1991 (15). This peptide is also called BPC 15, PL-10, PLD-116 (16) or PL14736 (17). The first studies with BPC 157 focused on its prominent beneficial effects on gastric and intestinal injuries induced by diverse ulcerogens (18). Later its beneficial effects on other organs such as the liver (16), pancreas (19), and heart (20) became also evident. BPC 157 was claimed to be 'cytoprotective' (21, 22) particularly in the gastrointestinal mucosa, and supporting epithelial integrity (23, 24).

BPC 157 has a strong anti-inflammatory activity in both acute and chronic inflammation models (25). In fact, preliminary results in clinical trials suggest that BPC 157 may become an important therapeutic tool for the treatment of inflammatory bowel disease (26). BPC 157 was shown to accelerate wound healing and to have a marked angiogenic effect (27). In addition, it significantly facilitates the healing of bone fracture in rats (15). This peptide also exhibits an osteogenic effect significantly improving the healing of segmental bone defect (28). BPC 157 accelerates the healing of transected rat Achilles tendon (29), and transected rat quadriceps muscle (30).

The purpose of the present study was to study the effect of BPC on inflammation and bone resorption in experimental periodontitis in rats.

MATERIALS AND METHODS

Animals

Experiments were carried out on male 300±50 g Charles River Wistar rats received from the breeding colony of Semmelweis University. The animals were kept in a 12-hour light/dark cycle and maintained on standard rat laboratory chow and tap water ad libitum. All procedures conformed to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. The study was approved by the Animal Ethics Committee of Semmelweis University.

Gingival blood flow measurements

1. Surgical procedures

Rats (n=7) were anesthetized with sodium pentobarbital (60 mg/kg body weight; intraperitoneally; CEVA Sanofi, France); and placed on a heated table. Body temperature was kept at around 37°C. Tracheotomy was performed and the animals were allowed to breathe spontaneously through the tracheal cannula. The right femoral artery and left femoral vein were cannulated. After surgery 500 IU/kg bodyweight heparin (Gedeon Richter Plc., Budapest, Hungary) was administered intravenously. Mean blood pressure (MBP, mmHg) was monitored continuously by a Haemosys computerized dataacquisition system through the femoral catheter using a pressure transducer connected to an electromanometer (Experimetria Ltd., Budapest, Hungary). The heart rate (HR) was determined by counting the pulsatory blood pressure signals (min^{-1}) by Haemosys.

2. Laser Doppler Flowmetry

Gingival blood flow (GBF) was measured by laser doppler flowmetry (LDF, Oxford Optronix Ltd, Oxford, UK) working at 780 nm. The flow rate was expressed in blood perfusion units (BPU). A straight laser Doppler probe (outer diameter: 0.9 mm) was directed to the papilla between the two upper incisal teeth using manipulator fixation. The probe did not touch the gingiva. The laser doppler flowmeter was connected to a personal computer (Haemosys System, Experimetria Ltd., Budapest, Hungary) for data acquisition, storage and analysis. The vascular resistance of the gingiva (GVR) was calculated as a ratio of MBP and GBF, and values were given in resistance (R) (mmHg/BPU).

3. Preparation and application of BPC 157

BPC 157 (GEPPPGKPADDAGLV, molecular weight: 1419 Da) is freely soluble in water at pH 7.0 and in saline. Peptide with 99% purity (assassed by high pressure liquid chromatography, HPLC, with the biologically inactive 1-des-Gly peptide as the impurity) was used. BPC 157 was applied intravenously at a dose of 10 µg/kg body weight. This dose has been shown *in vivo* to be protective in a gastric mucosal lesion model (25) and it has been shown to promote bone healing in rabbits (28).

Ligature induced experimental periodontitis

Rats were lightly anaesthetized with surgical doses of sodium pentobarbital. A sterile, 2-0 black braided silk thread was placed around the cervix of the lower left first molar and knotted mesially according to Lohinai *et al.* (6). On the buccal, lingual and distal side of the tooth the thread was located subgingivally, while on the mesial side it was situated supragingivally. After rats had recovered from anaesthesia they were allowed to consume commercial laboratory food and drink tap water ad libitum. Animals were divided into 3 groups.

Animals received the following treatments for 12 days: 0.9% saline, 100 ng/kg or 10 µg/kg BPC 157. Injections were administered intraperitoneally once per day, the first application was given 30 min after ligature placement, while the final application 24 h before the tissue harvest. On day 13, the animals were anaesthetized again as described above. The mandible and the gingiva around the bottom molars were excised. Gingival capillary permeability was studied by the Evans-blue extravasation technique (6), gingival morphological alterations were estimated by histological analysis, while alveolar bone resorption was investigated by microCT.

Evans-blue vascular permeability assay

To assess vascular permeability, animals (n=9-9) received 50 mg/kg Evans blue (Reanal, Hungary, dissolved in physiological saline at a concentration of 2.5%) *via* a femoral venous catheter. Five minutes later another cannula was introduced into the abdominal aorta toward the heart. Ten minutes after Evans blue administration the dye remaining in the gingivomucosal capillaries was removed by retrograde intraaortic injection of 40 ml isotonic saline solution. Then approximately 2-3 mm thick stripes were cut that included gingivomucosal tissue around the first molar, as well as gingiva both on the lingual and on the buccal side until the middle line of the second molar. The contralateral, non-ligature side served as control. Extravasated Evans blue in excised gingivomucosal tissue samples was extracted by incubation in 0.5 ml formamide for 48 h at room temperature. Evans blue concentration was determined by spectrophotometric measurement at 620 nm and expressed as µg/g gingivomucosal tissue as described earlier by our group (6).

Histological analysis

Tissue samples from the gingiva surrounding the mandibular first molars (n=3-3) were harvested on both sides. Samples were fixed by immersion in 3% paraformaldehyde as described before (31). Sections were stained with haematoxyline and eosin. Photomicrographs were taken using a transmitted light microscope (Olympus Vanox, Olympus, Tokyo, Japan).

Micro computed tomography

Rats (n=12) were anaesthetized again and the mandibles were excised, separated from the surrounding tissues and cut in half in a sagittal plane between the incisors and were processed. Alveolar bone resorption and alveolar bone morphometric parameters were imaged at an isotropic voxelsize of 10 µm, using a microCT Cone Beam 1172 SkyScan system (Skyscan, Kontich, Belgium) operating at a peak voltage of 100 kV and 100 µA with a 0.5 mm aluminium filter. Samples were rotated with a rotation step of 0.70 degrees and a frame averaging of 7

until 180 degrees. Three-dimensional reconstructions of the images were visualized using the NRecon software (SkyScan, Kontich, Belgium) with 0% beam hardening and 10% ring artefact correction. Global thresholding was performed by an experienced operator. Image datasets were analysed by the CT Analyser software (1.7.0.5, SkyScan, Kontich, Belgium) to evaluate bone volume (BV). Alveolar bone morphometric parameters were investigated between mesial and distal roots of the lower first molars, at half way of the root length (*Fig. 5C*). Alveolar bone resorption was estimated by calculating the distance between the cemento-enameljunction (CEJ) and the crista alveolaris around the mandibular first molars (*Fig. 4B*). Distances at mesial, buccal, mesiolingual and lingual positions were measured and expressed in mm (*Fig. 4A*). Alveolar bone resorption was also calculated under the furcation area. The distance between the furcation of the root and the surface of the interradicular bone at the same axis was given in mm (*Fig. 5A*). This measurement was taken on resliced mandibular cross section images at the midradicular plain of the lingual root.

Statistical analysis

Data are presented as mean±SEM. Statistical analysis was performed by repeated measurement of ANOVA and Bonferroni post hoc test and/or contrast analysis of compared groups. Paired (in group) and unpaired (between groups) Student t-test were used in microCT analysis. $p<0.05$ was considered statistically significant.

RESULTS

Acute effect of BPC 157 on gingival blood flow

First we investigated the acute effects of systemically applied BPC 157 on local and systemic haemodynamic parameters in healthy rats. Animals received 10 µg/kg BPC 157 intavenously. General and local haemodynamic parameters were recorded before and 15 minutes after treatment. BPC 157 had no significant effect on general haemodynamic parameters: mean blood pressure was 103±4 mmHg and 102±3 mmHg before and after treatment, respectively, not significant (N.S.), *Fig. 1A*, and heart rate was 385±11 min^{-1} and 407±13 min^{-1} before and after treatment, respectively, N.S., *Fig. 1B*. BPC 157 also had no effect on local haemodynamic parameters in gingiva (gingival blood flow was 530±71 BPU and 675±108 BPU before and after treatment, respectively, N.S., *Fig. 1C* and gingival vessel resistance was 0.22±0.03 mmHg/BPU and 0.17±0.03 mmHg/BPU before and after treatment, respectively, N.S., *Fig. 1D*).

Effect of BPC 157 on gingival inflammation in experimental periodontitis

Next we studied the effect of BPC 157 on gingival inflammation in experimental periodontitis. A ligature was placed around the cervix of the lower left first molar as described in Materials and Methods in 36 rats to induce unilateral periodontitis. Rats were then divided into three groups with 12 animals each. Animals received BPC 157 once daily for 12 days at 100 ng/kg body weight or at 10 µg/kg body weight in the low-dose and in the high-dose group, respectively, whereas the control group received physiological saline i.p. once daily for 12 days. Nine rats were randomly chosen from each group for gingival tissue vascular permeability measurements, whereas the remaining animals were used for soft tissue histology.

In ligature induced experimental periodontitis, the increase in vascular permeability in the gingival tissue proximal to the ligature is proportional to the extent of local inflammation. The increased vascular permeability can be detected by measuring the extravasation of Evans blue dye. From nine rats of each group, gingivomucosal tissue samples were collected from both the ligature and the non-ligature contralateral sides and vascular permeability was measured as described in Materials and Methods. We compared the vascular permeability on the non-ligature side and on the ligature side in each of the three groups. There was no significant difference among the three groups in the vascular permeabilities on the non-ligature (contralateral) sides. In the saline treated group, vascular permeability increased in response to ligature compared to the contralateral side (29.8±2.5 *vs*. 10.8±1.4 µg Evans blue/g tissue, respectively, $p<0.05$, *Fig*. *2*). Vascular permeabilty also increased in response to ligature in the low-dose BPC 157 group (24.9±5.5 *vs*. 10.9±1.4 µg Evans blue/g tissue, respectively, $p<0.05$, *Fig. 2*). However, in the high-dose BPC 157 group gingival capillary permeability did not significantly increase in response to ligature (12.6±0.8 *vs*. 8.3±0.6 µg Evans blue/g tissue, respectively, N.S., *Fig. 2*). In conclusion, intraperitoneally administered BPC 157 at a dose of 10 µg/kg but not 100 ng/kg prevents oedema formation that normally accompanies inflammation. Therefore, we used the higher dose in subsequent experiments.

Histology

Gingivomucosal tissue samples were taken both from the control non-ligature side and from the ligature side from three rats of the control group and of the high-dose BPC 157 group. Samples were processed for histology. There was no difference

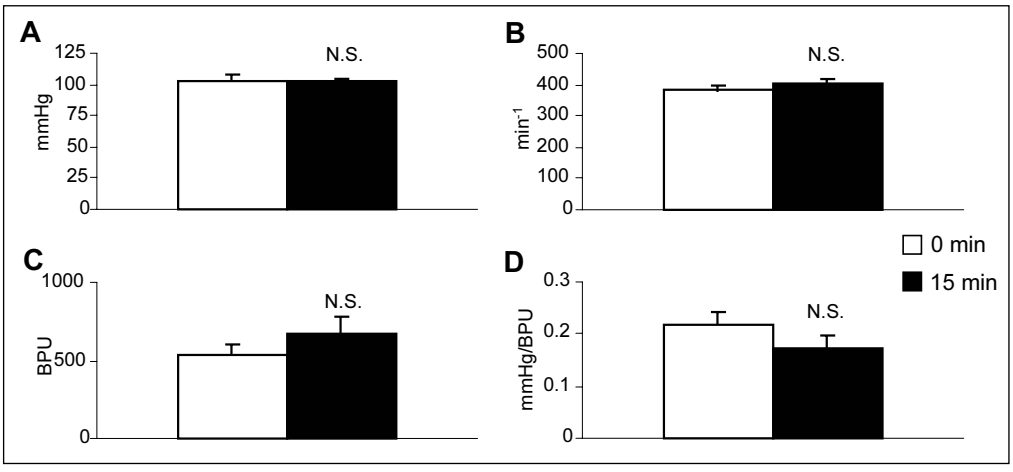

Fig. 1. Changes in general and local hemodynamic parameters before (0 min) and 15 min after systemic application BPC 157 (10 µg/kg i.v.) in healthy anaesthetized rats. General parameters are mean arterial blood pressure (A) and heart rate (B), local hemodynamic parameters are gingival blood flow (C) and gingival vascular resistance (D) were detected. (n=7, mean±SEM)

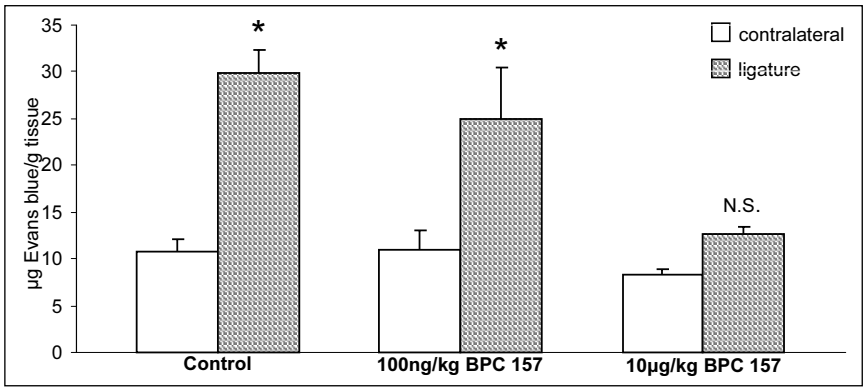

Fig. 2. Evans-blue extravasation during ligature-induced periodontitis and BPC 157 administration. Changes of vascular permeability in the ligature side and at the contralateral side were detected in saline treated (control), in low dose (100 ng/kg i.p.) and in high-dose (10 µg/kg i.p.) BPC treated rats. The non-ligatured (contralateral) sides were no significant difference among the three groups (saline treated (control), 100 ng/kg BPC, 10 µg/kg BPC treated). (* $p<0.05$, n=9-9, mean±SEM)

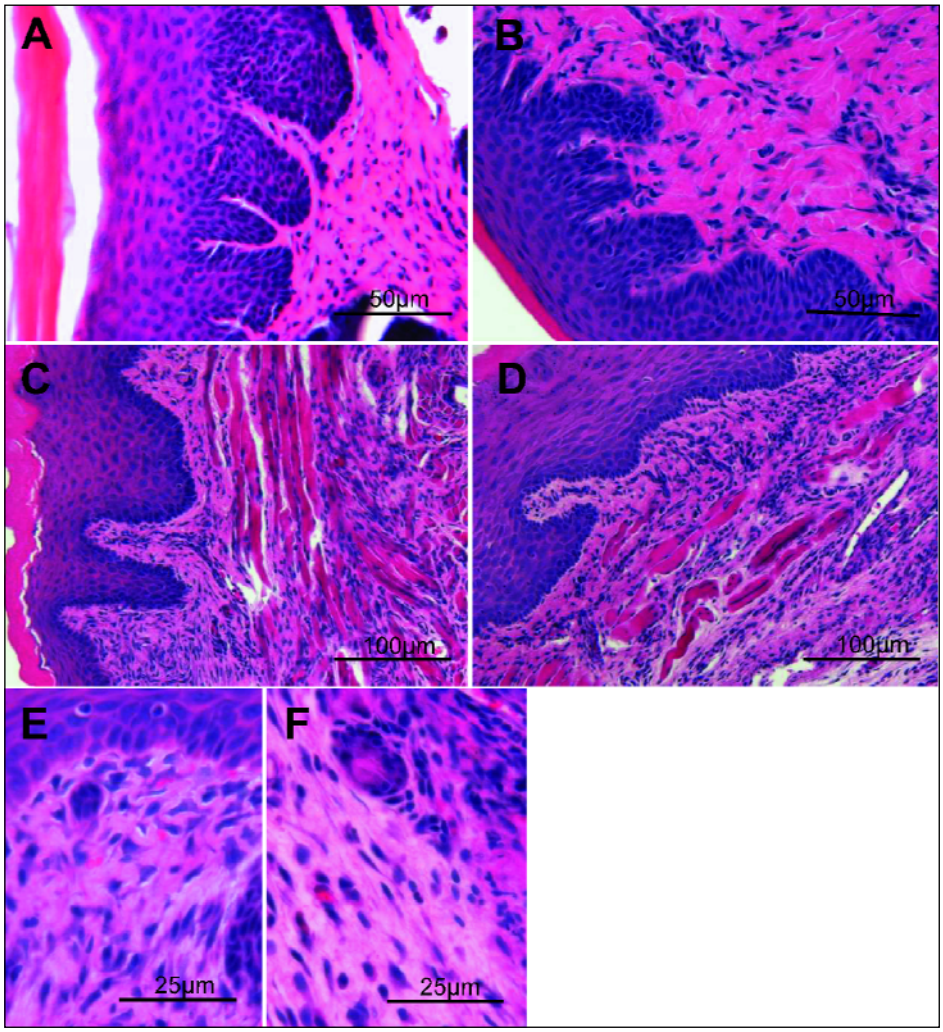

Fig. 3. Morphological changes during ligature-induced periodontitis and BPC 157 administration. Representative microscopical pictures show hematoxylin-eosin stained in gingivomucosal tissue. Animals treated with saline or BPC 157 (10 µg/kg i.p.) show no difference on the contralateral side (A: saline treated; B: BPC treated). On the ligature side saline treated (control) animals (C) show more vascular proliferation and signs of oedema than the BPC administrated ligatured rats (D). Likewise, ligature side of control group (E) shows more cellular elements than the ligature side of the BPC-treated rats (F).

in tissue morphology and cell types present in the gingiva on the non-ligature side between the saline treated and the BPC 157-treated rats (*Fig. 3*, control group: 3A and BPC 157 group: 3B). Samples show the histological characteristics of normal gingivomucosal sections. On the other hand, samples obtained from the ligature sides show the histological appearance of inflammation (*Fig. 3*, control group: 3C, 3E, BPC 157 group: 3D, 3F). Ligature side of the saline-treated group (*Fig. 3C*) showed more vascular proliferation and signs of oedema than the ligature side did from the BPC 157-administered group (*Fig. 3D*). In addition, there were more cellular elements at the saline group ligature side (*Fig. 3E*) than there were at the BPC 157 group ligature side (*Fig. 3F*).

Effect of BPC on alveolar bone loss in experimental periodontitis

To study bone loss in experimental periodontitis, mandibles were excised from rats treated with saline or 10 µg/kg BPC 157. Alveolar bone loss was studied using a microCT scanner. As a long-term consequence of ligature-induced periodontitis, not only tissue gingival inflammation measured by extravasation increased but there was also a considerable bone loss in the periodontium. As a result, the distance between the cemento-enamel junction and the alveolar crest enlarged. Our results, shown on *Fig. 4C*, clearly indicate an increase in bone loss on the ligatured side compared to the contralateral side, both in the saline-administered and in BPC 157-treated animals. Comparing

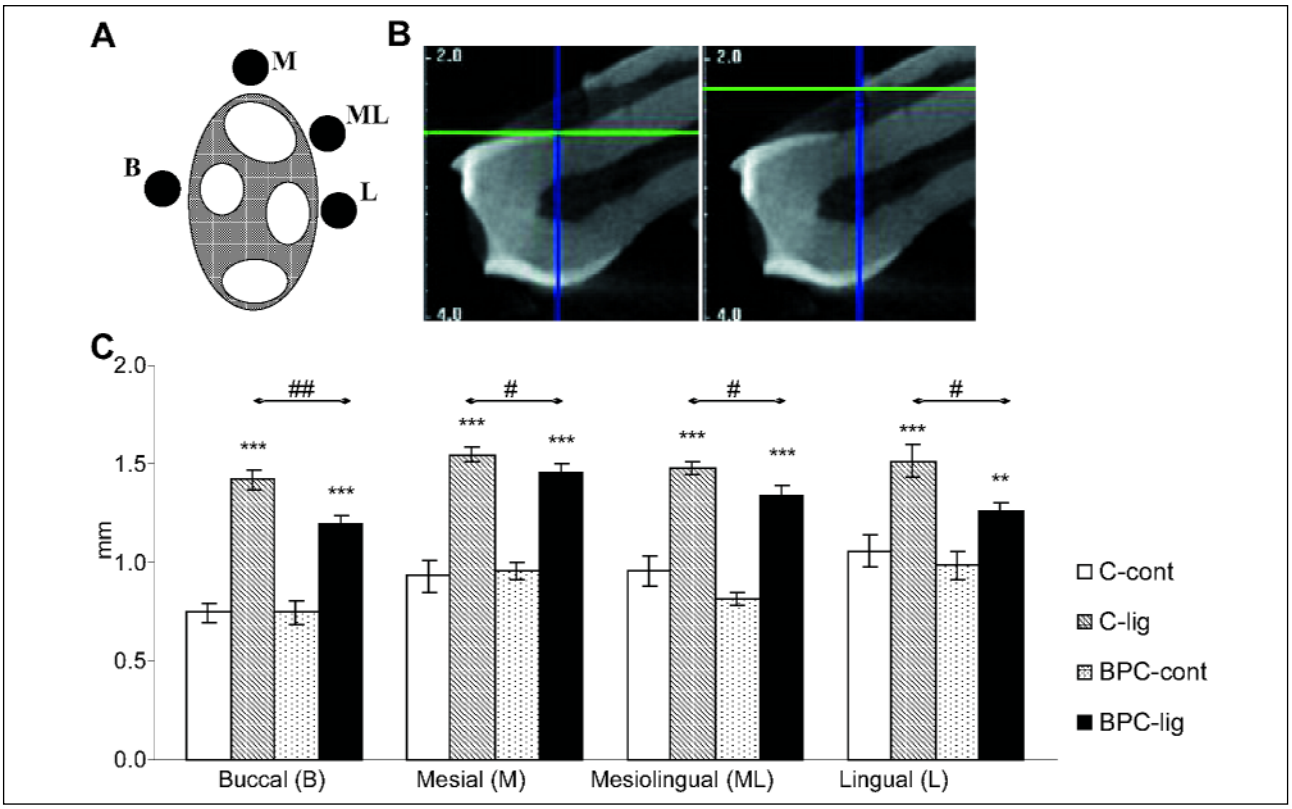

Fig. 4. MicroCT measurements on alveolar bone loss during ligature-induced periodontitis and BPC 157 administration. The schematic cross section (A) shows the rat first molar tooth with measurement points. (B: buccal, M: mesial, ML: mesiolingual, L: lingual). MicroCT reconstructed images (B) show distance between the cemento-enamel junction (CEJ) and the crista alveolaris. Changes of the distance between CEJ and crista alveolaris at the different sites of the rat first molar (C). The diagram shows data from control, saline treated group contralateral side (C-cont), control, saline treated group ligature side (C-lig), BPC 157 administered group contralateral side (BPC-cont) and BPC 157 administered group ligatured side (BPC-lig) (n=12-12; mean±SEM; $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$; ** $p<0.01$, *** $p<0.001$).

the ligatured samples, BPC 157 significantly decreased bone loss at the four locations tested. The distance between the cemento-enamel junction and the alveolar crest in control *vs*. BPC 157-treated animals was 1.42±0.05 *vs*. 1.19±0.04, respectively at the buccal side ($p<0.01$), 1.55±0.03 *vs*. 1.46±0.03, respectively at the mesial side ($p<0.05$), 1.48±0.03 *vs*. 1.34±0.05, respectively at the mesiolingual side ($p<0.05$), and 1.51±0.08 *vs*. 1.26±0.04, respectively at the lingual side ($p<0.05$). On the other hand, BPC 157 did not influence the distance between the cemento-enameljunction and the crista alveolaris around at the contralateral sides.

In addition to the measurements described above, we measured the distance between the furcation and the necrotized interradicular bone surface. Involvement of the furcation was recorded in each group. There is spongiosa in the interradicular bone in the furcation area. Lamina dura could not be detected in any of the groups. There was significant difference between the ligatured side of the saline-treated group and that of the BPC 157 treated group (0.62±0.07 *vs*. 0.42±0.03 mm, respectively, $p<0.01$). Compared to the contralateral side, bone loss significantly increased on the ligature side in both groups, whereas values measured on the contralateral sides were not significantly different between the two groups (*Fig. 5B*).

We also studied the changes in alveolar bone caused by inflammation using a microCT device, analyzing the spongiosa between the roots of the first molars, at half of the root length. Measuring further away from the inflamed bone surface, we detected no difference in bone volume in alveolar bone spongiosa between the control *vs*. the BPC group or between the contralateral *vs*. the ligatured side within treatment groups (*Fig. 5D*). Thus, our data suggest that in the experimental periodontitis model that we applied (*i.e.* the 13 day long ligature around the molars) the inflammatory process does not penetrate deep into the alveolar bone, and therefore does not affect the remaining spongy bone.

DISCUSSION

BPC 157 has been claimed to have anti-inflammatory and wound healing effects on multiple target tissues and organs (32). The purpose of the present study was to study the effect of BPC 157 on inflammation and bone resorption in experimental periodontitis in rats. The data obtained suggest that systemic application of BPC 157 does not alter the blood circulation in healthy gingiva, but chronic application of BPC 157 has potent antiinflammatory effects on the periodontal tissues in ligature induced periodontitis in rats. Therefore, this proof of concept study suggests that BPC 157 may represent a new peptide candidate in the treatment of periodontal disease.

When we studied the acute haemodynamic effects of BPC 157 on healthy rats, no changes were observed in gingival blood flow or in other investigated parameters even when BPC 157 was applied intravenously at 10 μg/kg. Likewise, in earlier studies BPC 157 was found to have no effect on blood pressure (33). In line with this, acute toxicology showed that BPC 157 even at a very high dose (2 g/kg) had no signs of toxic or lethal effects (30, 34-36). In healthy animals, it did not modify the psychopharmacologic profile either (37, 38). This peptide was

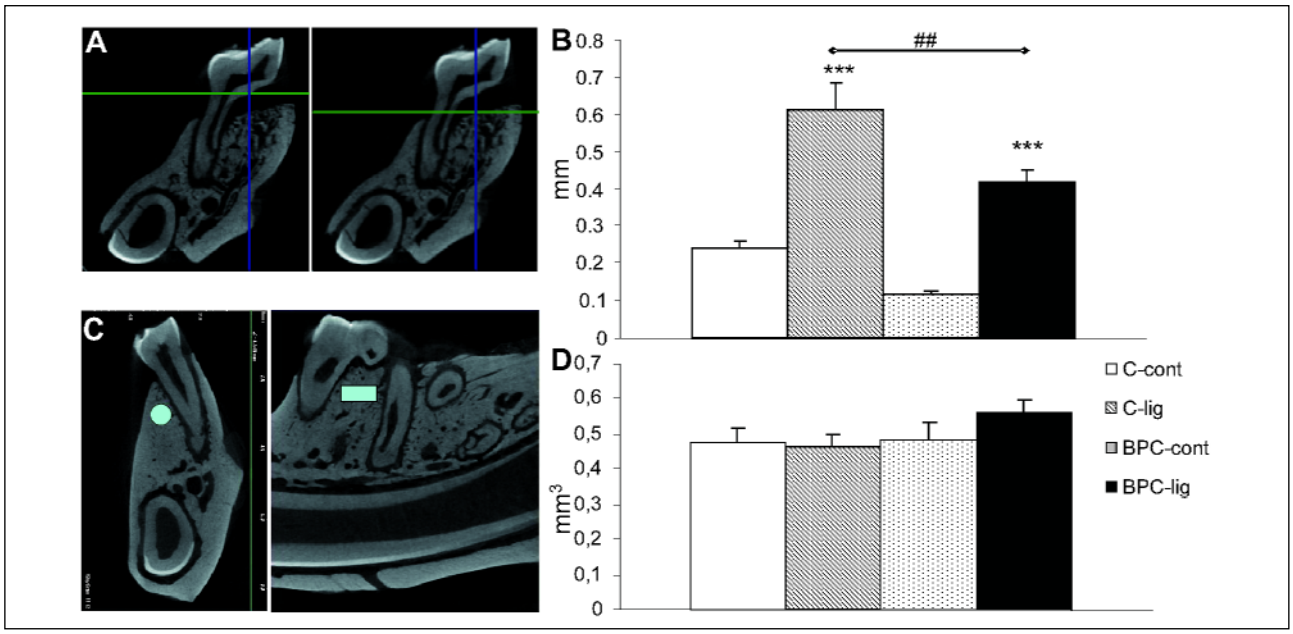

Fig. 5. MicroCT measurements on alveolar bone loss and bone volume changes during ligature-induced periodontitis and BPC 157 administration. MicroCT images (A) show the distance measurement between the bottom of furcation and the interradicular bone surface. The bone loss is proportional to the distance between the furcation and interradicular bone surface in periodontitis (B) ($^{##}$ $p<0.01$; *** $p<0.001$). Spongiosa volume deep down in bone, between the roots of the lower first molars, at half of the root length. Shaded ring and square mean the cylindrical shape of ROI (region of interest) (C). Values for bone volume in the ROI (D). The diagrams show saline treated group contralateral side (C-cont), saline treated group ligature side (C-lig), BPC 157 administered group contralateral side (BPC-cont) and BPC 157 administered group ligatured side (BPC-lig) (n=12-12, mean±SEM).

used alone, without carrier, and that this is an important advantage over the conventional peptides that have to use carrier (thereby peptide+carrier-complex) and mostly local use only, particularly in bone healing (28).

To study the effect of BPC 157 on periodontitis, the well established experimental model involving a ligature around the first molars was used (6, 9). As expected, in ligature induced experimental periodontitis, a considerable increase in vascular permeability in the gingival tissue was detected. This increase in extravasation of the Evans blue dye is a clear sign of tissue inflammation (39, 40). In addition, molar ligature also evoked the histological appearance of inflammation in the gingiva. Our findings are in line with other observations suggesting that the ligature induced experimental periodontitis is a useful and highly reproducible model for gingival/periodontal inflammation (41, 42).

The inhibitory effect of BPC on extravasation in the lower dose (100 ng/kg) fell short of significance, but the higher, 10 µg/kg dose of the peptide nearly completely abolished the extravasation induced by the 13-day ligature. Our histological observations confirmed the results obtained in functional experiments. BPC 157 in a dose that inhibited extravasation, also ameliorated the histological picture of inflammation. The inhibitory effect of BPC 157 on periodontal inflammation is not surprising taken into account that it has been reported by a number of laboratories to show a strong anti-inflammatory activity in both acute and chronic inflammation models (23, 25, 43). The mechanism of action of BPC 157 is not clear yet. It was shown to have multiple sites of action. BPC reduces the release of inflammatory mediators (*i.e.*, myeloperoxidase, leukotriene B_4, tromboxane B_2) (44), interacts with prostaglandin-dependent pathways (45, 46), has a direct protective and proliferative effect on target cells (18, 19), and modulates the release of nitric oxide (27). In addition, it has been claimed to promote new vessel formation (28), upregulation of the growth factors, as well as influencing other local factors (46, 47). The present study clearly shows that BPC 157 is effective in ameliorating experimental periodontitis but further studies are needed to identify the exact mediators of this action.

MicroCT is now widely and increasingly used to study bone metabolism in animals (48, 49). The great advantage of this technology is that it can process cross-sectional tomograms about 10 µm thick, and then build three-dimensional images using a computer. The microCT analysis was first used as a convenient method for histomorphometrical studies on long bones in ovariectomized rats and gene-deficient mice (50, 51). In addition it produceces high-quality imiges and enable accurate quantification of other mineralized tissues such as alveolar bone and tooth as well (52). MicroCT analysis can be used for measuring distance, area and volume, as well as density. Linear measurement can be used for example determine the distance of the crista alveolaris from the cemento-enamel junction as described recently (53). Loss of bone volume can be assessed using three-dimensional isoform display (54).

We used the microCT technology to study alveolar bone loss in our experimental conditions. We found that tooth ligation for 13 days not only increased gingival inflammation but also lead to a considerable bone loss around the tooth. As a result of the ligature, the distance between the cemento-enamel junction and the alveolar crest considerably enlarged. As expected, BPC 157 significantly decreased the bone loss around the ligatured molar. The enlargement of the distance between the cemento-enamel junction and the alveolar crest (crista alveolaris) is a clear sign of bone loss around the tooth. The change of this value was significantly but not completely decreased by BPC 157, *i.e.* the peptide did not completely abolish the effect of the ligature. Similarly the distance between the furcation and the resorbed interradicular bone surface was increased by the ligature, and

this was partly reversed by BPC 157. When we studied the changes deep down in the alveolar bone in response to ligature we found no significant difference in bone density. Measuring further away from the inflamed bone surface, we detected no difference in bone volume in alveolar bone spongiosa either in response to ligature or to BPC 157 treatment. Our data show that in our experimental periodontitis model (*i.e*. ligature for 13 days around the molars) the inflammatory process does not penetrate deep into the alveolar bone, and therefore does not affect the deep spongy bone. Our findings are in agreement with other recent observations using similar ligature models, which show a considerable increase of the distance between the cemento-enamel junction and the alveolar crest, regarded as a sign of alveolar bone loss (48, 52). In other experimental models, periodontitis induced by LPS and by bacterial infection also clearly resulted in alveolar bone destruction as detected by microCT (53, 54). The effect of BPC 157 has not been studied before in periodontal disease, our present observation is the very first one in this respect. But the peptide has been shown to have therapeutic healing effects in both bone and tendon injury models (28, 29, 55). Therefore, our studies confirm not only the antiinflammatory but also the tissue healing/preserving effects of BPC 157. However, further studies are necessary investigating the dose-dependence, the effective way of administration, and also the mechanism of action of BPC 157 to utilize the beneficial effect of the peptide in periodontitis.

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Conflict of interests: None declared.

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