Annals of Anatomy 205 (2016) 1–8

Contents lists available at ScienceDirect

Annals of Anatomy

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

RESEARCH ARTICLE

Somatostatin supports corneal wound healing in vivo

Ulrike Hampel[a,*], Paul Frömmling[a], Lars Bräuer[a], Ivonne Schaefer[b], Saadettin Sel[c], Detlev Holland[d], Friedrich Paulsen[a]

[a] Department of Anatomy II, Friedrich Alexander University Erlangen Nürnberg, Erlangen, Germany
[b] Department of Dermatology and Allergology, HELIOS Klinikum Erfurt, Germany
[c] University Medical Center for Ophthalmology, Ruprecht Karl University Heidelberg, Heidelberg, Germany
[d] Eye Clinic Bellevue, Kiel, Germany

ARTICLE INFO

Article history:
Received 2 December 2015
Received in revised form 2 January 2016
Accepted 6 January 2016

Keywords:
Somatostatin
Octreotid
Cornea
Migration
Proliferation
Wound healing
VEGF

ABSTRACT

Purpose: To evaluate the influence of somatostatin (SST) and its analog octreotid (Oct) on corneal wound healing processes.
Methods: The wound healing rate in C57BL/6 mice eyes under SST and Oct treatment was analyzed using an alkali-induced corneal wounding model. Effects of SST and Oct on cell proliferation, migration and quantified protein expression of vascular endothelial growth factor (VEGF) on human corneal epithelial cells (HCE, cell line) were evaluated by means of electric cell-substrate impedance sensing, scratch migration assays and ELISA. ERK1/2 and p38 phosphorylation was investigated by semi-quantitative western blot analysis.
Results: Ten nanograms per microliters of SST significantly accelerated the wound closure rate of corneal defects *in vivo*. SST and Oct had no influence on HCE cell proliferation and migration and did not activate ERK1/2 or p38 signaling in HCE cells. However, there was increased VEGF protein expression in cytosolic proteins and medium supernatants of HCE upon Oct stimulation for 24 h. One and 10 ng/ml Oct led to a 2.5-fold and 100 ng/ml Oct to a 4-fold upregulation of VEGF protein expression.
Conclusion: The data implicate that SST promotes corneal wound healing in a mouse model. However, using a HCE cell line *in vitro*, the wound healing mechanism does not seem to be supported by proliferation and migration processes or by activation of ERK1/2 and p38 signaling pathways. Other possible mechanisms could be the activation of other pathways and the induction of growth factors such as VEGF that modulate the observed corneal wound healing process.

1. Introduction

The maintenance and health of the ocular surface is regulated, among other factors, by hormones such as testosterone and estrogen (Sullivan, 2004). In 2008, the hormone somatostatin (SST) was detected in the tear film adding it to the list of putative hormones influencing the ocular surface (Minsel et al., 2009). Although SST amplificates were found in different tissues of the ocular surface and lacrimal apparatus, the main source is probably the lacrimal gland (Minsel et al., 2009). SST is a cyclic polypeptide consisting of either 14 or 28 amino acids (Patel et al., 1997) obtained by posttranslational modification of preprosomatostatin. Due to a stabilized conformation, SST analogs such as octreotid (Oct) are more potent and resistant to degradation and, therefore, more suitable for clinical applications (Bauer et al., 1982).

SST acts *via* 5 G protein-coupled receptors, named SSTR1-5 (Patel and Srikant, 1997). According to their pharmacological functions, SSTRs can be divided into SRIF1 and SRIF2 receptors. SRIF1 comprising SSTR2, 3 and 5, bind SST analogs (octreotid and lanreotid) with high affinity. In contrast SST analogs bind with lower affinity to SRIF2 receptors SSTR1 and 4. SSTR1, SSTR2 and SSTR5 were detected in lacrimal glands, nasolacrimal duct and conjunctiva (Minsel et al., 2009). Corneal tissue expresses SSTR1 and SSTR2, but not SSTR5 (Klisovic et al., 2001; Minsel et al., 2009). Detection of SST in tear fluid and expression of SST and its receptors in tissues of the ocular surface raise the question of its functional significance.

Signaling pathways activated by SST are mitogen-activated protein kinases (MAPK), amongst them the subclasses p38 mitogen-activated protein (MAP) kinases and extracellular-signal-regulated kinases (ERKs) (Hagemeister and Sheridan, 2008; Hanson et al., 2010; Li et al., 2005; Wang et al., 2011). Regarding the

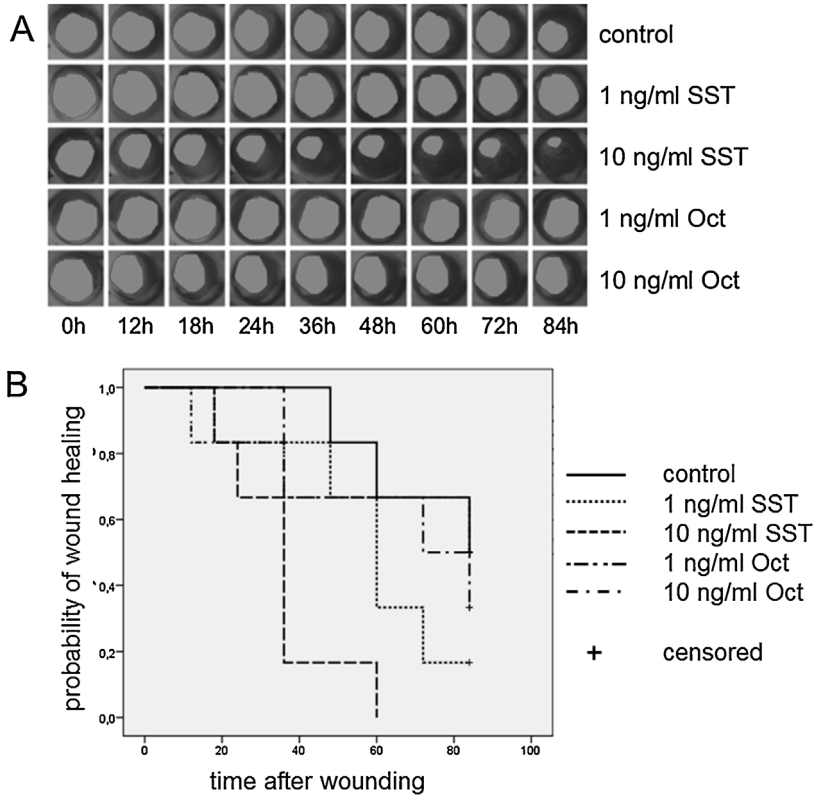
* Corresponding author. Tel.: +49 0 9131 8522266; fax: +49 0 9131 8522862.
E-mail addresses: uli.hampel@gmail.com (U. Hampel), friedrich.paulsen@fau.de (F. Paulsen).

http://dx.doi.org/10.1016/j.aanat.2016.01.001
0940-9602/

activation of p38 MAP kinase signaling at the ocular surface it has been shown that p38 MAP kinase signaling is necessary for migration of corneal epithelial cells into a defect area in the early phase of corneal wound healing (Saika et al., 2004). This is followed by a phase of elevated proliferation of cells to replace lost cells controlled by ERK1/2 signaling (Saika et al., 2004; Sharma et al., 2003).

Transparency of the cornea and therefore vision is impaired when vessels invade the cornea. Vascular endothelial growth factor (VEGF) stimulates angiogenesis of the cornea in a non-inflammatory mouse model (Kenyon et al., 1996) as well as vessel formation within the cornea under inflammatory conditions (Mimura et al., 2001; Philipp et al., 2000; Zheng et al., 2001) but VEGF and its receptors are also expressed and secreted on the healthy ocular surface including the cornea (Gebhardt et al., 2005). SST antagonized neovascularization of the cornea in a basic fibroblast growth factor induced model in mice (Wu et al., 2003). In rats, Oct inhibited corneal vessel formation (Danesi et al., 1997; Demir et al., 1999). However, the underlining mechanisms, e.g., *via* regulation of VEGF expression are unclear.

In this study we use a corneal alkali burn mouse model to elucidate the impact of SST and Oct on corneal wound healing *in vivo*. Afterwards we aimed to clarify whether migration and proliferation, important processes in wound healing, are influenced by SST and Oct. Furthermore, we questioned whether ERK and p38 MAP kinase signal transduction is activated by SST and Oct in corneal epithelial cells and, therefore, may influence wound healing processes. Impaired wound healing is often accompanied by neovascularization. We identify the role of SST and Oct in VEGF expression of corneal epithelial cells.



C Kaplan-Meier analysis (50% reduction of the wound area (t=84) in comparison to the wound area at the beginning of the experiments (t=0))

Log rank (p value)	1 ng/ml SST	10ng/ml SST	1 ng/ml Oct	10 ng/ml Oct
control	0,177	**0,004**	0,866	0,528
1 ng/ml SST		0,061	0,309	0,419
10 ng/ml SST			**0,045**	**0,013**
1 ng/ml Oct				0,680

Fig. 1. Corneal re-epithelialization upon alkali wounding and treatment with 1 ng/ml or 10 ng/ml SST or Oct. (A) Photographs show representative fluorescein-stained corneal wounds of the control, 1 ng/ml SST, 10 ng/ml SST, 1 ng/ml Oct and 10 ng/ml Oct groups at different time points during the healing process. The wound areas are shown in the images. (B) Kaplan–Meier curves show the probability of corneal wound healing at different time points in control, 1 ng/ml SST, 10 ng/ml SST, 1 ng/ml Oct and 10 ng/ml Oct groups. Censored cases are indicated by small vertical tick marks. (C) 50% reduction of the wound area ($t = 84$) in comparison to the wound area at the beginning of the experiments ($t = 0$) was used as event of the analysis. Corneal re-epithelialization was significantly enhanced by local application of 10 ng/ml SST.