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Gastric Pentadecapeptide BPC 157 Promotes Corneal Epithelial Defects Healing in Rats

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A B S T R A C T

We evaluated the role of human gastric pentadecapeptide BPC 157 in corneal epithelial defects healing in rats. 48 rats, in 4 groups (N=12). Total debridement of corneal epithelium preformed unilaterally and lesions stained and photographed. Animals medicated as follows: distilled water (control group) or BPC 157 2pg/ml, 2ng/ml, 2μg/ml, 2 drops/rat eye started immediately after injury induction, every 8 hours up to 40 hours (i.e., at 0, 8, 16, 24, 32, 40 h). Lesions were photographed before application or sacrifice (at 48 h). Defect area was analyzed using a special program. Through 48 hour period a steady recovery is noted in controls. Recovery was markedly accelerated in eyes on μg- or ng-topical regimen of BPC 157 ($p<0.05$). Of note, unlike control lesion present also after 48h, these lesions disappeared already following 40 h (μg) or 48 h (ng) post-injury. BPC 157 was shown to be effective in promoting corneal defects healing in rats. Results were dose dependent.

Key words: *BPC 157, corneal epithelial defects, rats*

Introduction

Epidermal growth factor (EGF)[1] and nerve growth factor (NGF)[2] promote corneal epithelial healing. However, most protein growth factors that might improve the healing are rapidly metabolized by the organism. Besides, for full effect, peptidergic agents frequently necessitate carriers' activity or addition. However, all special regimens remain inappropriate for routine use, and search for new agents for regular use is fully justified. Our focus is gastric pentadecapeptide BPC 157, currently in clinical trials for inflammatory bowel disease (PLD-116, Pliva). Recently, as a solution, it also stimulates corneal epithelial healing[3]. Therefore, we remove the entire epithelium, and this study further investigates the healing in severely impaired condition.

Possible recovery of completely denudated rat cornea is studied due to its healing effects on various tissues given systemically and/or locally[4–10]. Initially, BPC 157 opposes a variety of gastrointestinal lesions including stress-ulcer and thermal injury-gastric lesions[8,10], and thereafter it cures, besides corneal[3], different wounds (i.e., skin[6], colon-colon anastomosis[10], deep skin burn[4], segmental osteoperiosteal bone defects[5]). Also, it modulates NO-synthesis[6]. This may directly affect repair of connective tissues[6], as an ordered multistage process involving inflammation.

Besides, this gastric pentadecapeptide BPC 157 likely controls functions of collagen fragments[5]. Likewise, it has special relation over bone morphogenetic proteins (BMPs)[11]. It has high stability and shows no degradation in human gastric juice even for 24 h (unlike rapidly degraded h-TGF, and h-EGF)[9]. Finally, no carrier is used in previous[4–10] and present studies. Together, this stable pentadecapeptide highly resistant to otherwise inescapable degradation of peptides, presented with healing potential of its own[4–10], may be suitable for therapy of complex structures such as completely denudated rat cornea, even in particularly impaired healing condition.

Methods

Drugs

Pentadecapeptide BPC157 (GEPPPGLPAAAAGLV, M.W. 1419) (manufactured by Diagen, d.o.o., Ljubljana,

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Slovenia) is a partial of sequence of human gastric juice protein BPC, freely soluble in water at pH 7.0 and in saline. It was prepared as described before[4–10]. Peptide with 99% (HPLC) purity (1-des-Gly peptide as impurity), dissolved in distilled water was used in all of the experiments[4–10].

Experimental procedure

Male Wistar Albino rats, 200 g body weight, fed a stock diet with water ad libitum randomly assigned are used in all of the experiments. All of the experiments are approved by local Ethic Committee. Under deep anesthesia (ketamine [Ketalar, 50 mg/kg i.p.]) topical anesthetic tetracaine (2 drops; Tetrakain, Pliva, Croatia) to further inhibit possible eyelids reflex and to soften the corneal epithelium is given prior total debridement of corneal epithelium as described before[12]. Briefly, abrasion is made with the scalpel blade unilaterally under operating microscope, and the lesions stained with standard fluorescein solution for better visualization and photographed with a magnification camera.

Medication

Medication (distilled water for control group) or BPC 157 2pg/ml, 2ng/ml, 2µg/ml, 2 drops/rat eye) is started immediately after injury induction, every 8 hours up to 40 hours (i.e., at 0 h, 8 h,16 h, 24 h, 32 h, and 40 h).

Assessment of lesions and data analysis

The healing process was monitored by photography of the fluorescein stained cornea before application or sacrifice (48 hours following lesions induction). The erosion area (stained green) was determined by morphometrical analysis using a special program SFORM of VAMSTECH-Software Company (VAMSTECH, Zagreb, Croatia). The size of the de-eptihelized area is expressed as a percentage of the total corneal area.

Statistical analysis

The difference among groups was analysed with ANOVA (Analysis of Variance) and was considered significant at $p < 0.05$. The in-beetween group comparison was analysied with Tukey HSD test and was considered significant at $p < 0.05$.

Results

Total debridement of corneal epithelium leads to completely denudated cornea, confirmed by histological examination, and a complete fluorescein stained area in all rats before therapy. Through 48 hour period a steady recovery is noted in controls. However, a significant proportion (i.e. 15%) still remains green.

Compared with the control values, recovery is markedly accelerated in BPC treated groups (Figure 1). Since the earliest interval completely denudated rat corneas presented with positive outcome following µg- or ng-topical regimen of pentadecapeptide BPC 157. Of note, unlike control lesion present also after 48 hours, these lesions disappear already following 40 hours (µg) or 48 hours (ng) post-injury. (Figures 2–7).

Discussion

Regardless the general knowledge that the healing is always the same, corneal transparency provides particu-

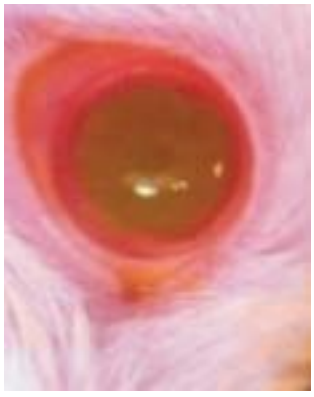

Fig. 2. Characteristic eye presentation in controls 8 hours after the entire epithelium removal. The defect area was stained green by flourescein while the 10 % of the remaining corneal surface showed spontaneous marginal epithelial recovery.

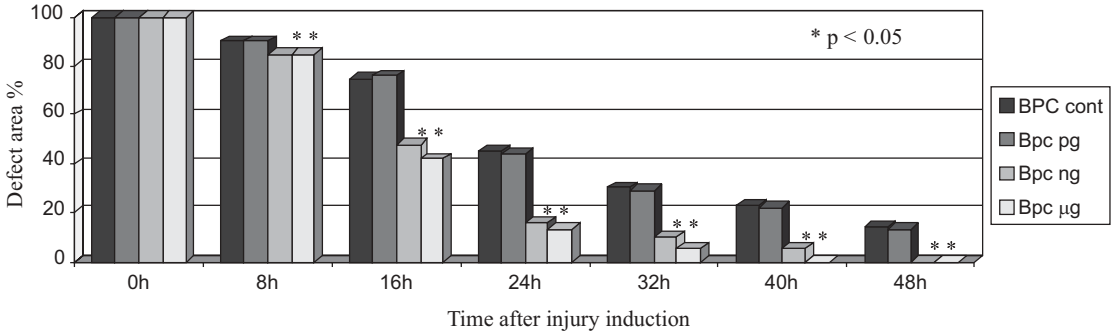

Fig. 1. BPC given in eye drops (µg and ng concentrations) immediately after injuries were induced, and in 8 hour intervals significantly improved corneal epithelial defects healing when compared to controls and pg BPC treated groups. (p<0.05).*

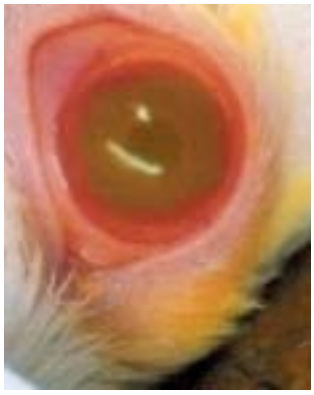

Fig. 3. Characteristic eye presentation in BPC 157 treated eye 8 hours after the entire epithelium removal. The defect area was stained green by flourescein while the 15 % of the remaining corneal surface showed promoted marginal epithelial recovery by BPC 157 administered in µg concentration solution. The defect area was significantly smaller compared to controls.

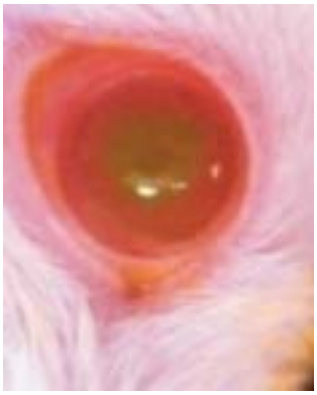

Fig. 4. Characteristic eye presentation in controls 32 hours after the entire epithelium removal. The defect area was stained green by flourescein while the 70 % of the remaining corneal surface showed spontaneous epithelial recovery coming from the edges.

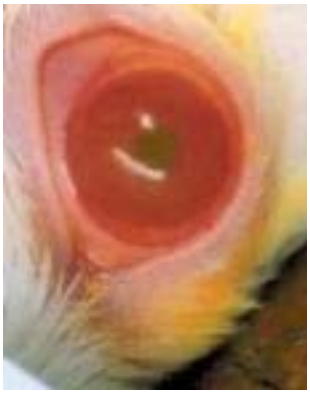

Fig. 5. Characteristic eye presentation in BPC 157 treated eye 32 hours after the entire epithelium removal. The defect area was stained green by flourescein while the 95 % of the corneal surface showed promoted epithelial recovery by BPC 157 administered in µg concentration solution. The defect area was significantly smaller compared to controls.

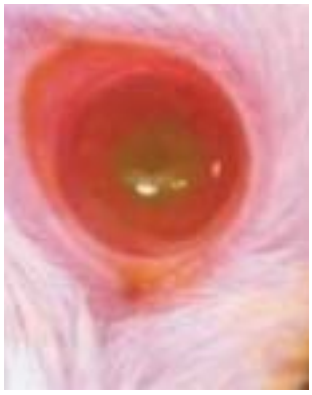

Fig. 6. Characteristic eye presentation in controls 40 hours after the entire epithelium removal. 77 % of the corneal surface showed spontaneous epithelial recovery.

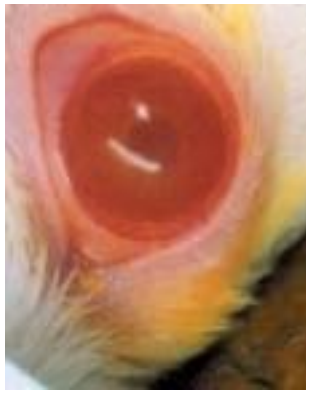

Fig. 7. Characteristic eye presentation in BPC 157 treated eye 40 hours after the entire epithelium removal. The corneal surface was completely healed showing a µg BPC 157 healing effect unlike controls which are still presenting with epithelium defect.

lar limitation. Namely, transparency is essential for the maintenance of visual function. Consequently, activated healing after anatomic barrier disruption presenting with remodeling processes predisposes the tissue to stromal ulceration and/or causes stromal opacification. Ultimately, the otherwise positive healing process paradoxically leads to irreversible visual deficit[2]. The transparency necessitates the flawless integrity of all its components: epithelium, stoma and endothelium[2]. This obviously provides particular requests to an agent suppose to accelerate complex corneal healing processes. Unlike our previous study confined to only small corneal lesion[3], the entire epithelium is removed, and the total cornea denudated. Like in the case of other tissues healing, pentadecapeptide BPC 157 alone (i.e., without any carrier) accelerates repair of corneal lesion. Therefore, since it accelerates also complex corneal healing and preserves transparency, it purposefully accelerates the healing process, presenting with no limitation due to angiogenesis shown in other models. Since acceleration is noted in different conditions (i.e., early phase [imme-

diate application following injury], as well as delayed phase [application following injury]), pentadecapeptide BPC 157 presented with a healing effect of its own.

BPC 157 could be efficient also *in vivo* as a growth--regulating factor. Besides, BPC 157 may alleviate all sequence of the healing events since these processes improved over control and/or standard agents values had already been shown in pentadecapeptide BPC 157 studies[4–10]. Specifically, besides angiogenesis in Szabo's angiogenesis model (i.e., synthetic sponge implantation[8], evidenced are advanced collagen, reticulin and blood vessels formation, increased tensile breaking force (skin incision wounds[6], deep partial thickness skin burns[4], raised bursting pressure (colon-colon anastomoses[10]. An advanced healing in special and complex conditions is also shown (i.e., healing of segmental osteoperiosteal bone defect that otherwise does not heal[5]. Therefore, the prominent improvement of otherwise delayed healing of completely denudated rat cornea is within the framework of its healing effect. Therefore, since it accelerates also complex corneal healing and preserves transparency, it purposefully accelerates the healing process, presenting with no limitation due to angiogenesis shown in other models. Since acceleration is noted in different conditions (i.e., early phase [immediate application following injury], as well as delayed phase [applications following injury]), pentadecapeptide BPC 157 presented with a healing effect of its own.

Corneal conditions may provide particular problems related to generally impair medication accessibility, and therapeutic efficacy. However, as mentioned, pentadecapeptide BPC 157 had been already seen to be effective besides given systemically (i.p. or i.m.)[4,5], also locally in alike complex structures healing. The impaired healing presented with deep partial thickness skin burn[4], non--union model[5] is consistently positively affected by local pentadecapeptide BPC 157 administration. The repair of completely denudated cornea is accelerated without carrier addition, like other healing effects[4–10], along with suggested unusual stability, and high resistance to otherwise highly degrading media[4,9].

Human gastric pentadecapeptide BPC157 when applied locally significantly speeds up the corneal epithelial defects healing in rats. The results were dose dependent.

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GASTRIČNI PENTADECAPEPTID BPC 157 UBRZAVA CIJELJENJE ROŽNIČNIH EPITELNIH EROZIJA U ŠTAKORA

SAŽETAK

Istraživan je utjecaj humanog gastričnog pentadekapeptida BPC 157 na zacijeljivanje rožničnih epitelnih defekata u štakora. Denudacija rožničnog epitela učinjena je unilateralno u 48 štakora, u četiri grupe (N=12), te su lezije obojene i fotografirane. Životinje su primale slijedeću terapiju: destiliranom vodom (kontrole) ili BPC 157 u konc.

2 pg/ml, 2 ng/ml, 2 μg/ml u dozi od 2 kapi po oku odmah nakon ozlijede, svakih 8 sati sve do 40 sati (0, 8, 16, 24, 32, 40 h). Lezije su fotografirane prije aplikacije i prije žrtvovanja (nakon 48 h). Veličina rožničnog defekta analizirana je specijalnim programom. U kontrola je uočena polagana epitelizacija kroz 48-satni period. Oporavak je bio znantno ubrzan u očima koje su primale lokalnu terapiju s BPC 157 u μg- ili ng koncentracijama ($p < 0,05$). Za razliku od kontrola, lezije su u cijelosti epitelizirale nakon 40 h (μg) ili 48 h (ng) nakon ozlijede. Ovisno o dozi, BPC 157 pokazao se efektivan u stimuliranju epitelizacije rožnice u štakora.