European Journal of Pharmacology 834 (2018) 77–83

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Translational pharmacology

Expression of active matrix metalloproteinase-9 as a likely contributor to the clinical failure of aclerastide in treatment of diabetic foot ulcers

Trung T. Nguyen[a], Derong Ding[a], William R. Wolter[b], Matthew M. Champion[a], Dusan Hesek[a], Mijoon Lee[a], Rocio L. Pérez[a], Valerie A. Schroeder[b], Mark A. Suckow[b,1], Shahriar Mobashery[a], Mayland Chang[a,*]

[a] Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, USA
[b] Freimann Life Sciences Center and Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, USA

ARTICLE INFO

Keywords:
Matrix metalloproteinase-9
Aclerastide
Diabetic foot ulcers
Reactive oxygen species
Angiotensin II

ABSTRACT

Chronic wounds are a complication of diabetes. Treatment for diabetic foot ulcers is complex with little clinical recourse, resulting in 108,000 lower-limb amputations annually in the United States alone. Matrix metalloproteinases (MMPs) play important roles in the pathology and in the repair of chronic wounds. We previously identified active MMP-8 and MMP-9 in wounds of diabetic mice and determined that MMP-8 accelerates wound repair, while MMP-9 is the culprit for the diabetic wound being refractory to healing. Aclerastide, a peptide analog of angiotensin II, recently failed in phase III clinical trials for treatment of diabetic foot ulcers. We demonstrate herein that treatment of wounds of diabetic mice with aclerastide results in elevated levels of reactive oxygen species and of active MMP-9, which is likely an important contributor to the failure of aclerastide in clinical trials.

1. Introduction

Diabetes affects over 30 million individuals in the United States (Centers for Disease Control and Prevention, 2017). A challenging problem in diabetes is the inability of wounds to heal, with 25% of diabetic patients developing diabetic foot ulcers (DFUs) over their lifetimes (Rice et al., 2014). The standard-of-care for DFUs is debridement, off-loading, and treatment of wound infections with antibiotics. There is a single FDA-approved drug, becaplermin (Regranex™); however, it is not the standard-of-care in DFU treatment due to its modest efficacy and black-box warning (Margolis et al., 2005; Ziyadeh et al., 2011). The dearth of treatment options for DFUs results in 108,000 lower-limb amputations annually in the United States (Centers for Disease Control and Prevention, 2017) and prognosis with approximately 50% mortality within one year of amputation (Fortington et al., 2013).

Matrix metalloproteinases (MMPs) play important roles in both the pathology and repair of chronic wounds (Chang, 2016; Nguyen et al., 2016). The molecular basis of why the diabetic wound is recalcitrant to healing and which MMP(s) accelerates wound healing is not fully understood (Nguyen et al., 2016). There are 24 human MMPs and each MMP exits in three forms (Nguyen et al., 2016), of which only the active unregulated MMPs play catalytic roles in physiological and pathophysiological processes. The majority of the methods for profiling MMPs cannot distinguish among the three forms of MMPs (Fisher and Mobashery, 2010). In addition, these methods often require screening for a specific MMP rather than "fishing" out and identifying the active MMP(s) that plays critical roles in repair and disease.

To address this challenge, we developed an affinity resin (Hesek et al., 2006) that binds only to active MMPs, which are then identified by mass spectrometry (Gooyit et al., 2014). Using this affinity resin we identified active MMP-8 and MMP-9 in wounds of *db/db* mice (Gooyit et al., 2014). We documented that MMP-9 was detrimental to wound healing and that MMP-8 played a beneficial role in repairing the wound (Gao et al., 2015; Gooyit et al., 2014). We also identified a small-molecule selective MMP-9 inhibitor referred to as ND-336 that accelerated diabetic wound healing (Gao et al., 2015). These findings for the first time identified MMP-9 as a target in DFUs, one that addressed the molecular basis of disease, rather than merely serving a palliative purpose.

We were keenly interested in a new drug for diabetic wound healing, aclerastide (NorLeu^3-angiotensin(1–7) or DSC127), which

* Corresponding author.
E-mail address: mchang@nd.edu (M. Chang).
[1] Present address: Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA.

https://doi.org/10.1016/j.ejphar.2018.07.014
Received 29 March 2018; Received in revised form 5 July 2018; Accepted 12 July 2018
0014-2999/ © 2018 Elsevier B.V. All rights reserved.

entered phase III clinical trials in 2013. Aclerastide is a peptide analog of angiotensin II, a growth factor (Vukelic and Griendling, 2014) that plays an important role in wound healing (Yahata et al., 2006). Aclerastide had shown superior efficacy over becaplermin in the *db/db* mouse model of wound healing (n = 5 mice per group), when given topically at 0.1 mg/wound/day for 5 days starting immediately after injury (Rodgers et al., 2003), but in November of 2015 it failed in phase III clinical trials for the treatment of DFUs, where it was applied once daily for several weeks.

Angiotensin II mediates cell growth by stimulating NADPH oxidase (Griendling and Ushio-Fukai, 2000), producing reactive oxygen species after tissue injury (Mehta and Griendling, 2007). Reactive oxygen species is known to promote activation of MMP-9 (Gu et al., 2002) via NF-κB regulation. We theorize that aclerastide might stimulate formation of reactive oxygen species, which would in turn upregulate MMP-9. In light of the detrimental role that we have documented for MMP-9 in diabetic wounds, we wondered if a contributor to the clinical failure could be the effect of aclerastide on MMP-9 upregulation.

2. Materials and methods

2.1. Compounds

Aclerastide (NorLeu^3-angiotensin(1–7)) was custom-synthesized by GenScript; purity 98.6% by HPLC. Peptide sequence Asp-Arg-NorLeu-Tyr-Ile-His-Pro was confirmed by MS/MS analysis on a Bruker micrOTOF/Q2 mass spectrometer. High-resolution mass spectra were measured using a Bruker micrOTOF/Q2 mass spectrometer in electrospray ionization (ESI). HRMS $[M + H]^+$ calcd for $C_{42}H_{65}N_{12}O_{11}$ 913.4890; found 913.4894. ND-336 was synthesized as previously described (Gao et al., 2015).

2.2. Animal model

Female *db/db* mice (BKS.Cg-*Dock7*^{m} $^{+/+}$ *Lepr*^{db}/J, 8-weeks old, about 40 g body weight) were purchased from Jackson Laboratory. Animals were fed 501 Laboratory Rodent Diet and were given water ad libitum. The mice were housed in polycarbonate cages (one animal per cage) containing corncob bedding (The Andersons Inc.) and maintained at 72 ± 2°F with a light/dark cycle of 12/12 h. All animal studies were conducted in accordance with the National Institutes of Health guide for the care and use of laboratory animals, and with approval and oversight by the Institutional Animal Care and Use Committee at the University of Notre Dame. All animal studies complied with the ARRIVE guidelines.

Mice were shaved in the dorsal area, prepared for aseptic surgery, and anesthetized with isoflurane in early morning. A single 8-mm diameter full-thickness excisional wound was created on the dorsal area using a biopsy punch (Miltex) and it was covered with Tegarderm dressing (3 M Company). The next day (day 1), the animals were randomly assigned to different experimental groups. A sample size of n = 7 animals per group was estimated to have 80% power to give 20% improvement in wound closure, with a statistical significance of 0.05. Body weight was monitored before and during the experiments; no significant weight loss (≥ 20% body weight) was observed. Mice were excluded from the studies if body weight was < 32 g or blood glucose was < 250 mg/dl, indicating that the animals were not diabetic. At specific time points or at the end of the study, mice were euthanized by isoflurane inhalation overdose, followed by cervical dislocation.

2.3. Aclerastide and ND-336 study

This study consisted of three groups of *db/db* mice (37.5 ± 3 g body weight): n = 13 mice for vehicle, n = 12 mice for aclerastide, n = 12 mice for ND-336 as positive control. Aclerastide and ND-336 were dissolved in water at a concentration of 1.0 mg/ml; the vehicle consisted of distilled sterile water. The aclerastide, ND-336, and vehicle solutions were sterile-filtered and stored at 4 °C. Dosing solutions were prepared freshly every 2 days and warmed to room temperature before dosing. Mouse wounds were topically administered 100 µl of aclerastide, ND-336, or vehicle solutions once a day in the morning for 14 days. The dose of aclerastide and ND-336 was equivalent to 0.1 mg/wound/day. Treatments were started one day post-injury (day 1) in the order: vehicle, aclerastide, and ND-336. Wounds were measured on days 0, 7, 10, and 14. Mice (n = 3 per group) were killed on day 7, the wounds were harvested and frozen in liquid nitrogen and stored at − 80 °C for gelatin zymography and affinity resin analysis.

Table 1
Quantification of active MMPs in mouse wound samples[a].

Q1 Precursor *m/z*	Q3 Product *m/z*	MMP-8 Peptide
873.92 $[M+2H]^{2+}$	959.56 $[M+H]^+$ y9	[C]GVPDSGDFLLTPGSPK
873.92 $[M+2H]^{2+}$	1218.64 $[M+H]^+$ y12	
873.92 $[M+2H]^{2+}$	**1430.72 $[M+H]^+$ y14**	
534.76 $[M+2H]^{2+}$	639.32 $[M+H]^+$ y5	DISNYGFPR
534.76 $[M+2H]^{2+}$	753.37 $[M+H]^+$ y6	
534.76 $[M+2H]^{2+}$	**840.40 $[M+H]^+$ y7**	
500.26 $[M+2H]^{2+}$	618.35 $[M+H]^+$ y6	FFSLAETGK
500.26 $[M+2H]^{2+}$	**705.38 $[M+H]^+$ y7**	
500.26 $[M+2H]^{2+}$	852.45 $[M+H]^+$ y8	
		MMP-9 Peptide
832.94 $[M+2H]^{2+}$	1033.57 $[M+H]^+$ y9	AFAVWGEVAPLTFTR
832.94 $[M+2H]^{2+}$	1090.59 $[M+H]^+$ y10	
832.94 $[M+2H]^{2+}$	**1276.67 $[M+H]^+$ y11**	
622.34 $[M+2H]^{2+}$	704.41 $[M+H]^+$ y6	GSPLQGPFLTAR
622.34 $[M+2H]^{2+}$	**761.43 $[M+H]^+$ y7**	
622.34 $[M+2H]^{2+}$	889.49 $[M+H]^+$ y8	
549.00 $[M+3H]^{3+}$	642.4 $[M+H]^+$ y6	LGLGPEVTHVSGLLPR
549.00 $[M+3H]^{3+}$	979.6 $[M+H]^+$ y9	
549.00 $[M+3H]^{3+}$	**767.4 $[M+H]^+$ b8**	

[a] Quantification was done using three peptides per proteinase. In the first quadrupole (Q1), precursor *m/z* ions are selected and fragmented in second quadrupole (Q2), where a specific fragment is monitored in the third quadrupole (Q3). Denoted in bold in Q3 are the fragments used as 'quantifier' for each proteinase. The fragments in Q3 are assigned with conventional peptide fragmentation nomenclature. [C] = alkylated cysteine.

2.4. Wound measurements

The mice were briefly anesthetized with isofluorane via inhalation and the wounds were photographed using a Nikon D5300 camera mounted on a tripod at a fixed distance; a ruler was included in the photographic frame. Wound areas were calculated using NIH ImageJ software (version 1.51). The wound closure analysis was performed separately by two experimenters, one of whom was blinded. Wound healing is reported as percent change in wound area relative to day 0. Wounds were measured and analyzed consecutively by animal number.

2.5. In vivo imaging for reactive oxygen species

A separate study was conducted with 15 *db/db* mice: n = 6 for vehicle and aclerastide, and n = 3 for ND-336. Excisional wounds were treated topically with vehicle, aclerastide, or ND-336 as described earlier once a day for 2 days. Mice were anesthetized with 2% isoflurane via inhalation on days 1 and 2 after wound infliction. The mice (n = 3 per group per time point) were intraperitoneally injected with 200 µl of L-012 (Soares et al., 2016) (Wako Chemicals) dissolved in PBS at 5 mg/ml. The images were acquired immediately, and at 5-min intervals using a Xenogen IVIS Lumina instrument (Caliper Life Sciences), controlled with *Living Image* software (v 3.0). The bioluminescent images were analyzed and quantified by *ImageJ* software version 1.51. Mice (n = 3 per group) were euthanized on days 1 and 2 for affinity resin analysis.

Download English Version:

https://daneshyari.com/en/article/8528950

Download Persian Version:

https://daneshyari.com/article/8528950

Daneshyari.com