European Journal of Pharmacology 764 (2015) 346–352

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Molecular and cellular pharmacology

The pro-healing effect of exendin-4 on wounds produced by abrasion in normoglycemic mice

Stefano Bacci [a], Annunziatina Laurino [b], Maria Elena Manni [b], Elisa Landucci [c], Claudia Musilli [b], Gaetano De Siena [c], Alessandra Mocali [d], Laura Raimondi [b,*]

[a] Department of Clinical and Experimental Medicine, Research Unit of Histology and Embriology, University of Florence, 50139 Florence, Italy
[b] Department of NEUROFARBA, Section of Pharmacology, University of Florence, 50139 Florence, Italy
[c] Department of Health Sciences, Section of Pharmacology, University of Florence, 50139 Florence, Italy
[d] Department of Experimental and Clinical Biomedical Sciences, Section of Experimental Pathology and Oncology, University of Florence, 50134 Florence, Italy

ARTICLE INFO

Article history:
Received 28 April 2015
Received in revised form
25 June 2015
Accepted 26 June 2015
Available online 8 July 2015

Keywords:
Wound healing
Exendin-4
Exendin-4(9-39)
Angiogenesis
Glucagon-like type 1 receptor

ABSTRACT

Experimental evidence suggested that Exendin-4 (Exe4), an agonist at glucagon like receptor-1 (GLP-1R), promoted tissue regeneration. We aimed to verify the effect of Exe4, in the absence or in the presence of Exendin-4(9-39), an antagonist at GLP-1R, on the healing of abraded skin.

Two wounds (approximately 1.1 × 1.1 cm^2; namely "upper" and "lower" in respect of the head) were produced by abrasion on the back of 12 mice, which were then randomly assigned to receive an intradermal injection (20 μl) of

Group 1: saline (NT) or Exe4 (62 ng) in the upper and lower wound respectively;

Group 2: Exendin-4(9-39) (70 ng) in the upper and Exendin-4(9-39) (70 ng) and, after 15 min, Exe4 (62 ng) in the lower wound.

Wounds were measured at the time of abrasion (T0) and 144 h (T3) afterward taking pictures with a ruler and by using a software. The inflammatory cell infiltrate, fibroblasts/myofibroblasts, endothelial cells and GLP-1R expression, were each labeled by immunofluorescence in each wound, pERK1/2 was evaluated by Western-blot in wound lysates.

At T3, the percentage of healing surface was 53% and 92% for NT and Exe4 wounds respectively and 68% and 79% for those treated with Exendin-4(9-39) and Exendin-4(9-39)+Exe4 respectively. Exe4, but not Exendin-4(9-39) induced quantitative increase in fibroblasts/myofibroblasts and vessel density when compared to NT wounds. This increase was not evident in wounds treated with Exendin-4(9-39)+Exe4.

Exe4 promotes wound healing opening to the possible dermatological use of this incretin analogue.

1. Introduction

Exendin-4 (Exe4) is a natural peptide sharing 53% homology with Glucagon-like peptide-1 (GLP-1), the insulinotropic intestinal peptide belonging to the incretin hormones. Exe4 activates GLP-1 receptor (GLP-1R) thus mimicking most of GLP-1 effects including the ability to induce receptor phosphorylation and internalization (Roed et al., 2013), two mechanisms ensuring a continuous recycling from intracellular stores towards the plasma membrane (Widmann et al., 1995). Since the insulinotropic activity of GLP-1 was found dysregulated in diabetic patients (Meier and Nauck, 2010), a synthetic version of Exe4, exenatide, became recently part of an innovative anti-diabetic therapy (incretin analogues). The advantages for diabetic patients taking this drug include almost the null risk of experiencing hypoglycemia, the reduction of body weight gain, the protection against cardiovascular and neurological complications, the amelioration of foot ulcer healing and an improvement of psoriasis (He et al., 2013; Mannucci and Dicembrini, 2012; Drucker and Rosen 2011). Until now, it is unknown whether these effects are secondary to the correction of hyperglycemia or if they depend on activation of extra-pancreatic GLP-1R thus opening the possibility that Exe4 may have clinical usefulness beyond the correction of hyperglycemia.

Small chemical modifications on Exe4 primary structure produced Exendin-4(9-39), a peptide which is devoid of intrinsic activity at the GLP-1R (Al-Sabah et al., 2014) and consequently considered to be an antagonist. The binding of Exe4 and of

Abbreviations: GLP-1, Glucagon like peptide-1; GLP-1R, Glucagon like peptide-1 receptor; Exe4, Exendin-4

* Corresponding author. Fax: +39 0554271280.
E-mail address: laura.raimondi@unifi.it (L. Raimondi).

http://dx.doi.org/10.1016/j.ejphar.2015.06.056
0014-2999/

Exendin-4(9-39) at the GLP-1R would be mutually exclusive (Donnelly, 2012). Overall, Exe4 and Exendin-4(9-39), represent important experimental tools for investigating the pharmacology of GLP-1R.

Recent experimental evidence suggest a possible role for Exe4 in the promotion of tissue regeneration. It was reported that Exe4 was able to stimulate endothelial cell migration (Kang et al., 2013), to inhibit apoptosis (Favaro et al., 2012; Khang et al., 2013), and to reduce inflammation and oxidative stress in myocardial post-conditioning (Du et al., 2014) Interestingly, all these effects were prevented by Exendin-4(9-39).

We hypothesized a possible role for Exe4 in wound healing, a pro-survival event consisting of functionally distinct and temporally overlapping processes involving the coordinated activity of multiple cell types s, orchestrated by growth factors, cytokines and extracellular matrix components to avoid the chronicization of the wound and the risk of life treating sepsis (Gurtner et al., 2008; Schultz et al., 2011).

Since GLP-1R is expressed at mouse skin (List et al., 2006), we decided to investigate the differences, if any, of treating wounds by abrasion with saline (NT) or with a single high dose of peptides which interact at GLP-1R including Exe4, an agonist, Exendin-4(9-39), an antagonist or a combination of both.

2. Material and methods

2.1. Animals

Male mice (CD1 strain) from the Harlan lab (MI, Italy) were used. Five mice were housed per cage and placed in the experimental room 24 h before use. The animals were kept at 23 ± 1 °C with a 12 h light–dark cycle (light on at 07:00 h) and were fed a standard laboratory diet with water ad libitum. Experiments and animal use procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 80-23, revised 1996). The experimental protocols were approved by the Animal Care Committee of the Department of Pharmacology, University of Florence, in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS no. 123) and the European Communities Council Directive of 24 November 1986 (86/609/EEC). The authors further attest that all efforts were made to minimize the number of animals used and their suffering.

2.2. Experimental protocol

Mice (35.33 ± 1.45 g body weight; means ± S.E.M of 12 mice) were anesthetized with a single intra-peritoneal injection of ketamine (80 mg/kg body weight)/xylazine (10 mg/kg body weight). After surgical anesthesia, the back of these mice was shaved and disinfected with 70% ethanol.

Two circular wounds (approximately 1.1 × 1.1 cm^2) at a distance of about 1 cm apart, were produced by one single researcher on the back of 12 animals, referred as “upper” and “lower” depending on their location with respect to the head of the mouse (see Fig. 1; Immonen et al., 2014). Dermabrasion was performed using commercial sandpaper (KWH Mirka Ltd., Jeppo, Finland; grain size 68 μm). The animals were exposed to dermabrasion for about 15 s until the complete removal of the epidermis but avoiding deep wounds. During the treatment the animals were kept on a bed at a constant temperature (37.5 °C) to reduce stress due to anesthesia. Immediately after, a small amount of Streptosil was applied topically to prevent the onset of infections. The wound was left uncovered during the whole period of experiments. All the animals

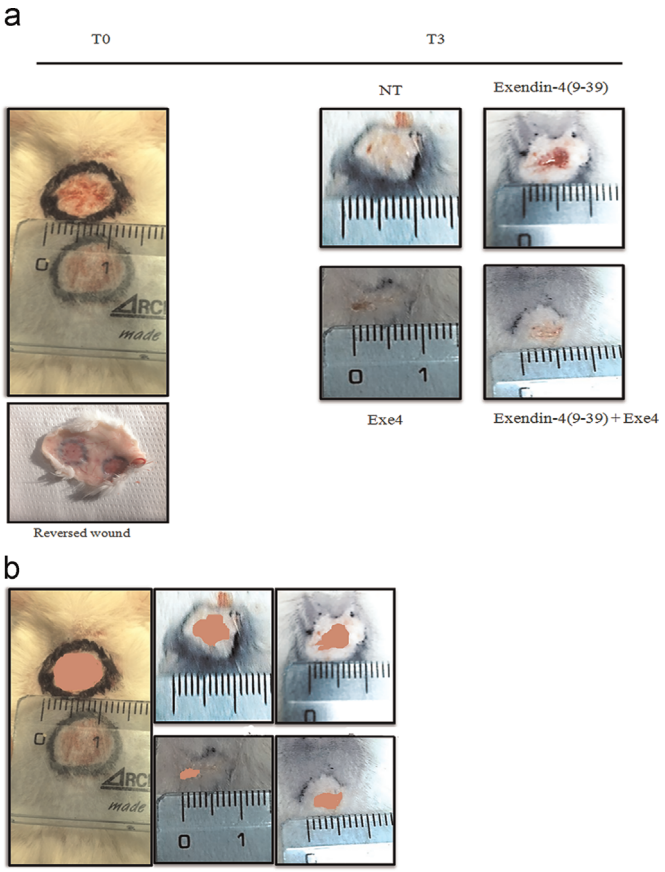

Fig. 1. The effect of treatment on wound healing. Wounds from abrasion were produced on the back of normoglycemic mice and injected intradermically with 20 μl of the drugs as described in Section 2. Panel A: pictures from mice after abrasion (T0) and at the end of the experiment (T3). Panel B: pictures of wounds from mice killed at T0 and T3 whose area was filled by a software (pixels).

were then housed individually to prevent traumatic damage to the wounds by other mice.

Another researcher was assigned to treat animals as it follows: mice were randomly divided in two groups and their wounds received the following treatments:

Group 1 ($n=6$): 20 μl of saline solution (NT) or of Exe4 (62 ng; Sigma-Aldrich, St. Louis, MO, USA) were injected in the dermis of the upper and the lower wound respectively;

Group 2 ($n=6$): 20 μl of Exe4(9-39) (Sigma-Aldrich, St. Louis, MO, USA) or Exe4(9-39) followed by 20 μl of Exe4 (62 ng; Sigma) after 15 min in the dermis of the upper and the lower wound respectively.

The intradermal route was chosen because the epithelium was discontinuous and inflamed after abrasion. The dose of Exe4 used (62 ng) was similar to that used by Du et al. (2014), it was within the safety range in rodents (FDA, Application no. 21-773) and it aimed to produce a drug reserve at the site of the injury. The presence of a small swelling at the injection site was taken as an indication that the treatment was performed successfully.

Two animals from both groups were killed by cervical dislocation at the time of abrasion (T0). Pictures were taken by using a ruler to measure the initial size of the wounds. The remaining animals from Group 1 and 2 ($n=4$) were killed 144 h (T3) after abrasion. Pictures were again taken using a ruler to measure the size of the wounds. The wounds removed always included the complete epithelial margins and the scab, when present. Each wound portion was codified and stored at −80 °C until used for biochemical and histological evaluations. All measurements were performed at the end of the experiment in blinded fashion by