ELSEVIER

Burns 31 (2005) 310–315

BURNS

www.elsevier.com/locate/burns

The stable gastric pentadecapeptide BPC 157, given locally, improves CO_2 laser healing in mice

M. Bilic[a,b,c], Z. Bumber[a,b,c], A. Boban Blagaic[a,b,c], L. Batelja[a,b,c], S. Seiwerth[a,b,c], P. Sikiric[a,b,c,*]

[a]*Department of Pharmacology, Medical Faculty, University of Zagreb, Salata 11, P.O. Box 916, 10000 Zagreb, Croatia*
[b]*Department of Pathology, Medical Faculty, University of Zagreb, 10000 Zagreb, Croatia*
[c]*Department of Otorhinolaryngology, Medical Faculty, University of Zagreb, 10000 Zagreb, Croatia*

Accepted 5 October 2004

Abstract

The stable gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV; mol. wt. 1419), which is at present in phase II clinical trials for the treatment of inflammatory bowel disease, has been shown to counteract healing impairment by systemic corticosteroids in burned mice, both in vivo and in vitro, in the absence of carrier or protease inhibitor. Because of the particular healing problems associated with laser use, we have now studied the effect of pentadecapeptide BPC 157 on CO_2 laser injuries (Sharplan 1075 laser: 20 W, distance 12.5 cm, spot size 0.8 mm and exposure time 1 s) created on the dorsal skin of anaesthetised male NMRI–Hannover mice. The injury was either not treated or treated by topical application of a thin layer of neutral cream containing pentadecapeptide BPC 157 (1 μg, 1 ng or 1 pg (dissolved in saline)/g) or vehicle only, once daily, with the first application 60 min after injury and the last 24 h before killing (1, 7 and 21 days after the laser application). BPC 157 consistently improved healing after the CO_2 laser injury, both macroscopically and microscopically. The effect was produced with a simple method of application and favourable peptide stability (no carrier), and confirms the effectiveness of an ointment containing 1 μg BPC 157 (dissolved in saline)/g neutral cream.

Keywords: Stable gastric pentadecapeptide BPC 157; Cream; Laser injury; Improved healing; Mice

1. Introduction

While laser therapy is extensively used in wound healing and tissue repair, and promotes the production of various healing growth factors (e.g., vascular endothelial growth factor) [1], it also impairs specific parts of the healing process [2–4], to which there is as yet no satisfactory solution. Recent evidence [5–8] suggests that a stable gastric pentadecapeptide BPC 157 (PL-10, PLD-116, PL-14736; Pliva, Croatia), which shows promise and an absence of toxicity in clinical trials for the treatment of inflammatory bowel disease [9], and in the promotion of healing in ulcers and wounds (e.g., [5–8,10–15]), might also be useful in healing laser lesions. In particular, having remarkable stability [16], pentadecapeptide BPC 157, unlike other growth-factor peptides [17,18], consistently improved burn healing in mice (both microscopically and as measured by tensiometry), when given systemically or locally, and when applied alone, without carrier, counteracted the impairment of burn healing induced by systemic corticosteroids [5,6]. It also had other effects on corticosteroid-challenged animals. In post-burn stress gastric lesions, BPC 157 had an anti-ulcer effect of its own in non-corticosteroid treated mice and potentiated the anti-ulcer effect observed in mice treated with 6α-methylprednisolone. Pentadecapeptide BPC 157 also inhibited corticosteroid immunosuppression in vitro: splenic cells from burned animals treated with 6α-methylprednisolone had lower reactivity to mitogen than those of control, healthy animals, but the use of BPC 157 (1 μg/g cream) returned this reactivity to control values [5].

* Corresponding author. Tel.: +385 1 4566 833/34; fax: +385 1 492 00 50.
E-mail address: sikiric@mef.hr (P. Sikiric).

0305-4179/$30.00
doi:10.1016/j.burns.2004.10.013

These findings led us to study the effect of topical applications of pentadecapeptide BPC 157 on CO_2 laser injuries in mice.

2. Materials and methods

2.1. Drugs

Pentadecapeptide BPC157 (GEPPPGKPADDAGLV; mol. wt. 1419) (manufactured by Diagen, d.o.o., Ljubljana, Slovenia) is a partial sequence of the human gastric juice protein BPC. It is freely soluble in water at pH 7.0 and in saline. It was prepared as described before [5–8,10–15]. Peptide with 99% purity (1-des-Gly peptide as impurity) (as measured by high-performance liquid chromatography) dissolved in saline [5–24,28] was used in all of the experiments. Neutral cream (Belobaza; Ljekarna Zagreb, Croatia) was purchased from commercial sources.

2.2. Animals

Randomly assigned male mice (NMRI–Hannover, 30 g body wt.) housed individually in separate cages, with free access to food and water, and given no special fluid resuscitation after injury induction, were used in all experiments. The local animal research committee approved the protocol. Ten mice were used in each of the experimental groups described next.

2.2.1. Experimental protocol

A CO_2 laser (Sharplan 1075; 20 W, distance 12.5 cm, spot size 0.8 mm and exposure time 1 s) was used to create an injury on the dorsal skin of anaesthetised mice [5,6]. The injury was then either not treated or treated by the topical application of a thin layer of neutral cream containing either pentadecapeptide BPC 157 at various concentrations, or nothing (vehicle only). As in our earlier studies on burned mice [5,6], the medication was applied once daily, with the first application 60 min after injury and the last 24 h before killing. Pentadecapeptide BPC 157 creams (prepared as 1.0 μg, 1 ng or 1 pg (dissolved in distilled water)/g commercial neutral cream, without carrier or peptidase inhibitor) were applied topically in a thin layer; controls received either a topical application of commercial neutral cream (Belobaza) or no treatment. Lesions were assessed 1, 7 and 21 days after the injury.

2.2.2. Lesion assessment

In general, lesions were assessed as in our earlier studies [5,6]. Macroscopically, gross lesion severity was gauged by the depth of the wound crater (mm), the area of necrosis (day 1) and the wound area (days 1, 7 and 21) (in mm^2). Microscopically, the factors investigated included oedema, blood vessel formation and their total diameter, the number of preserved hair follicles, reticulin and collagen formation, the number of inflammatory cells and the number of mice with complete re-epithelisation (for details, see [5,6]). Standard histological sections were stained with haematoxylin–eosin (for vessels, oedema, necrosis and inflammatory cells), Gomori silver (for reticulin) and Van Gieson (for collagen). The factors assessed were, according to day of killing, as follows. Day 1: oedema, number and diameter of blood vessels, inflammatory cells and reticulin; day 7: number and diameter of blood vessels, necrosis, number of vital follicles, reticulin and collagen; day 21: number and diameter of blood vessels, epithelisation and collagen. In particular, oedema, angiogenesis and inflammatory cells were assessed according to our recent investigation [5,6]. Necrosis was scored on a modified Suzuki scale (magnification ×25) [5,6]. The number of preserved hair follicles was counted in three microscopic fields (magnification ×25) situated in the middle of the injured area and in the deepest layer of follicles. The development of epithelisation was expressed as the ratio of the number of animals to the number with incomplete epithelisation for each group [5,6]. Reticulin and collagen were described as percentages in two microscopic fields (magnification ×40) at both margins of the injured area between the deepest follicles and the muscular layer.

Mice subjected to the same initial procedures (i.e., careful shaving of the back 24 h before experimentation to avoid skin damage; anaesthesia at the time of experimentation), but not injured by laser, were used as a sham-operated group.

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

2.3. Statistical analysis

Fisher's exact probability test (two-tailed) was used on data for the presence/absence of re-epithelisation. Non-parametric analysis of variance (Kruskall–Wallis one-way ANOVA by ranks) and the post hoc Wilcoxon rank-sum test were used for differences in depth of wound crater, necrotic and wound area, oedema, blood vessel formation and their total diameter, number of preserved follicles, reticulin and collagen formation, and number of inflammatory cells. A Bonferroni downward adjustment of the α-level was made to compensate for multiple comparisons, based on the number of comparisons needed for full evaluation of each experiment.

3. Results

A consistent injury was induced in all mice subjected to laser, but this was modified dose-dependently if they had been treated with pentadecapeptide BPC 157 cream (Tables 1–3). Mice injured and then either untreated or treated with vehicle only showed no difference in any