Pentadecapeptide BPC 157 cream improves burn-wound healing and attenuates burn-gastric lesions in mice

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Abstract

The effects of the gastric pentadecapeptide BPC 157 were investigated when administered topically or systemically in burned mice. This agent is known to have a beneficial effect in a variety of models of gastrointestinal lesions, as well as on wound or fracture healing. Deep partial skin thickness burns (1.5 × 1.5 cm) covering 20% of total body area, were induced under anesthesia on the back of mice by controlled burning and gastric lesions were assessed 1, 2, 3, 7, 14 and 21 days following injury. The first application of BPC 157 was immediately following burning, and thereafter, once daily, until 24 h before sacrifice. In the initial experiments, exposure to direct flame for 5 s, the BPC 157 was applied at 10 μg or 10 ng/kg b.w. intraperitoneally (i.p.) by injection or alternatively, topically, at the burn, as a thin layer of cream (50 μg of BPC 157 dissolved in 2 ml of distilled water was mixed with 50 g of commercial neutral cream (also used as local vehicle-control)), while silver sulfadiazine 1% cream was a standard agent acting locally. Others received no local medication: they were treated i.p. by injection of distilled water (distilled water-control) or left without any medication (control). In subsequent experiments involving deeper burns (direct flame for 7 s), BPC 157 creams (50 μg, 5 μg, 500 ng, 50 ng or 5 ng of BPC 157 dissolved in 2 ml of distilled water was mixed with 50 g of commercial neutral cream), or vehicle as a thin layer of cream, were applied topically, at the burn. Compared with untreated controls, in both experiments, in the BPC 157 cream-treated mice all parameters of burn healing were improved throughout the experiment: less edema was observed and inflammatory cell numbers decreased. Less necrosis was seen with an increased number of capillaries along with an advanced formation of dermal reticulin and collagen fibers. An increased number of preserved follicles were observed. Two weeks after injury, BPC 157 cream-treated mice completely reversed the otherwise poor re-epithelization ratio noted in the untreated control or mice treated with vehicle only. Tensiometry investigation showed an increased breaking strength and relative elongation of burned skin, while water content in burned skin decreased. This was, however, not the case with the vehicle or silver sulfadiazine. Relative to the control values, in silver sulfadiazine cream-treated mice, only collagen fiber formation was increased, in addition to a decreased inflammatory cell number. Relative to control values, BPC 157 given i.p. decreased the number of inflammatory cells, lowered water content in burned skin, and raised breaking strength and relative elongation of burned skin during tensiometry. Through the experimental period, gastric lesions were continuously noted in all thermally injured mice left without local medication and they were consistently attenuated only by BPC 157 treatments: either given i.p. (at either dose), or given locally (at either concentration). Other treatments (i.e. local treatment with silver sulfadiazine cream or neutral cream in mice subjected for 5 s to direct flame), led to only poor, if any attenuation. This stable gastric pentadecapeptide appears to be active and gives a stimulation to burn healing at the defect site. The agent may act by causing an upregulation of the growth factors, as well as influencing other local factors. © 2001 Elsevier Science Ltd and ISBI. All rights reserved.

Keywords: Pentadecapeptide BPC 157; Cream; Burn-wound healing; Burn-gastric lesions; Collagen/BMP-6; Mice

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1. Introduction

Deep partial skin thickness burns may be used in a reproducible animal model to study burn wound healing and attenuation of acute gastric mucosal lesions in response to antiulcer agent therapy [1–3]. An essential failure of healing is a common problem in both mucosal ulcer and skin wound therapy (for review see Ref. [4]). This study aimed to investigate the effects on burn wound healing and thermal injury-gastric lesions of the gastric pentadecapeptide BPC 157 (GEPPPGKPADDALVM.W. 1419 [4]). BPC 157 is known to have a beneficial effect in a variety of gastrointestinal lesion models [5–9], including stress-ulcer [10,11], and a healing effect in different wound models [12–14]. BPC 157 also stimulates healing of segmental osteoperiosteal bone defects when given locally either percutaneously into the bone defect, or applied intramuscularly [15]. BPC 157 solution was shown to stimulate corneal wound healing [16].

Conditions of healing in burns are complex as a result of vascular damage caused by burn. Dermal vascular changes following thermal trauma result in fibrin deposition and vascular occlusion at the site directly destroyed by thermal energy and changes in dermal haemodynamics and blood vessel morphology in the areas of dermis surrounding the burn area [17]. In addition, a particular problem for peptide application is to find a carrier that does not limit their efficacy at the wound site (i.e. EGF, TGF-β) [18–20]. In previous experiments [5–16], BPC 157 was effective without a special carrier. Its particular property of stability (i.e. not degraded in human gastric juice for 24 h [21]) may be a relevant point. BPC 157 may be suitably stable in a simple commercial vehicle (i.e. neutral cream) providing a suitable presence at the defect site and able to influence the potential upregulation of the growth factors, as well as other local factors, acting to promote burn healing.

This study as a first step in burn wound therapy and burn injury-gastric lesion-studies also used topical application of BPC 157 at the site of the burn, administration at a concentration 1 μg/1 g of vehicle, once daily. Pentadecapeptide BPC 157 as an intraperitoneal (i.p.) application was also used. Silver sulfadiazine cream 1% was used as a standard agent acting locally [22]. The next step was the analysis of the influence of this local medication on thermal injury-induced gastric lesions and the healing of the burn wounds itself if given daily topically at the site of the stronger burn wounds also in lower concentrations.

2. Materials and methods

2.1. Drugs

BPC 157 (manufactured by Diagen, d.o.o., Ljubljana, Slovenia) is a partial sequence of human gastric juice protein BPC, freely soluble in water at pH 7.0 and in saline. It was prepared as described before [5,6,11–16]. Peptide with 99% (HPLC) purity (1-des-Gly peptide as impurity), dissolved in saline [5,6,11–16] was used in all of the experiments. Silver sulfadiazine cream 1% (Dermazin, Lek, Slovenia), and neutral commercial cream (Belobaza, Ljekarna Zagreb, Croatia) were commercially purchased.

2.2. Experimental protocol

2.2.1. Animals

Randomly assigned male mice (NMRI-Hannover, 30 g b.w.), maintained individually in separate cages, with free access to food and water, and no special fluid resuscitation after injury induction, were used in all experiments, as approved by the local animal research committee. In the investigations, 10 mice were used in each of the experimental groups.

2.2.2. Lesion induction and assessment

Deep partial skin thickness burns were induced on the back of animals by controlled burning (1.5 × 1.5 cm or 20% of total body area), under ether anesthesia. The anesthetized animals were exposed to direct flame through a 1.5 × 1.5 cm window in an asbestos network, for 5 or 7 s, using a modification of previously described methods [3–5,23,24]. This procedure resulted in no mortality. Burn healing parameter assessment includes histological investigation, water content assessment in burn skin, and tensiometry studies (i.e. determination of breaking strength, and relative elongation of the burned part of skin) [20,25]. Gastric lesions (means ± S.E.M., mm) were assessed as described previously [5,11]. The assessment was at 1, 2, 3, 7, 14 or 21 days following injury.

2.2.3. Histological assessment

The parameters investigated included edema, blood vessel formation, and their total diameter, number of preserved follicles, reticulin and collagen formation, number of inflammatory cells, and number of mice with complete re-epithelization [3,23,24]. Standard histological slices were stained using hematoxylin–eosin (blood vessels, edema, necrosis, inflammatory cells), Gomori silver stain (reticulin) and Van Gieson staining (collagen). The assessed parameters according to day of sacrifice were as follows: day 1: edema, number and diameter of blood vessels, inflammatory cells and reticulin, number of vital follicles; days 2 and 3: as for day 1 with necrosis as well; day 14: number and diameter of blood vessels, epithelization, reticulin and collagen; day 21: number and diameter of blood vessels, epithelization and collagen.
Specifically, edema has been determined by measuring the distance from the muscular layer to the surface of burned area (mag. 2 ×, μm). Angiogenesis has been determined by measuring the number and sum of diameters of blood vessels. Two microscopic fields (mag. 6.3 ×) in the central part of the burned area were analysed to determine this parameter. Inflammatory cells have been counted in three visual fields in the middle portion of the burned area (mag. 40 ×) between the deepest layer of follicles and the muscular layer of the skin. Necrosis has been scored using a modified Suzuki scale (mag. 25 ×) [26]: Grade 1: necrosis within the epidermal layer; Grade 2: necrosis up to the deepest layer of hair follicles; Grade 3: necrosis exceeding the deepest layer of hair follicles; Grade 4: necrosis exceeding the muscular layer. The number of preserved follicles has been counted in three microscopic fields (mag. 25 ×) in the middle of the burned area and examining the deepest layer of hair follicles. Development of epithelization has been expressed as the number of animals with complete epithelization versus incomplete epithelization ratio for each group, respectively. Retinol and collagen have been determined as percentages in two microscopic fields (mag. 40 ×) on each margin of the burned area between the deepest follicles and the muscular layer.

Mice subjected to the same procedure (i.e. careful shaving of the back area 24 h previously to avoid skin damage, anaesthetized at the time of burning, etc.) but not exposed to direct flame, were used as the sham burn group. However, no pathology was produced in sham burned mice.

All microscopic measurements were carried out by three independent blinded observers and no significant differences were noted between their individual assessments. For morphometrical analysis, a special program SFORM (VAMS-Software Company, Zagreb, Croatia) was used.

2.2.4. Tensiometry investigation
Measurement of tensile breaking force and relative elongation of the burned part of the skin was carried out in separate experiments, immediately after sacrifice, at days 7 and 14 as described [20,25] by a special device (Technical Faculty, University of Zagreb, Croatia) using formulas: breaking force (N/mm²) = force (N)/cross-section skin burned area (mm²); relative elongation of burned part of skin = length burned skin immediately before breaking/initial length of burned skin.

VSFB2.2.5. Assessment of water content
In separate experiments, a 20 × 20 mm specimen of full-thickness tissue from burned sites of the back and a 20 × 20 mm specimen from the upper back (non-damaged) were taken for weight determination after sacrifice, at day 1, 2, or 3. The tissues biopsies were placed in a drying chamber (58 °C) until stable weight was reached for approximately 48 h [27]. The wet/dry ratio of each specimen was calculated and compared to control values.

2.2.6. Medication protocol
Generally, 10 mice per experimental group were used and medication was given once daily (first application immediately after burn-injury, last application 24 h before sacrifice).

Initial experiments involved injury induced by direct flame for 5 s, with BPC 157 cream (1 μg/1 g of vehicle, specifically 50 μg of BPC 157 dissolved in 2 ml of distilled water mixed with 50 g of commercial neutral cream) given locally, on the burn as a thin layer of cream. Likewise, BPC 157 (10 μg or 10 ng/kg) given by i.p. injection was also used. Silver sulfadiazine cream 1% (Dermazin, Lek, Slovenia) was given as a standard agent acting locally. An equal amount of neutral commercial cream (Belobaza, Belupo, Croatia) was given locally on the burn (local vehicle-control), an equal volume of saline was given for i.p. injection (5.0 ml/kg i.p.) or no medication (zero-control) was used.

To increase the level of the injury, and to ascertain the effect of BPC 157, given at various concentrations topically, burn was induced by direct flame for 7 s. As before, BPC 157 was applied locally, at the burn, as a thin layer of cream (50 μg, 5 μg, 500 ng, 50 ng or 5 ng of BPC 157 dissolved in 2 ml of distilled water was mixed with 50 g of commercial neutral cream). Control animals received no medication (zero-control), or an equal amount of neutral commercial cream (Belobaza, Belupo, Croatia) locally at the burn (local vehicle-control).

2.3. Statistical analysis
Fisher’s exact probability test two-tailed (presence/absence of re-epithelization in mice), non-parametric analysis of variance (ANOVA) (Kruskall–Wallis one-way ANOVA by ranks) and post-hoc Wilcoxon rank-sum test (necrosis, edema, blood vessel formation, and their total diameter, number of preserved follicles, retinol and collagen formation, number of inflammatory cells, breaking tensile force, relative elongation of burned part of skin, gastric lesions size (sum of longest lesions diameters) in mice) were used for statistical analysis, with a downward adjustment of the α-level to compensate for multiple comparisons. The differences were considered to be significant at P < 0.01 (5 s expo-
sure to direct flame) or $P < 0.008$ (7 s exposure to direct flame).

3. Results

3.1. Healing of thermal burn wounds

3.1.1. Microscope assessment of BPC 157 regimens and silver sulfadiazine cream in mice exposed to direct flame for 5 s

A consistent damage was induced in controls which received an equal volume of saline (5.0 ml/kg i.p.) or mice with no medication. Since these were not different, they are shown together (control). Mice treated with only cream vehicle (Belobaza) showed no difference with respect to controls, and the same poor re-epithelization ratio was observed in the untreated controls or in mice treated with only cream vehicle. Compared with the controls, the BPC 157 cream-treated mice showed improvement in all parameters of burn healing throughout the experiment. Less edema was observed and inflammatory cell numbers were lower. Necrosis was not so extensive and an increased number of capillaries along with an advanced formation of dermal reticulin and collagen fibers and an increased number of preserved follicles were seen. Two weeks after injury, BPC 157 cream-mice completely reversed the otherwise poor re-epithelization ratio noted in the untreated control or mice treated with only cream vehicle (after a 3-week period, the re-epithelization was also completed in controls (data not shown)). This was, however, not the case with silver sulfadiazine cream-mice. Relative to the control values, in silver sulfadiazine cream-mice, only collagen fiber formation was increased (7th, 14th, 21st post-injury day), besides a decrease in inflammatory cell numbers at the third post-injury day. Compared with the control, BPC 157 given i.p. decreases the number of inflammatory cells seen at the third post-injury day, but other parameters were not affected (Tables 1 and 3).

3.1.2. Microscope assessment. Various concentrations of BPC 157 cream in mice exposed to direct flame for 7 s

A consistent and even more pronounced damage was induced in controls (Tables 2 and 4). Mice treated with vehicle only showed no difference with respect to controls, and the same poor re-epithelization ratio was present in the untreated control or mice treated with only cream vehicle. Compared with the controls, the BPC 157 creams regularly improved wound healing parameters in mice. With respect to the control values, and the lower concentrations used (1 µg, 100 ng, 10 ng, 1 ng or 100 pg/g vehicle), parameters of burn healing were improved throughout the experiment. Less edema was observed (1 µg–100 pg/g vehicle) and inflammatory cell numbers reduced (1 µg–100 ng/g vehicle). Less extensive necrosis was seen (1 µg or 1 ng/g vehicle), an increased number of capillaries (1 µg–100 pg/g vehicle), an advanced formation of dermal reticulin (1 µg or 1 ng/g vehicle) and collagen fibers (1 µg or 1 ng/g vehicle) were observed, and an increased number of preserved follicles (1 µg or 1 ng/g vehicle). At 2 weeks after injury, poor re-epithelization ratio otherwise noted in the untreated control was completely reversed in mice treated with 1 µg and 100 ng BPC 157/g vehicle. Thus, it seems that some of the parameters of burn healing are sensitive to concentrations as low as 1 ng or 100 pg/g vehicle, while others need higher concentrations.

3.1.3. Measurement of water content

Water content of burned skin was assessed as described before [26]. In the experiments using mice subjected to direct flame either for 5 or 7 s, cream-vehicle-treated mice showed no difference with respect to controls, while a lower water content was observed in mice that received the BPC 157 (1 µg/g) cream, when they were assessed at the earliest post-injury interval (i.e. day 1). Interestingly, in mice subjected to direct flame for 5 s, compared with the control, BPC 157 given 10 µg/kg i.p. decreased water content at the first post-injury day. On the other hand, with respect to controls, silver sulfadiazine cream had no effect (Tables 3 and 4).

Table 1

<table>
<thead>
<tr>
<th>Burned mice 10 mice/group; 2 weeks after injury</th>
<th>Complete epithelization</th>
<th>Incomplete epithelization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Silver sulfadiazine cream</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td><strong>BPC 157</strong></td>
<td></td>
<td></td>
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<tr>
<td>1.0 µg/g vehicle cream</td>
<td>9*</td>
<td>1*</td>
</tr>
<tr>
<td>10 µg/kg i.p.</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>10 ng/kg i.p.</td>
<td>2</td>
<td>8</td>
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</table>

Burn induced by 5 s exposure to direct flame, once daily medication. Epithelization is expressed as the ratio between the number of mice with complete epithelization versus number of mice with incomplete epithelization for each group, 2 weeks after burn induction. Medication (first application immediately after burn-injury, last application 24 h before sacrifice) was given once daily. The effects of a various pentadecapeptide BPC 157 regimens (topically applied in a cream, or given i.p.), silver sulfadiazine cream, or a vehicle (neutral cream (Belobaza)).

* $P < 0.01$, at least versus control.
Burn induced by 7 s exposure to direct flame, once daily medication. Epithelization is expressed as the ratio between the number of mice with complete epithelization versus number of mice with incomplete epithelization for each group, 2 weeks after burn induction. Pentadecapeptide BPC 157 (50 μg, 5 μg, 500 ng, 50 ng or 5 ng of BPC 157 dissolved in 2 ml of distilled water was mixed with 50 g of commercial neutral cream), or a vehicle (neutral cream (Belobaza)).

* P<0.008, at least versus control.

3.1.4. Tensiometry investigation

3.1.4.1. Mice subjected to direct flame for 5 s. Measurement of tensile breaking force: In comparison with control animals at days 7 and 14, tensile breaking force showed higher values in mice treated topically with BPC 157 cream (1 μg/g vehicle). Likewise, at day 14, higher values were noted in those which received i.p. pentadecapeptide BPC 157 application (10 μg or 10 ng/kg). In animals treated topically with silver sulfadiazine cream 1%, or vehicle, neutral cream, Belobaza, the observed values correspond with the values shown in controls (Fig. 1A).

Relative elongation of the burned part of skin: Compared with the control values at days 7 and 14, the higher values were consistently noted in groups treated with BPC 157 i.p. (10 μg/kg or 10 ng/kg), as was observed in animals treated topically with 1 μg/g BPC 157 cream. On the other hand, topical application of the vehicle (Belobaza) or silver sulfadiazine cream led to values not different from control.

3.1.4.2. Mice subjected to direct flame for 7 s. Measurement of tensile breaking force: At days 7 and 14, assessment of tensile breaking force showed that topical application of vehicle did not change the control values. On the other hand, a higher tensile breaking force than in controls was observed in groups that received BPC 157 creams 1 μg/g (days 7 and 14) or 100 ng/g (day 14) (Fig. 1B). The effectiveness of both 1 μg/g or 100 ng/g observed at day 14 indicates an increased activity of wound healing mechanisms to increase mechanical strength during the experiment. Of note, an essentially potentiated activity (i.e. directly, repeated topical applications and/or triggering growth factors, as well as other local factors) may be associated with an additional activity of the lower doses potentially postponed (i.e. seen at later period(s), if they had not been effective earlier). Interestingly for longer-term therapeutic use, this situation was also seen in some microscopically observed parameters of burn healing (see also Table 4), as well as in the case of gastric lesion attenuation (see later).

Relative elongation of the burned part of skin: Compared with the control values at days 7 and 14, higher values were consistently noted in groups treated topically with 1 μg/g or 100 ng/g pentadecapeptide BPC...
Fig. 1. Tensiometry assessment. Breaking tensile force (N/mm²) (force (N)/cross-section skin burned area (mm²)) (left). Relative elongation of burned part of skin (Δlength of burned skin immediately before breaking/initial length of burned skin) (right). Means ± S.E.M. Medication (first application immediately after burn-injury, last application 24 h before sacrifice) was given once daily. (A) Injury induced by direct flame for 5 s, at day 0, days following injury. The effects of various BPC 157 regimens (topically applied in a cream, or given i.p.), silver sulfadiazine cream, or a vehicle (neutral cream (Belobaza)). $P < 0.01$, at least versus control. (B) Injury induced by direct flame for 7 s, once daily medication. Pentadecapeptide BPC 157 (50 µg, 5 µg, 500 ng, 50 ng or 5 ng of BPC 157 dissolved in 2 ml of distilled water was mixed with 50 g of commercial neutral cream), or a vehicle (neutral cream (Belobaza)). $P < 0.008$, at least versus control.
Medication (first application immediately after burn-injury, last application 24 h before sacrifice) was given once daily. The effects of various BPC 157 regimens (topically applied in a cream, or given i.p.), silver sulfadiazine cream, or a vehicle (neutral cream (Belobaza)). The parameters compared with the control, assessed as described in the text, are: E, edema; W, water content; Bvn, blood vessel number; Bvd, blood vessel density; /100 pg/g cream; BPC/p54 or g cream/p161. The parameters influenced by BPC 157 application, either topical or systemic.

3.2. Gastric lesions in burned mice

3.2.1. BPC 157 regimens (topically applied in a cream, or given i.p.) and silver sulfadiazine cream in mice exposed to overt flame for 5 s

Deep partial skin thickness burns consistently induced reproducible acute gastric mucosal lesions [15,28]. Accordingly, in the experiments using 5-s exposure to flame, gastric lesions are present throughout the entire experimental period in all thermally injured mice left without local medication. Interestingly, when compared to controls, BPC 157 given once daily i.p., attenuates the lesion at both early (i.e. at post-burn day 3) and later stages (i.e. at post-burn days 14 and 21). Likewise, this antiulcer effect is even more prominent with BPC 157 cream 1 μg/g vehicle; it is evident from post-burn day 3 to the end of the experiment. Silver sulfadiazine cream decreased stomach damage at two points, at post-burn days 7 or 21, but not before, whereas the vehicle treatment temporarily attenuated gastric lesions at post-burn day 3 (Fig. 2A).

3.2.2. Various concentrations of BPC 157 cream in mice exposed to direct flame for 7 s

With the deep partial skin thickness burns-model [15,29] a longer exposure to direct flame (i.e. 7 s) produced even larger thermal-injury-related gastric lesions. As may be expected, in comparison with the control, vehicle treatment was completely ineffective against this exaggerated gastric lesion (Fig. 2B). On the other hand, a prominent antiulcer effect was demonstrated following topical application of BPC 157 creams. Interestingly, against the background of the inevitable larger gastric lesion seen regularly in the controls, all concentrations of BPC 157 creams counteract gastric lesion development. Shown through the whole experimental period, this antiulcer effect is consistent with the attenuation of stress and other gastrointestinal lesion, noted in the previous BPC 157 studies using systemic application [5,10,11].

4. Discussion

The benefit of creams containing BPC 157 on burn wound and burn-induced stress gastric ulcers may be considered from both local (i.e. healing of the burn-wound) [3,23,24] and systemic (i.e. interruption of the negative chain of events that otherwise inevitably lead to stress stomach lesions) [1,2] points of view. The
gastric pentadecapeptide BPC 157 appears to have a special relationship to bone morphogenetic protein-6 precursor (BMP-6), a molecule known to be involved in keratinocyte differentiation and wound healing [29,30]. This molecule is also found in the intestine [31] and has been consistently implicated in recent molecular investigation of BPC-157 patterns [32]. In its structure it shows analogy to several regions of bone morphogenetic protein-6 precursor (BMP-6), and numerous fragments of BMP-6 precursor molecule are contained within the consensus sequence of BPC 157 molecule. In addition to the molecular similarity to BMP-6, the fragments of BPC 157 also correspond to various collagen fragments, important in the wound healing [33]. Consequently, the similarity to collagen fragments may explain the fact that BPC 157 has strong osteogenic effects [15,32], but does not require the addition of collagen matrix for its effects [15,32] unlike other peptides [20,29,30].

Re-epithelization, a desired final result, likely appears to be enhanced due to advanced reticulin and collagen formation, less edema and necrosis, improved blood vessel formation and more preserved follicles noted in BPC 157 cream-treated mice. This finding is also supported in the experiments using various concentrations of BPC 157 creams, and substantiated by the demonstration of increased tensile breaking force, or increased relative elongation of the burned part of skin during tensiometry. Dermal vascular changes following thermal trauma include fibrin deposition and vascular occlusion at the site directly destroyed by thermal energy and changes in dermal haemodynamics and blood vessel morphology in the areas of dermis surrounding the burn area [17]. Since it is effective when given topically, BPC 157 may be present directly at the site of burn injury in sufficiently high concentration permitting an effective stimulation of all parameters of burn healing, and possibly preventing vascular occlusion. To support this, an increase in the number of capillaries (1st to 14th post-injury day) was noted in the BPC 157 cream-treated mice, along with its effect on angiogenesis [14] and NO-system modulation [34]. Consequently, besides a local attenuation of the burn injuries, it may promptly accelerate healing of gastric lesions following local absorption. This shows, compared with control values, different patterns of healing in BPC 157 and silver sulfadiazine cream regimens. A marked attenuation of burn-produced gastric lesions was seen along with accelerated burn wound healing involving all parameters when BPC 157 creams were used for topical treatment. Alternatively, with the silver sulfadiazine cream, only a few parameters of burn healing were improved (i.e. collagen) in parallel with a relatively weak and delayed attenuation of thermal injury-induced gastric lesions. As observed in other wound healing models [12,13,15] systemic application of BPC 157 in burn healing models increased breaking strength and relative elongation of the burned part of skin during tensiometry, and reduced water content in burned skin.

Supporting both attenuation of burn-induced gastric lesions and promotion of burn-healing with very low concentrations of the BPC 157 creams, all the beneficial effects of the BPC 157 had been previously described in different experimental models [12–16]. These included an angiogenesis model (with synthetic sponge implantation [14]), accelerated collagen, reticulin and blood vessel formation, increased tensile breaking force (skin incision wounds [12]), raised bursting pressure (colon–colon anastomoses [13]), and an accelerated healing in special and complex conditions (healing of chronic segmental osteoperiosteal bone [15]). Burn injury induces a pronounced inflammatory reaction, involving various vasoactive mediators such as histamine [35], serotonin [36], leukotrienes [37] and prostaglandins [38] with the resulting effects on vascular permeability and edema [39]. In relation to the events in burn patients [40], BPC 157 strongly inhibited the effect of calcium ionophore A23187 [9,41,42], known to induce histamine release from mast cells and tissues (for review, see Ref. [43]), and it reduced the A23187 promoted-release of leukotriene and other inflammatory mediators (i.e. MPO, TXB2) as noted in inflamed animal tissues and human blood [9,41,42]. Thus, along with the verified effects of BPC 157, a parallel inhibition of their damaging overactivity in burned tissue is very likely.

Finally, taking the present findings together with previous reports [4–16,21,34], this suitably stable gastric pentadecapeptide can achieve an adequate concentration to give a significant stimulus to burn wound healing at the defect site. Due to its structural characteristics, it may potentially provide an upregulation of growth factors, as well as other local factors. This triggering is not unexpected since the pentadecapeptide is stable and not degraded in human gastric juice even for 24 h, unlike other peptides, i.e. human-EGF, human-TGF [21]. The observed effectiveness of this pentadecapeptide applied without a special carrier, dissolved in distilled water, in a simple commercial vehicle, is a major advantage over its relationship with the added carrier [18,19]. For instance, besides the previously mentioned limitations for BMP-6 and TGF-β [20,29,30]), only topical application of EGF ointment containing a protease inhibitor has ameliorative systemic effects [19]. Possibly, the lack of otherwise present serum proline peptidases required for the systemic
cleaving of proline containing peptide bonds [44] may further promote this local BPC 157 stability and its prolonged therapeutic potential after topical application.

5. Conclusion

In summary, when compared with the control values, the activity of the pentadecapeptide BPC 157 on inflammatory cells, edema, reticulin, collagen, necrosis, blood vessel formation, number of preserved follicles, re-epithelization and stress-gastric lesion attenuation, tensile breaking strength, and water content in burned skin, suggests that this substance may act to give a more accelerated healing in burns of treated mice. In addition, BPC 157 appears to attenuate stress-induced gastric lesions. This stable gastric pentadecapeptide may reach an adequate concentration to give a significant stimulation to burn healing at the defect site, without special carrier. The mechanisms by which it acts are unclear but may be linked with its particular structural characteristics and may involve it in exerting an upregulation of growth factor synthesis, as well as influences on other local inflammatory factors.
References


