Gastric Pentadecapeptide BPC 157 Promotes Corneal Epithelial Defects Healing in Rats

Ratimir Lazić¹, Nikica Gabrić¹, Iva Dekaris², Damir Bosnar², Alenka Boban-Blagač³ and Predrag Sikirić³

¹ Eye Clinic »Svjetlost«, Zagreb, Croatia
² University Department of Ophthalmology, General Hospital »Sveti Duh«, Zagreb, Croatia
³ Department of Pharmacology, School of Medicine, University of Zagreb, Zagreb, Croatia

ABSTRACT

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 performed 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 healing³. 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 locally⁴–¹⁰. Initially, BPC 157 opposes a variety of gastrointestinal lesions including stress-ulcer and thermal injury-gastric lesions⁸,¹⁰, and thereafter it cures, besides corneal⁵, different wounds (i.e., skin⁶, colon-colon anastomosis¹⁰, deep skin burn¹, segmental osteoperiosteal bone defects⁸). Also, it modifies NO-synthesis⁵. This may directly affect repair of connective tissues⁵, as an ordered multistage process involving inflammation.

Besides, this gastric pentadecapeptide BPC 157 likely controls functions of collagen fragments⁵. 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). Finally, no carrier is used in previous⁴–¹⁰ and present studies. Together, this stable pentadecapeptide highly resistant to otherwise inescapable degradation of peptides, presented with healing potential of its own⁴–¹⁰, 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,
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 before4–10. Peptide with 99% (HPLC) purity (1-des-Gly peptide as impurity), dissolved in distilled water was used in all of the experiments4–10.

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 before12. 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-epithelized 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-between group comparison was analysed 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-
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. The transparency necessitates the flawless integrity of all its components: epithelium, stoma and endothelium. 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, 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...
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 studies4–10. Specifically, besides angiogenesis in Szabo’s angiogenesis model (i.e., synthetic sponge implantation), evidenced are advanced collagen, reticulin and blood vessels formation, increased tensile breaking force (skin incision wound), deep partial thickness skin burns, raised bursting pressure (colon-colon anastomoses). An advanced healing in special and complex conditions is also shown (i.e., healing of segmental osteoperiosteal bone defect that otherwise does not heal). 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.), also locally in alike complex structures healing. The impaired healing presented with deep partial thickness skin burn, non-union model is consistently positively affected by local pentadecapeptide BPC 157 administration. The repair of completely denuded cornea is accelerated without carrier addition, like other healing effects4–10, along with suggested unusual stability, and high resistance to otherwise highly degrading media.

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

REFERENCES

2 pg/ml, 2 ng/ml, 2 µg/ml u dozi od 2 kapi po oku odmah nakon ozljede, 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 znatno 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 ozljede. Ovisno o dozi, BPC 157 pokazao se efektivan u stimuliranju epitelizacije rožnice u štakora.