

Clarke, Modet & Cº

COLOMBIA

P-3487

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 12-154938- -00008-0000

SUPERINTENDENCIA DE INDUSTRIA Y
DIVISION DE NUEVAS CREACIONES
E. S. D.

Fecha: 2014-09-25 14:33:47 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 523 RESPUESTA Folios: 49

Re: Expediente No. **12-154.938**
Solicitud de patente a nombre de **UNIVERSITY OF SOUTHERN CALIFORNIA**

Yo, SILVANA MARIA LOPEZ DIAZ identificada como aparece al pie de mi firma, domiciliada en la Carrera 11 No. 86-53, piso 6, obrando como apoderada sustituta de acuerdo con la sustitución presentada el 11 de julio de 2013 de la sociedad UNIVERSITY OF SOUTHERN CALIFORNIA, domiciliada en California, Estados Unidos de América, solicitante de la patente de la referencia, en respuesta al oficio No. **6333** notificado por fijación en lista el 3 de junio de 2014 relacionado con concepto técnico de fondo, y teniendo en cuenta el memorial presentado el 21 de agosto de 2014, mediante el cual se solicitó una prórroga de 30 días para dar respuesta al referido oficio, me permito manifestar:

1. Con fundamento en la facultad que confiere el artículo 34 de la Decisión 486 al solicitante para modificar la solicitud de patente en cualquier momento del trámite, presento junto con este memorial nuevo capítulo reivindicatorio, el cual consta de 8 reivindicaciones y reemplaza completamente el capítulo presentado anteriormente. En el nuevo capítulo reivindicatorio se realizaron las siguientes modificaciones:

- 1.1 Como primera medida, se modificó la antigua reivindicación 1, con el fin de definir el péptido por su estructura, es decir por el número de secuencia
- 1.2 Asimismo, se eliminaron las antiguas reivindicaciones 2, 7, 8 y 9, con el fin de dar concisión al capítulo reivindicatorio.

En cuanto a estas modificaciones, me permito decir que las mismas no amplían de ninguna manera la materia objeto de la presente solicitud y se realizaron con el ánimo de superar las objeciones de claridad y nivel inventivo planteadas por el examinador en su concepto técnico.

En cuanto al número de reivindicaciones, atentamente nos permitimos decir que no se adjunta tasa por reivindicaciones adicionales, toda vez que el nuevo capítulo reivindicatorio tiene menor número de cláusulas que el originalmente presentado y en esencia es el mismo objeto pero más limitado y definido con mayor claridad.

1/49

Clarke, Modet & C^o

COLOMBIA

1. Claridad de la solicitud

Respecto a las objeciones de claridad, el solicitante manifiesta que mediante la presentación del nuevo capítulo reivindicatorio quedan subsanadas la totalidad de las objeciones, de modo tal que las nuevas reivindicaciones proporcionan claridad y concisión a la invención descrita, siguiendo las recomendaciones realizadas por el examinador en su concepto técnico.

Como primera medida, es deseo del solicitante aclarar que el capítulo reivindicatorio que se debe ser considerado corresponde al presentado en el radicado No. 12-154938-00005-0000, presentado el 11 de julio de 2013 en respuesta a la oposición de la sociedad PROCAPS S.A., el cual contiene 12 reivindicaciones. La anterior aclaración considerando que, aunque el examinador en el oficio No. 6333 notificado por fijación en lista el 3 de junio de 2014 informa que se evaluarán 12 reivindicaciones, y no 18 reivindicaciones como se presenta originalmente, en la sección 4 del mismo documento indica objeciones a términos que ya se encuentran en dicho capítulo reivindicatorio. La presente respuesta al referido oficio se realizó considerando que el examinador evaluó el capítulo reivindicatorio correcto, y cometió únicamente un error al indicar el número de radicado de la solicitud.

Habiendo aclarado lo anterior, el solicitante aclara que la nueva reivindicación 1 fue modificada de tal forma que se incluyó el número de secuencia del péptido. Adicionalmente, como podrá notar el examinador, el presente capítulo reivindicatorio no contiene términos ambiguos, tales como “aproximadamente” y “al menos”, de tal forma que es posible distinguir la invención del estado de la técnica.

Por otra parte, se indica al examinador que se eliminaron las antiguas reivindicaciones 2, 7, 8 y 9, con el fin de dar concisión a la materia reclamada.

En relación con la falta de concisión del capítulo reivindicatorio, en cuanto a la materia reclamada en la nueva reivindicación 1, que indica que la formulación farmacéutica comprende (a) y “(b) un péptido que comprende Nle3 A(1-7) (SEQ ID NO:1)”, y la materia reclamada en la nueva reivindicación dependiente 2, que describe que “el péptido consiste de Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO: 1)”, es deseo del solicitante aclarar el término “comprende” es un término no limitativo, es decir con este término se indica por ejemplo que el péptido puede incluir aminoácidos adicionales. Por su parte, el término “consiste de” es un término limitativo, indicando que el péptido no contiene aminoácidos adicionales. De esta forma, la nueva reivindicación 2 hace referencia limita las características de la formulación farmacéutica descrita en la reivindicación 1, y por tanto tiene un alcance distinto. A este respecto, es relevante citar la GUIA PARA EXAMEN DE SOLICITUDES DE PATENTE DE INVENCION Y MODELO DE UTILIDAD emitida en enero de 2014:

“Cuando una reivindicación comprenda una expresión de transición de tipo “cerrado”, el examinador interpretará que los productos o procedimientos tan sólo comprenden los elementos expuestos en la reivindicación. Así, si una reivindicación trata de “un producto consistente

Clarke, Modet & C^o

COLOMBIA

únicamente en A, B y C”, el examinador interpretará que el producto tan sólo comprende los elementos A, B y C; de manera que un producto del estado de la técnica que tiene A, B, C y D o cualquier otro elemento no anula la novedad, y por tanto, la considerará nueva.

- Cuando una reivindicación comprenda una expresión de transición de tipo “abierto”, el examinador interpretará que los productos o procedimientos incluyen también elementos no mencionados en la reivindicación. Así, si una reivindicación trata de “un producto que comprende A, B y C”, el examinador interpretará que el producto comprende los elementos A, B, C y cualquier otro elemento no mencionado en la reivindicación; de manera que un producto del estado de la técnica que tiene A, B, C y D o cualquier otro elemento anula la novedad, y por tanto, la considerará no nueva” (paginas 74-75, sección 2.6.5.2) (El subrayado es nuestro)

De esta forma, es clara la diferencia que existe entre el alcance de las presentes reivindicaciones 1 y 2.

En relación con el título de la solicitud, el cliente manifiesta una modificación de este a “*FORMULACIONES TIPO HIDROGEL QUE COMPRENDEN EL PÉPTIDO Nle3 A(1-7) EN COMBINACIÓN CON HIDROXIETILCELULOSA*”.

En consecuencia, nos permitimos informar que las nuevas reivindicaciones 1 a 9 se ajustan a las disposiciones del artículo 30 de la Decisión 486.

2. Nivel Inventivo

El examinador establece en su concepto técnico la falta de nivel inventivo de las antiguas reivindicaciones 1 a 12 a la luz de los documentos “Rodgers Et al. 2005 *Journal of Peptide Research*, Blackwell publishing Ltd. Oxford, 66 (1), 41-47” (D1), “US-NIH 2009, NTC00796744” (D2), y “Matuszewska et al. 1994. *Pharm. Res.* 11(1), 65-71” (D3).

De este modo, nos permitimos indicar que el artículo 18 de la Decisión 486 establece que “*Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica*”.

Lo anterior significa que el análisis de nivel inventivo **NO** es una comparación de los objetos de las anterioridades y la solicitud en estudio. El análisis de nivel inventivo **debe demostrar** la obviedad de la invención y establecer cómo ésta no contiene un salto cualitativo, que demuestre su altura inventiva, teniendo en cuenta que se hace **necesario demostrar** que la invención analizada carece de nivel inventivo y hubiese sido obvia para una persona versada en la materia.

Considerando la anterior definición, es deseo del solicitante presentar argumentos que demuestran el nivel inventivo de la presente invención a la luz de las anterioridades citadas.

Clarke, Modet & C^o

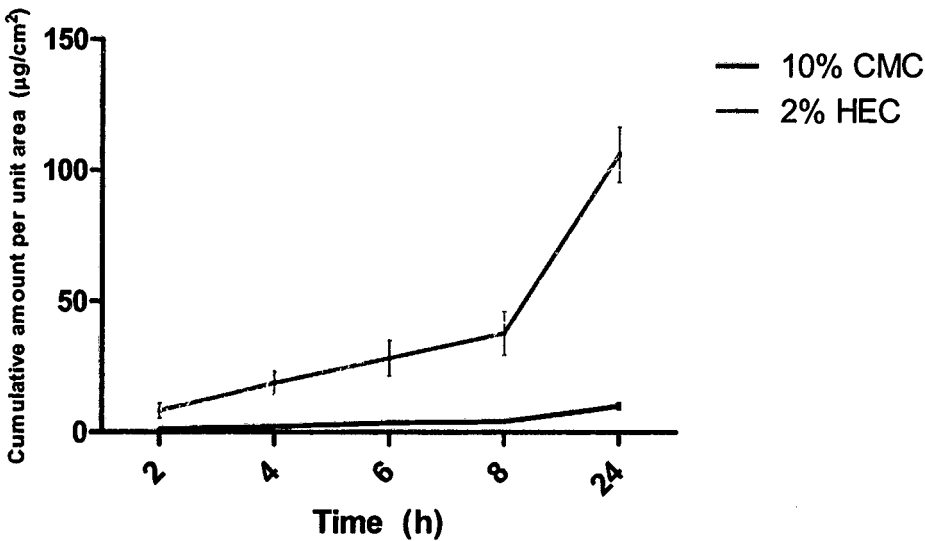
COLOMBIA

El examinador indica en su reporte técnico que *“dentro de la descripción no hay ningún ejemplo que muestre un efecto técnico inesperado al usar como vehículo HEC y no CMC”*. A este respecto, es deseo del solicitante indicar que en el capítulo descriptivo originalmente presentado se indica claramente que *“proporcionó un aumento de 10 veces en la liberación del péptido durante un período de 24 horas a partir de formulaciones tales como los que comprenden 10% de carboximetilcelulosa (CMC), un resultado que sería inesperado para los expertos en la técnica. Además, los datos muestran que las matrices de HEC son más biocompatibles que las formulaciones probadas de HPMC y CMC”*.

Lo anterior significa que, contrario a lo indicado por el examinador, si hay evidencia en el capítulo descriptivo originalmente presentado en relación con el efecto técnico inesperado que se logra a través de la elección de HEC en lugar de CMC.

Adicional a lo anterior y con el fin de aportar evidencia a la afirmación incluida en el capítulo descriptivo en relación con el efecto técnico inesperado, se incluye a continuación datos experimentales en los que se compara el HEC con el CMC.

Durante estudios para determinar el vehículo de liberación óptima, se encontró que el HEC manifiesta una cinética de liberación que permite diez veces más fármaco liberado en un periodo de 24 horas. Se calcularon las tasas de liberación, como una medida de la potencial biodisponibilidad, siendo significativamente diferente las tasas de liberación para diferentes vehículos. La liberación acumulada (*“Cumulative amount per unit area (µg/cm²)”*) fue significativamente mayor para 2% HEC ($106 \pm 4.7 \mu\text{g}/\text{cm}^2$) que para 10% CMC ($10.1 \pm 1.2 \mu\text{g}/\text{cm}^2$) en un periodo de 24 horas.



Así, en la figura anterior se muestra una comparación de la liberación de péptido (*“fármaco”*) en un periodo de 24 horas usando dos formulaciones que comprenden distintos vehículos, específicamente 2% HEC (línea azul) y 10% CMC (línea negra), respectivamente. Como se observa claramente, la formulación que comprende 2% HEC presenta una tasa de liberación de $106 \pm 4.7 \mu\text{g}/\text{cm}^2$, mientras que la formulación que comprende 10% CMC presenta una tasa de liberación de $10.1 \pm 1.2 \mu\text{g}/\text{cm}^2$. Por lo tanto, las formulaciones que comprenden HEC proporcionan una liberación del

4

Clarke, Modet & Cº

COLOMBIA

fármaco diez veces mayor en un periodo de 24 horas que las formulaciones que comprenden CMC, como las del estado de la técnica. Particularmente, es relevante indicar que las formulaciones inventivas de HEC proporcionan una diez veces mayor tasa de liberación con una concentración que es únicamente una quinta parte de la concentración de HMC en las formulaciones que lo contienen (2% HEC vs. 10% CMC). Consecuentemente, concentraciones menores de HEC en las formulaciones deben mostrar mayor liberación de fármaco. Para una persona del oficio normalmente versada en la técnica es evidente que la formulación reclamada puede usarse en todos los rangos reclamados. Estos resultados son sorprendentes e inesperados.

Considerando todos los anteriores argumentos, es claro que la materia objeto de la presente invención no resulta obvia a la luz de los documentos del estado de la técnica citados por el examinador y del efecto técnico demostrado anteriormente.

Por lo tanto, se demuestra que la presente invención tiene nivel inventivo en concordancia con los lineamientos del artículo 18 de la Decisión 486/00.

Adicional a los argumentos anteriormente presentados, es deseo del solicitante indicar que las solicitudes correspondientes en Estados Unidos de América y en Europa han sido concedidas (US 8,536,231 B2; US 8,207,233 B1; US8,207,234 B1; EP 2536422). Copia de las patentes US 8,536,231 B2, US 8,207,233 B1, US8,207,234 B1 y EP 2536422 se presentan como anexo al presente memorial.

Así, aunque el solicitante reconoce el principio de independencia de cada Oficina de Patente, resulta sorprendente que otras Oficinas hayan reconocido el cumplimiento de los requisitos de patentabilidad de la presente invención, mientras únicamente la Oficina de Patentes en Colombia se aparta de dicho criterio unánime, sin considerar que las definiciones de novedad, nivel inventivo y aplicación industrial son conceptos universales.

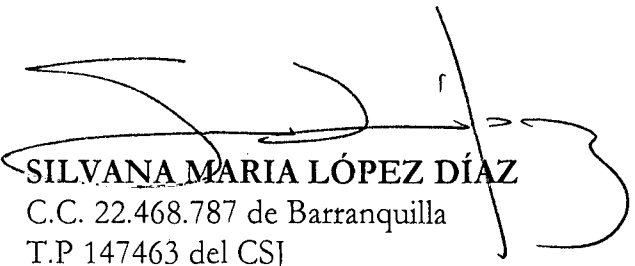
Habiendo atendido cabalmente las objeciones presentadas por ese despacho, atentamente solicitamos se conceda el privilegio de patente de invención a la solicitud en estudio o si el señor examinador considera que subsisten razones para objetar la presente invención, rogamos expedir otro examen de fondo con los debidos motivos y razones que sustenten su objeción con el fin de que nuestro mandante tenga la posibilidad de exponer sus argumentos con base en la interpretación de los artículos 45 de la Decisión 486 -el cual otorga la facultad al examinador para emitir los exámenes de fondo-, 35 y 59 del Código Contencioso Administrativo que señala que: *"habiéndose dado oportunidad a los interesados para expresar sus opiniones, y con base en las pruebas e informes disponibles, se tomará la decisión que será motivada al menos en forma sumaria si afecta a particulares"* y *"(.) deberá proferirse la decisión definitiva. Esta se motivará en sus aspectos de hecho y de derecho, y en los de conveniencia si es el caso"*, respectivamente (El subrayado es nuestro).

Clarke, Modet & Cº

COLOMBIA

En consecuencia, solicitamos a ese despacho pronunciarse en relación con todos los argumentos presentados en este memorial dando estricta aplicación al principio del debido proceso consagrado en el artículo 29 de la Constitución Nacional.

Señor Superintendente,



SILVANA MARIA LÓPEZ DÍAZ

C.C. 22.468.787 de Barranquilla

T.P 147463 del CSJ

notificaciones@clarkemodet.com.co

Clarke, Modet & Cº

COLOMBIA

7

ANEXOS

- Patente US 8,536,231 B2
- Patente US 8,207,233 B1
- Patente US 8,207,234 B1
- Concesión Patente EP 2536422 y reivindicaciones

REIVINDICACIONES

1. Una formulación farmacéutica, que comprende:
 - (a) de 0.5% a 4% de HEC sobre una base en peso (mg)/volumen (ml), o sobre una base peso/peso (mg); y
 - (b) un péptido que comprende Nle3 A(1-7) (SEQ ID NO:1);caracterizada porque el péptido está presente en una concentración de 0.03 % a 1% sobre una base en peso (mg)/volumen (ml), o sobre una base de peso/peso (mg).
2. La formulación farmacéutica de la reivindicación 1, caracterizada porque el péptido consiste de Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO: 1).
3. La formulación farmacéutica de la reivindicación 1, caracterizada porque comprende de 1% a 3% de HEC sobre una base en peso (mg)/volumen (ml), o sobre una base de peso/peso (mg).
4. La formulación farmacéutica de la reivindicación 1, caracterizada porque comprende 2% de HEC sobre una base en peso (mg)/volumen (ml), o sobre una base de peso/peso (mg).
5. La formulación farmacéutica de la reivindicación 1, caracterizada porque la formulación comprende una formulación de hidrogel.
6. La formulación farmacéutica de la reivindicación 2, caracterizada porque comprende de 1% a 3% de HEC sobre una base en peso (mg)/volumen (ml), o sobre una base de peso/peso (mg).
7. La formulación farmacéutica de la reivindicación 2, caracterizada porque comprende 2% de HEC sobre una base en peso (mg)/volumen (ml), o sobre una base de peso/peso (mg).
8. La formulación farmacéutica de la reivindicación 1, caracterizada porque la formulación comprende una formulación de hidrogel.



US008536231B2

(12) **United States Patent**
Rodgers et al.

(10) **Patent No.:** **US 8,536,231 B2**
(45) **Date of Patent:** ***Sep. 17, 2013**

(54) **METHODS FOR TREATING DIABETIC FOOT ULCERS**

(76) Inventors: **Kathleen E. Rodgers**, Long Beach, CA (US); **Gere S. diZerega**, San Luis Obispo, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(h) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **13/467,253**

(22) Filed: **May 9, 2012**

(65) **Prior Publication Data**

US 2012/0329729 A1 Dec. 27, 2012

Related U.S. Application Data

(63) Continuation of application No. 13/364,061, filed on Feb. 1, 2012, now Pat. No. 8,207,233.

(60) Provisional application No. 61/438,780, filed on Feb. 2, 2011.

(51) **Int. Cl.**

A61K 38/08 (2006.01)

A61K 47/38 (2006.01)

C07K 7/14 (2006.01)

C07K 17/04 (2006.01)

C07K 17/12 (2006.01)

(52) **U.S. Cl.**

USPC 514/928; 424/488; 530/316; 930/40

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,015,629 A 5/1991 DiZerega et al.
5,629,292 A 5/1997 DiZerega et al.
5,716,935 A 2/1998 DiZerega et al.
5,834,432 A 11/1998 DiZerega et al.
5,955,430 A 9/1999 DiZerega et al.
6,096,709 A 8/2000 DiZerega et al.
6,110,895 A 8/2000 DiZerega et al.
6,165,978 A 12/2000 DiZerega et al.
6,177,407 B1 1/2001 Rodgers et al.
6,248,587 B1 6/2001 Rodgers et al.
6,258,778 B1 7/2001 Rodgers et al.
6,335,195 B1 1/2002 Rodgers et al.
6,444,646 B1 9/2002 DiZerega et al.
6,455,500 B1 9/2002 Rodgers et al.
6,455,501 B1 9/2002 DiZerega et al.
6,475,988 B1 11/2002 Rodgers et al.
6,482,800 B1 11/2002 Rodgers et al.
6,498,138 B1 12/2002 Rodgers et al.
6,566,335 B1 5/2003 Rodgers et al.
6,730,775 B1 5/2004 DiZerega et al.
6,762,167 B1 7/2004 Rodgers et al.
6,821,953 B1 11/2004 DiZerega et al.
6,916,783 B2 7/2005 Rodgers et al.
7,022,675 B2 4/2006 DiZerega et al.
7,118,748 B1 10/2006 Rodgers et al.
7,173,011 B2 2/2007 Rodgers et al.
7,176,183 B2 2/2007 DiZerega et al.
7,338,938 B2 3/2008 Rodgers et al.
7,744,927 B2 6/2010 Rodgers et al.

7,776,828 B2 8/2010 Rodgers et al.
7,786,085 B2 8/2010 Rodgers et al.
8,207,233 B1 * 6/2012 Rodgers et al. 514/6.9
8,207,234 B1 * 6/2012 Rodgers et al. 514/6.9
2009/0227507 A1 9/2009 Rodgers et al.

FOREIGN PATENT DOCUMENTS

WO 98/33813 8/1998
WO 99/31125 6/1999
WO 99/40107 8/1999
WO 01/44270 6/2001
WO 01/98325 12/2001
WO 2006/092668 9/2006
WO 2009/075881 6/2009
WO 2011/120032 3/2011
WO 2012/021578 2/2012

OTHER PUBLICATIONS

Carney, et al. (2008) "Could nuslatide acetate be the future drug of choice for diabetic foot ulcers and fracture repair?" Expert Opin Pharmacother, 9(15): 2717-2726.
Balingit, et al. (2012) "NorLeu3-A(1-7) stimulation of diabetic foot ulcer healing: Results of a randomized, parallel-group, double-blind, placebo-controlled phase 2 clinical trial," Wound Rep Reg, 20:482-490.
XP-002680220, Safety and Efficacy Study of DSC127 in Treating Subjects with Diabetic Ulcers, 2010.
Matuszewska, et al. (1994) "Acidic Fibroblast Growth Factor: Evaluation of Tropical Formulations in a Diabetic Mouse Wound Healing Model," Pharmaceutical Research, 11(1):65-71.
American Diabetes Association, Consensus development conference on diabetic foot wound care. Diabetes Care 1999; 22:1354-1360.
Pecoraro RE, Reiber GE, Burgess EM, Pathways to diabetic limb amputation: basis for prevention. Diabetes Care 1990; 13:513-521.
Rodgers KE, Roda N, Felix JC, Espinoza T, Maldonado S, diZerega GS. Histological evaluation of the effects of angiotensin peptides on wound repair in diabetic mice. Experimental Dermatology 2003; 12(6):784-790.
Rodgers K, Xiong S, Felix J, Roda N, Espinoza T, Maldonado S, diZerega GS. Development of angiotensin (1-7) as an agent to accelerate dermal repair. Wound Repair Regen 2001; 9:238-250.
Rodgers KE, Espinoza T, Felix J, Roda N, Maldonado S, diZerega GS. Acceleration of healing, reduction of fibrotic scar, and normalization of tissue architecture by an angiotensin analogue, Norleu3-A (1-7). Plast Reconstr Surg 2003; 111:1195-1206.
Wagner, FJ. A classification and treatment program for diabetic, neuropathic, and dysvascular foot problems. Am Acad of Orthopaedic Surgeons. Instructional Course Lecture 1979; 28:143-165.
Foster AV, Eaton C, McConville DO, Edmonds ME. Application of OpSite film: a new and effective treatment of painful diabetic neuropathy. Diabetes Med 1994; 11(8):768-772.
Sheehan P, Jones P, Caselli A, Giurini JM, Veves A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. Diabetes Care 2003; 26:1879-1882.
Rodgers, KE, Abiko M, Girgis W, St. Amand KM, Campeau JD, diZerega GS. Acceleration of dermal tissue repair by Angiotensin II. Wound Repair Regen 1997; 5:175-183.
Rodgers, KE, DeCherney AH, St. Amand KM, Dougherty WR, Felix JC, Girgis W, diZerega GS. Histologic alterations in dermal repair after thermal injury: effects of topical angiotensin II. Burn Care and Rehabilitation 1997; 18:381-388.

(Continued)

Primary Examiner — David Romeo

(74) Attorney, Agent, or Firm — McDonnell Boehnen Hulbert & Berghoff LLP

(57) **ABSTRACT**

The present invention provides methods and pharmaceutical formulations for treating diabetic foot ulcers.

27 Claims, 3 Drawing Sheets

9

(56)

References Cited

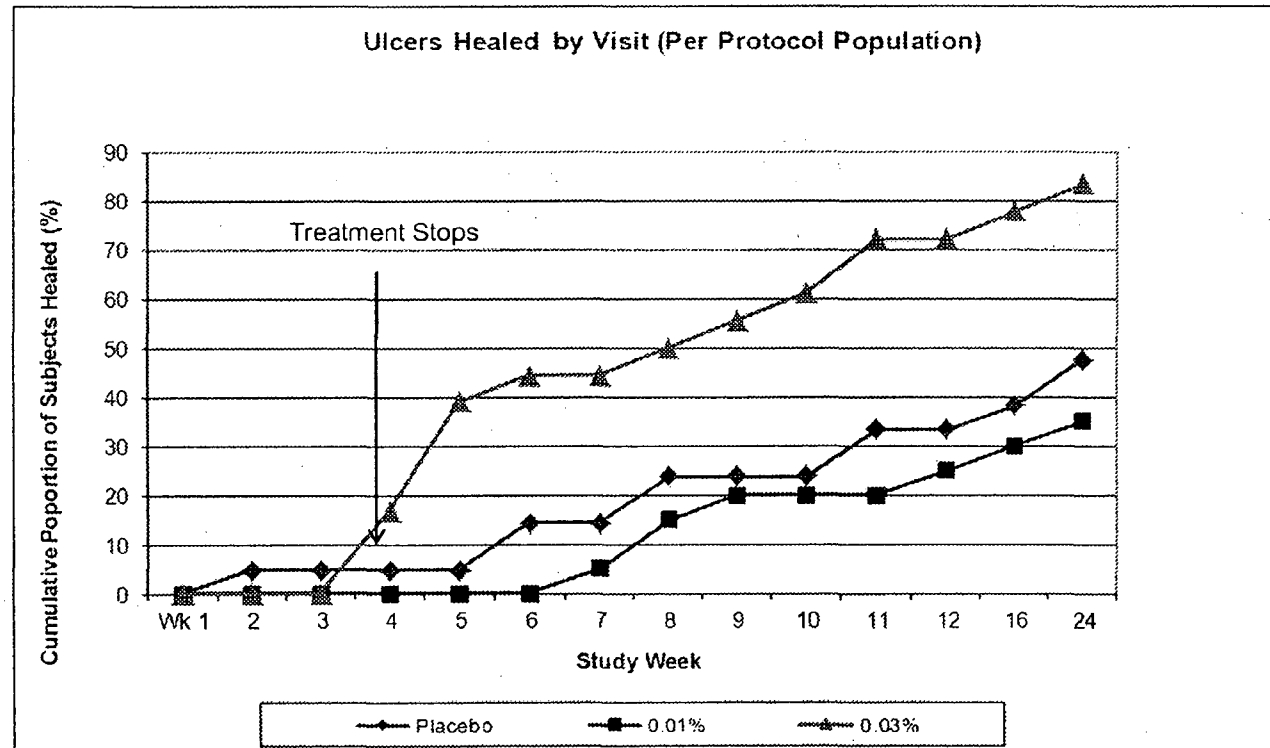
OTHER PUBLICATIONS

- Okuyama N, Roda N, Guerrero A, Dougherty W, Nguyen T, diZerega GS, Rodgers KE. Effect of angiotensin II on the viability, vascularity of random flaps in a rat model. *Annals Plastic Surgery Res* 1999; 68:913-918.
- Rodgers KE, Ellefson DD, Espinoza T, Roda N, Maldonado S, diZerega GS. Effect of NorLeu3-A (1-7) on scar formation over time after full thickness incision injury in the rat. *Wound Repair Regen* 2005; 13:309-317.
- Santos RA, Brosnihan KB, Chappell MC, Pesquero J, Chernicky CL, Greene LJ, and Ferrario CM. Converting enzyme activity and angiotensin metabolism in the dog brainstem. *Hypertension* 1988; 11(suppl 1):I-53-I-57.
- Santos RAS, Brosnihan KB, Jacobsen DW, DiCorleto P, and Ferrario CM. Production of Ang-(1-7) by human vascular endothelium. *Hypertension* 1992; 19(suppl II):II-56-II-61.
- Santos et al. Characterization of a new angiotensin antagonist selective for angiotensin-(1-7): evidence that the actions of angiotensin-(1-7) are mediated by specific angiotensin receptors. *Brain Res Bull* 1994; 35:293-298.
- Yamamoto K, Chappell MC, Brosnihan KB, Ferrario CM. In vivo metabolism of angiotensin I by neutral endopeptidase (EC 3.4.24.11) in spontaneously hypertensive rats. *Hypertension* 1992; 19:692-696.
- Chappell MC, Tallant EA, Brosnihan KB, Ferrario CM. Processing of angiotensin peptides by NG108-15 neuroblastoma X glioma hybrid cell line. *Peptides* 1990; 22:375-380.
- Chappell MC, Jacobsen DW, Tallant EA. Characterization of angiotensin II receptor subtypes in pancreatic acinar AR42J cells. *Peptides* 1995; 16:741-747.
- Chappell MC, Tallant EA, Brosnihan KB, Ferrario CM. Conversion of angiotensin I to angiotensin-(1-7) by thimet oligopeptidase (E.C. 3.4.24.15) in vascular smooth muscle cells. *J Vasc Biol Med* 1995; 5:129-137.
- Daemen MJAP, Lombardi DM, Bosman FT, Schwartz SM. Angiotensin II induces smooth muscle cell proliferation in the normal and injured rat arterial wall. *Circ Res* 1991; 68:450-56.
- Dzau VE, Pratt R, Gibbons G, Schunkert H, Lorell B, Ingelfinger J. Molecular mechanism of angiotensin in the regulation of vascular and cardiac growth. *J Mol Cell Cardiol* 1989; 21 [Suppl III]:S7.
- Naftilan AJ, Pratt RE, Dzau VJ. Induction of platelet-derived growth factor A-chain and c-myc gene expression by Angiotensin II in culture rat vascular smooth muscle cells. *J Clin Invest* 1989; 83:1419-24.
- Kawahara Y, Sunako M, Tsuda T, Fukazaki H, Fukumoto Y, Takai Y. Angiotensin II induces expression of the c-fos gene through protein kinase C activation and calcium ion mobilization in cultured vascular smooth muscle cells. *BBRC* 1988; 150:52-9.
- Mangiarua EI, Palmer VL, Lloyd LL, McCumbee WD. Platelet-derived growth factor mediates angiotensin II-induced DNA synthesis in vascular smooth muscle cells. *Arch Physiol Biochem* 1997; 105(2):151-7.
- Su EJ, Lombardi DM, Wiener J, Daemen MJ, Reidy MA, and Schwartz MA. Mitogenic effect of angiotensin II on the rat carotid arteries and type II or III mesenteric microvessels but not type I mesenteric microvessels is mediated by endogenous basic fibroblast growth factor. *Circ Res* 1998; 82:321.
- Rodgers LC, Driver VR, Armstrong DG. Assessment of the diabetic foot. In Krasner DL, Rodeheaver GT, Sibbald RG eds. *Chronic Wound Care: A Clinical Source Book for Healthcare Professionals*. 4th ed. Malvern PA: HMP Communications, 2007: 549-556.
- Bolton L, McNees P, van Rijswijk L et al. Wound healing outcomes using standardized care. *JWOCN* 2004; 31:65-71.
- Stouffer GA, Owens GK. Angiotensin II induced mitogenesis of spontaneously hypertensive rat derived cultured smooth muscle cells is dependent on autocrine production of transforming growth factor-B. *Circ Res* 1992; 70:820-28.
- Koibuchi Y, Lee WS, Gibbons GH, Pratt RE. Role of transforming growth factor B-1 in the cellular growth response to Angiotensin II. *Hypertension* 1993; 21:1046-50.
- Rodgers, et al., (2006) *J. Peptide Res.*, 66(suppl. 1):41-47.

* cited by examiner

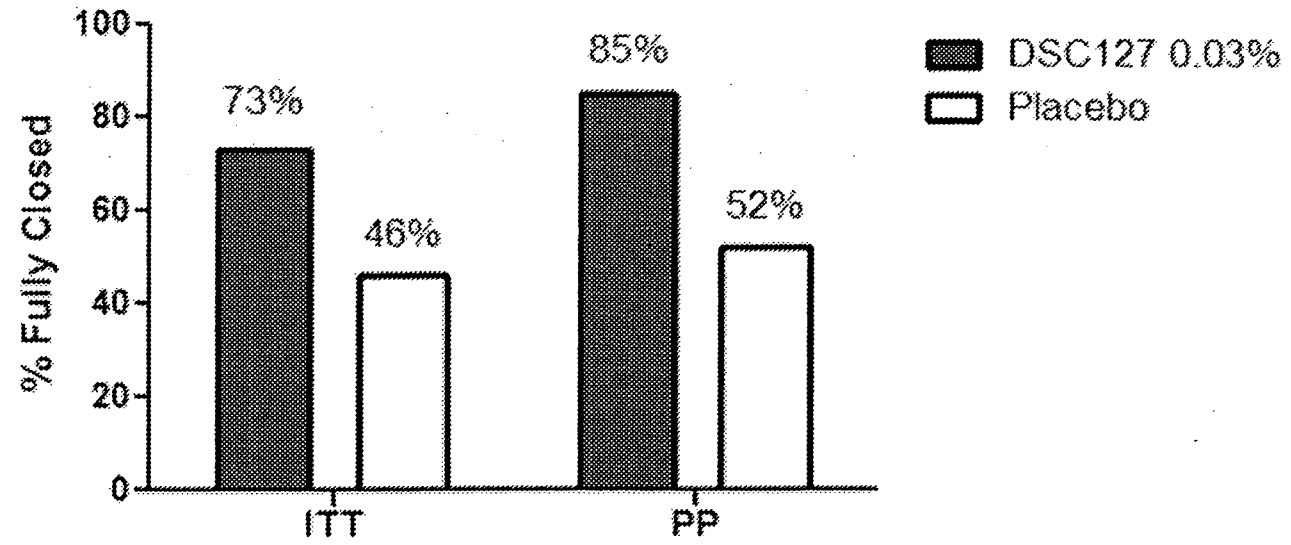
10

Figure 1



12

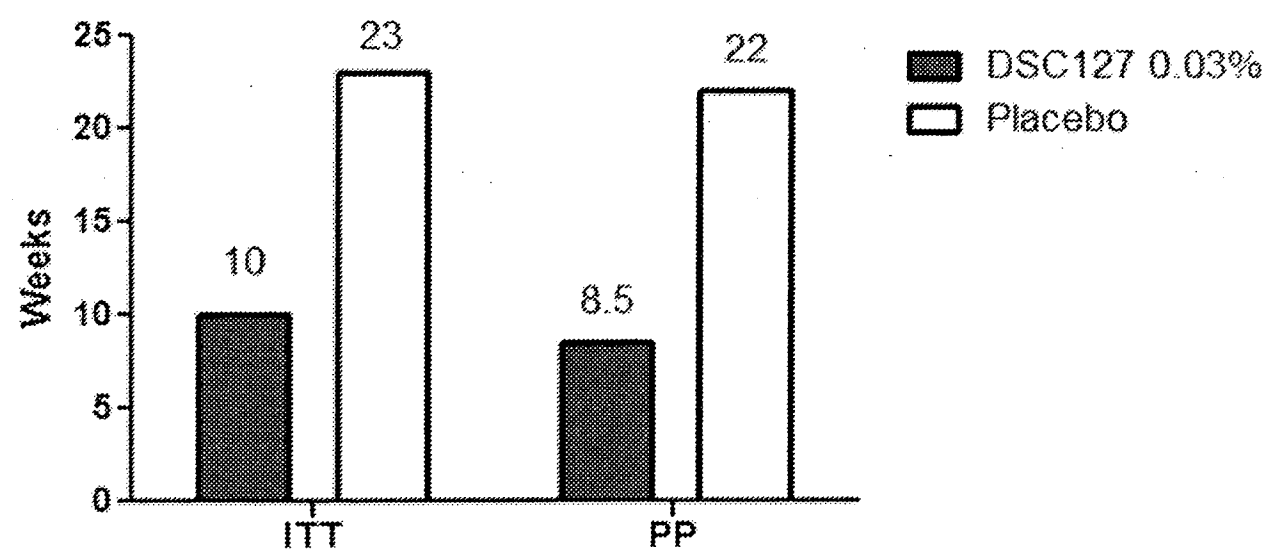
Figure 2



12

13

Figure 3



13

1

METHODS FOR TREATING DIABETIC FOOT
ULCERS

CROSS REFERENCE

This application is a continuation of U.S. application Ser. No. 13/364,061 filed Feb. 1, 2012 which claims priority to U.S. Provisional Patent Application Ser. No. 61/438,780 filed Feb. 2, 2011, incorporated by reference herein in their entirety.

BACKGROUND

Diabetes is common, disabling and deadly. In the U.S., diabetes has reached epidemic proportions. According to the American Diabetes Association, about 24 million people (8% of the total U.S. population) have diabetes, and nearly two million new cases are diagnosed in people aged 20 years or older each year. If current trends continue, 1 in 3 Americans will develop diabetes at some point in their lifetime, and those with diabetes will lose, on average, 10-15 years of life expectancy. Importantly, up to 25% of people with diabetes will develop a diabetic foot ulcer, resulting in 3 million diabetic foot ulcers annually in the U.S. alone. More than half of all foot ulcers will become infected, thus requiring hospitalization, and 1 in 5 will require an amputation that carries a high risk of mortality.

Without question, diabetes puts tremendous economic pressure on the U.S. healthcare system. Total costs (direct and indirect) of diabetes have reached \$174 billion annually, and people with diagnosed diabetes have medical expenditures that are over two times higher than medical expenditures for people without diabetes. Hospitalization costs alone are \$16,000 to \$20,000 for a patient with a diabetic foot ulcer, and direct and indirect costs of an amputation range from \$20,000 to \$60,000 per patient. A recent study by researchers at the University of Chicago suggested that treatment costs for diabetes in the United States would reach \$336 billion by the year 2034. Advanced, cost-effective treatment modalities for diabetes and its co-morbidities, including diabetic foot ulcers, are in great need, yet in short supply, globally. According to the American Diabetes Association, by the year 2025 the prevalence of diabetes is expected to rise by 72% to 324 million people worldwide.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides methods for treating diabetic foot ulcers, comprising administering to a human patient suffering from a diabetic foot ulcer an amount of a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof, effective to treat the diabetic foot ulcer. In one embodiment, the peptide comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In another embodiment, the peptide consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In various embodiments, the diabetic foot ulcer is one caused, at least in part, by neuropathy and resulting pressure; the diabetic foot ulcer comprises one or more calluses; and the diabetic foot ulcer is a chronic ulcer. In a further embodiment, the chronic foot ulcer has not responded to any other treatment.

In another embodiment, the peptide is administered topically. In a further embodiment, the peptide is administered as a topical formulation forming a continuous film covering the entire area of the diabetic ulcer. In a still further embodiment, the peptide is administered in a hydrogel formulation. In another embodiment, the peptide is administered at a concen-

2

tration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In a further embodiment, the peptide is administered in a gel formulation that about 0.5% to about 4% hydroxyethyl cellulose (HEC) on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

All embodiments of the first aspect of the invention can be combined unless the context dictates otherwise.

In a second aspect, the present invention provides pharmaceutical formulations, comprising:

(a) about 0.5% to about 4% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis; and

(b) a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof;

wherein the peptide is present at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

In one embodiment, the peptide comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In another embodiment, the peptide consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In a further embodiment, the pharmaceutical formulation comprises about 1% to about 3% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In a still further embodiment, the pharmaceutical formulation comprises about 2% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In another embodiment, the formulation comprises a hydrogel formulation. All embodiments of the second aspect of the invention can be combined unless the context dictates otherwise.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a graph showing the ulcers healed by visit in the Per Protocol (PP) patient population taking NorLeu³-A(1-7).

FIG. 2 is a graph showing the percentage of fully healed ulcers (100% wound closure) in the patient population taking NorLeu³-A(1-7) (also referred to as "DSC127").

FIG. 3 is a graph showing the median time to fully healed ulcers (100% wound closure) in the patient population taking NorLeu³-A(1-7) (also referred to as "DSC127").

DETAILED DESCRIPTION OF THE INVENTION

All references cited are herein incorporated by reference in their entirety. Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991, Academic Press, San Diego, Calif.), "Guide to Protein Purification" in *Methods in Enzymology* (M. P. Deutscher, ed., (1990) Academic Press, Inc.); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990, Academic Press, San Diego, Calif.), *Culture of Animal Cells: A Manual of Basic Technique*, 2nd Ed. (R. I. Freshney, 1987, Liss, Inc. New York, N.Y.), *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E. J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, Tex.).

As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. "And" as used herein is interchangeably used with "or" unless expressly stated otherwise.

All embodiments of any aspect of the invention can be used in combination, unless the context clearly dictates otherwise.

c4

In a first aspect, the present invention provides methods for treating diabetic foot ulcers, comprising administering to a human patient suffering from a diabetic foot ulcer an amount of a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof, effective to treat the diabetic foot ulcer.

As demonstrated in the examples that follow, the inventors have demonstrated that the methods of the invention can be used to treat diabetic foot ulcers, and provide substantial improvement over standard therapies.

Nle3A(1-7) (or NorLeu³-A(1-7); also referred to as "DSC127") is a peptide consisting of the amino acid sequence Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1). In various embodiments, the peptide administered to the human patient may comprise or consist of Asp-Arg-Nle-Tyr-Ile (SEQ ID NO:2), Asp-Arg-Nle-Tyr-Ile-His (SEQ ID NO:3), or most preferably Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salts thereof. Nle3A(1-7) or salts thereof may be chemically synthesized or recombinantly expressed, each of which can be accomplished using standard methods in the art.

In one embodiment, the peptide, or salt thereof, is administered at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In various further embodiments, the peptide, or salt thereof, is administered at a concentration of about 0.03% to about 0.75%; about 0.03% to about 0.5%; about 0.03% to about 0.25%; about 0.03% to about 0.1%; about 0.03% to about 0.075%; about 0.03% to about 0.05%; and about 0.03%; all on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

The human patient may be suffering from Type I diabetes or Type II diabetes, and has a foot ulcer, defined as an open wound anywhere on the foot (heel, mid-foot, and forefoot).

As used herein, "treating" a diabetic foot ulcer includes

- (a) limiting the progression in size, area, and/or depth of the foot ulcer;
- (b) reducing size, area, and/or depth of the foot ulcer;
- (c) increasing rate of healing and/or reducing time to healing;
- (d) healing of the foot ulcer (100% epithelialization with no drainage); and
- (e) decreased incidence of amputation or slowing in time to amputation.

The foot ulcer may be caused by any underlying pathology, including but not limited to neuropathy, trauma, deformity, high plantar pressures, callus formation, edema, and peripheral arterial disease. In preferred embodiments, the human diabetic foot ulcer is one caused, at least in part, by neuropathy and resulting pressure (weight bearing on the extremity due to lack of feeling in the foot). As is known to those of skill in the art, human diabetic foot ulcers tend to be due to neuropathy and pressure, which differs significantly from, for example, murine acute wounds. In a further preferred embodiment, the diabetic foot ulcer comprises one or more calluses.

In a further embodiment, the diabetic foot ulcer is a chronic ulcer. As used herein, a "chronic" foot ulcer is one that has been present for at least 7 days with no reduction in size; preferably at least 14 days; even more preferably, present at least 21 or 28 days with no reduction in size. In a further preferred embodiment that can be combined with any of these embodiments, the chronic foot ulcer has not responded (ie: no reduction in size, area, and/or depth of the foot ulcer; no healing of the foot ulcer) to any other treatment.

The peptide or salt thereof may be administered by any suitable route, preferably via topical administration. In one embodiment, the methods of the invention can comprise administering a topical formulation as often as deemed

appropriate, ie: once per day, twice per day, etc. The methods may further comprise administration of the peptide, or salt thereof for as longed as deemed desirable by an attending physician, for example, until healing of the ulcer. For administration, it is preferred that the topical formulation form a continuous film covering the entire area of the ulcer, including the margins. In a preferred embodiment, the topical formulation is applied with a thickness of approximately 0.25 to 2 mm; preferably 0.5 to 1.5 mm; preferably about 1 mm in thickness.

In one embodiment, the topical administration comprises administration in a formulation selected from the group consisting of hydrogels, creams, ointments, pastes, and lotions. The formulations may be applied in any suitable manner, which may include any wound dressings to seal in the formulation deemed appropriate by the human patient or caregiver. Exemplary such dressings, include, but are not limited to, semipermeable films, foams, hydrocolloids, and calcium alginate swabs.

The methods may further comprise debridement in and around the wound in combination with administration of the peptide and formulations thereof. Debridement of all necrotic, callus, and fibrous tissue is typically carried for treatment of diabetic foot ulcers. Unhealthy tissue is sharply debrided back to bleeding tissue to allow full visualization of the extent of the ulcer and to detect underlying abscesses or sinuses. Any suitable debridement technique can be used, as determined by an attending physician. The wound can then be thoroughly flushed with sterile saline or a non-cytotoxic cleanser following debridement.

In another embodiment, the topical formulation comprises about 0.5% to about 4% hydroxyethyl cellulose (HEC) on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In various further embodiments, the topical formulation may comprise about 1% to about 3% HEC, or about 2% HEC, on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. These formulations comprising low percentage HEC (ie: 2%) matrices provided a 10-fold increase in peptide release over a 24 hour period from formulations such as those comprising 10% carboxymethylcellulose (CMC), a result that would be unexpected to those of skill in the art. Furthermore, the data show that the HEC matrices are more biocompatible than HPMC and CMC formulations tested.

The peptides, or salt thereof may be administered together with one or more (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer. In some embodiments, the buffer in the pharmaceutical composition is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The peptides may be administered with a lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain embodiments, the peptides may be administered with a preservative e.g. benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the peptides may be administered with a bulking agent, like glycine. In yet other embodiments, the peptides may be administered with a surfactant e.g., polysorbate-20, polysorbate-40, polysorbate-60, polysorbate-65, polysorbate-80 polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleate, or a combination thereof. The peptides may be administered with a tonicity adjusting agent, e.g., a compound that renders the formulation substantially isotonic or isoosmotic with human blood.

5

Exemplary tonicity adjusting agents include sucrose, sorbitol, glycine, methionine, mannitol, dextrose, inositol, sodium chloride, arginine and arginine hydrochloride. In other embodiments, the peptides may be administered with a stabilizer, e.g., a molecule which, when combined with the peptide substantially prevents or reduces chemical and/or physical instability of the protein of interest in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride, paraben, and combinations of methyl paraben and propyl paraben.

In all aspects and embodiments of the invention, suitable acids which are capable of forming salts with the peptides include, but are not limited to, inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid and the like; and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid and the like. Suitable bases capable of forming salts with the peptides include, but are not limited to, inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide and the like; and organic bases such as mono-, di- and tri-alkyl and aryl amines (e.g., triethylamine, diisopropyl amine, methyl amine, dimethyl amine and the like) and optionally substituted ethanol-amines (e.g., ethanolamine, diethanolamine and the like).

The peptides or salts thereof can further be derivatized to provide enhanced half-life, for example, by linking to polyethylene glycol. The peptides or salts thereof may comprise L-amino acids, D-amino acids (which are resistant to L-amino acid-specific proteases in vivo), a combination of D- and L-amino acids, and various "designer" amino acids (e.g., β -methyl amino acids, Co-methyl amino acids, and α -methyl amino acids, etc.) to convey special properties.

The polypeptides may be the sole active agent in the pharmaceutical composition, or the composition may further comprise one or more other active agents suitable treating diabetic foot ulcers, such as antibiotics. The methods may be used in conjunction with other foot ulcer therapies, including but not limited to negative pressure wound therapy, total contact casts, removable cast walkers, half-shoes, becaplermin, infection control, and hyperbaric oxygen therapy.

The methods may include any other embodiments as disclosed in the example that follows. Such embodiments may be used in any combination in the methods of the invention, unless the context clearly dictates otherwise.

In a second aspect, the present invention provides pharmaceutical formulations, comprising:

(a) about 0.5% to about 4% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis; and

(b) a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof;

wherein the peptide is present at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

The pharmaceutical formulations are demonstrated herein to be particularly effective for treating diabetic foot ulcers, such as chronic diabetic foot ulcers that are not effectively treated using standard therapies.

In one embodiment, the peptide comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In another embodiment, the peptide consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof.

In one embodiment, the peptide, or salt thereof, is present in the formulation at a concentration of about 0.03% to about

6

1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In various further embodiments, the peptide, or salt thereof, is present in the formulation at a concentration of about 0.03% to about 0.75%; about 0.03% to about 0.5%; about 0.03% to about 0.25%; about 0.03% to about 0.1%; about 0.03% to about 0.075%; about 0.03% to about 0.05%; and about 0.03%; all on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

In a further embodiment, the pharmaceutical formulation comprises about 1% to about 3% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. These formulations comprising low percentage HEC (ie: 2%) matrices provided a 10-fold increase in peptide release over a 24 hour period from formulations such as those comprising 10% carboxymethylcellulose (CMC), a result that would be unexpected to those of skill in the art. Furthermore, the data show that the HEC matrices are more biocompatible than HPMC and CMC formulations tested. In a still further embodiment, the pharmaceutical formulation comprises about 2% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In another embodiment, the formulation comprises a hydrogel formulation.

In one embodiment, the formulation is topical gel-based formulation selected from the group consisting of hydrogels, creams, ointments, pastes, and lotions. The formulation is administered so as to form a continuous film covering the entire area of the ulcer, including the margins, without running off. In a preferred embodiment, the topical formulation is applied with a thickness of approximately 0.25 to 2 mm; preferably 0.5 to 1.5 mm; preferably about 1 mm in thickness. In another non-limiting embodiment the formulation is applied at approximately 0.075 mL per cm² surface area.

In all aspects and embodiments of the invention, suitable acids which are capable of forming salts with the peptides include, but are not limited to, inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid and the like; and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid and the like. Suitable bases capable of forming salts with the peptides include, but are not limited to, inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide and the like; and organic bases such as mono-, di- and tri-alkyl and aryl amines (e.g., triethylamine, diisopropyl amine, methyl amine, dimethyl amine and the like) and optionally substituted ethanol-amines (e.g., ethanolamine, diethanolamine and the like).

The pharmaceutical formulations may further comprise (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer. In some embodiments, the buffer in the pharmaceutical formulations is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The pharmaceutical formulations may also include a lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain embodiments, the pharmaceutical formulations includes a preservative e.g. benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the pharmaceutical formulations includes a bulking agent, like glycine. In yet other embodiments, the pharmaceutical formulations includes a surfactant e.g., polysorbate-20, polysorbate-40, polysorbate-60, polysorbate-65, polysorbate-80

16

7

polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleate, or a combination thereof. The pharmaceutical formulations may also include a tonicity adjusting agent, e.g., a compound that renders the formulation substantially isotonic or isoosmotic with human blood. Exemplary tonicity adjusting agents include sucrose, sorbitol, glycine, methionine, mannitol, dextrose, inositol, sodium chloride, arginine and arginine hydrochloride. In other embodiments, the pharmaceutical formulations additionally includes a stabilizer, e.g., a molecule which, when combined with a protein of interest substantially prevents or reduces chemical and/or physical instability of the protein of interest in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride.

The peptides or salts thereof can further be derivatized to provide enhanced half-life, for example, by linking to polyethylene glycol. The peptides or salts thereof may comprise L-amino acids, D-amino acids (which are resistant to L-amino acid-specific proteases in vivo), a combination of D- and L-amino acids, and various "designer" amino acids (e.g., β -methyl amino acids, α -methyl amino acids, and $N\alpha$ -methyl amino acids, etc.) to convey special properties.

The peptide may be the sole active agent in the pharmaceutical composition, or the composition may further comprise one or more other active agents suitable for treating diabetic ulcers.

The formulations may include any other embodiments as disclosed in the example that follows. Such embodiments may be used in any combination in the formulations of the invention, unless the context clearly dictates otherwise.

EXAMPLE

Randomized, Parallel-Group, Double-Blind,
Placebo-Controlled Phase 2 Clinical Trial to
Evaluate the Safety and Effectiveness of
NorLeu³-A(1-7) in Treating Subjects with Diabetic
Ulcers

The study was designed as a randomized, parallel-group, double-blind, placebo-controlled, multi-center trial. After 14-days of best-of-care to evaluate ulcer healing and ensure the wounds were chronic, four-weeks of active treatment were then followed by eight weeks of observation and assessment. The study compared the effects of two concentrations of NorLeu³-A(1-7) and placebo, measuring both clinical efficacy and safety. Sustained tissue integrity was evaluated for all subjects during a follow-up period lasting 12 weeks after complete wound closure.

Subjects were randomized in a 1:1:1 ratio to one of the three treatment groups:

Group 1: Placebo Vehicle Control without NorLeu³-A(1-7) (also referred to as "DSC127") (2% Hydroxyethyl Cellulose (HEC) with 0.1% methyl paraben, 0.02% propyl paraben)

Group 2: 0.03% NorLeu³-A(1-7) in Vehicle

Group 3: 0.01% NorLeu³-A(1-7) in Vehicle

The four-week treatment period required daily application of the treatment to the wound site. The first application each week was at the clinic and for the remainder of the week the patient self-administered the treatment.

8

If wound healing occurred during the treatment or assessment periods a final assessment visit was conducted and the integrity was assessed 4 and 12 weeks later (usually weeks 16 and 24 of the study).

Inclusion Criteria

The following patients were considered eligible for participation in the study:

- 1) Male or female ambulatory subjects who were at least 18 years of age at screening
- 2) At start of Screening Period and upon enrollment into the study, had at least one chronic non-healing Wagner Grade 1 or Grade 2 (ulcers of partial or full thickness and not involving bone, tendon or capsule (probing to tendon or capsule), and that have no sign of infection or myelitis) plantar neuropathic diabetic ulcer between 1.0-6.0 cm² on the midfoot or forefoot, including the toes but excluding the heel. Non-healing is defined as present for a minimum of one month but not longer than ten months with less than 30% reduction in size in response to treatment (non-study treatment, but including off-loading) during the Screening Period. If more than one ulcer is present that meets the inclusion criteria, the larger was studied and treated according to the protocol. Non-study ulcers were treated according to institutional best practice, using per protocol offloading.
- 3) Have an Ankle Brachial Index (ABI) greater than 0.7 for neuroischemic or greater than 0.8 for neuropathic DFU.
- 4) Have a Tissue Oxygen Pressure (TcPO₂) greater than 40 mm Hg or great toe systolic pressure greater than 50 mmHg to ensure healing potential.
- 5) Have Type I or Type II diabetes under metabolic control as confirmed by a glycosylated hemoglobin (HbA1c) of less than or equal to 12%, and a serum creatinine level of no greater than 3 mg/dL obtained within 3 months of study enrollment.
- 6) Assess the baseline level of neuropathy of the foot using Semmes-Weinstein filaments. Patients were considered to have site specific neuropathy sufficient for loss of protective sensation (LOPS) if they were unable to feel a #5.07 monofilament applied to at least 5 of the following 7 sites (28) on the study foot:
 - plantar to toes and metatarsals 1, 3 and 5 (3 sites)
 - plantar to midfoot medial and lateral (2 sites)
 - plantar heel (1 site)
 - dorsal distal first interspace (1 site)
- 7) Female subjects of child-bearing potential must have negative pregnancy test at the time of initiation of study therapy.
- 8) Female subjects of child-bearing potential must have been willing to use a medically acceptable method of birth control, such as Essure®, hormonal contraception (oral pills, implantable device or skin patch), intrauterine device, tubal ligation or double barrier, during the treatment and assessment period of study participation.
- 9) Ability and willingness to understand and comply with study procedures and to give written informed consent prior to enrollment in the study or initiation of study procedures.

Exclusion Criteria

If a subject met any of the following criteria, he or she was excluded from the study:

- 1) Has a known hypersensitivity to any of the study medication components.
- 2) Exposure to any investigational agent within 30 days of entry into study.
- 3) Females who are pregnant or nursing.

- 4) Females unwilling to use a medically acceptable method of birth control, such as Essure®, hormonal contraception (oral pills, implantable device or skin patch), intrauterine device, tubal ligation or double barrier, during the treatment and assessment period of study participation.
- 5) Active malignant disease of any kind A subject, who has had a malignant disease in the past, was treated and is currently disease-free, may be considered for study entry.
- 6) Chronic renal insufficiency (serum creatinine during screening is greater than 3.0 g/dL obtained within 3 months of study enrollment).
- 7) Chronic liver dysfunction evidenced by transaminase levels greater than twice normal.
- 8) Receiving hemodialysis or chronic ambulatory peritoneal dialysis (CAPD) therapy.
- 9) Resting blood pressure (at the time of the initial visit of the Screening Period) which exceeds 160 systolic and/or 90 diastolic mmHg on 3 consecutive readings at least 15 minutes apart.
- 10) Prior radiation therapy of the foot with the ulcer under study.
- 11) Current use of corticosteroids (within past 8 weeks), immunosuppressants (within past 8 weeks).
- 12) Known to be HIV positive.
- 13) Subjects whose ulcer was primarily ischemic in etiology as diagnosed by an ABI of <0.7 or great toe systolic pressure <40 mmHg or TcPO₂<40 mmHg in the supine position and <40 mmHg while sitting, measured on the forefoot with electrode set at 44 C.
- 14) Sickle-cell anemia, Raynaud's or other peripheral vascular disease.
- 15) Current history of drug abuse.
- 16) Subjects receiving a biologic agent to include growth factors and skin equivalents (Regranex™, Apligraf™, or Dermagraft™) in the past 30 days.
- 17) Subjects with uncontrolled diabetes defined as a glycosylated hemoglobin (HbA_{1c})>12%, or a serum creatinine level of greater than 3 g/dL obtained within 3 months of study enrollment determined on two separate occasions at least 3 weeks apart.
- 18) Subject with an ulcer which is determined to be clinically infected and requires topical antimicrobials or agents known to affect wound healing or has been taking systemic antibiotics for more than 7 days for any reason.
- 19) Subject with a Wagner Grade 3 or higher DFU, deep abscess or infection of the joint or tendon, or gangrene or osteomyelitis.
- 20) An EKG with a marked baseline prolongation of QT/QTc interval (e.g., repeated demonstration of QTc interval >450 milliseconds (ms))

A total of 80 subjects were enrolled in the study; 27 were randomized to the 0.03% dose of NorLeu³-A(1-7), 25 were randomized to placebo and 28 were randomized to the 0.01% dose. All subjects also received best standard of care, which included debridement, wound cleansing, application of an occlusive dressing and standardized proper off-loading.

Results

Recent results from a Phase 2 clinical trial with NorLeu³-A(1-7) in patients with diabetic foot ulcers showed that the proportion of study ulcers healed by 12 weeks as defined by 100% epithelialized with no drainage, as well as all secondary endpoints. The double-blind, placebo-controlled, multi-center clinical trial randomized 80 subjects to receive one of two doses of NorLeu³-A(1-7) (0.03% and 0.01%) or vehicle placebo (2% hydroxyethyl cellulose in phosphate buffer with 0.1% methyl paraben and 0.02% propyl paraben) control, in

addition to best standard of care (which included debridement). The drug was well-tolerated and there were no significant adverse events associated with NorLeu³-A(1-7) treatment.

In the Intent-to-Treat (ITT) population (all subjects receiving any study medication and from whom any post-baseline data are available): Results show that 54% of the diabetic wounds treated with 0.03% (high dose) of NorLeu³-A(1-7) achieved 100% closure in 12 weeks or less, compared with 33% of patients receiving placebo control, and 30% of patients receiving the 0.01% dose (low dose) of NorLeu³-A(1-7). Based on odds ratio analysis, patients treated with NorLeu³-A(1-7) 0.03% were 2.3 times more likely to have their wounds heal completely as compared to patients treated with placebo/standard of care.

In the Per Protocol (PP) population (all patients that did not have a major protocol violation affecting efficacy): Results show that 65% of the diabetic wounds treated with 0.03% dose of NorLeu³-A(1-7) achieved 100% closure in 12 weeks or less, compared with 38% of patients receiving placebo control, and 28% of patients receiving the 0.01% dose of NorLeu³-A(1-7). See FIGS. 1-3. Based on odds ratio analysis, patients treated with NorLeu³-A(1-7) 0.03% were 3.0 times more likely to have their wounds heal completely as compared to patients treated with placebo/standard of care.

The high dose of NorLeu³-A(1-7) well exceeded the trial's primary endpoint measurement target of an improvement of 15 percentage points in complete healing of wounds within the 12-week duration of the study for both the ITT (21% increase) and PP (27% increase) populations compared with placebo.

The trial was not powered for statistical significance, however there was a statistically significant (p=0.049) 50% improvement in the rate of healing in the PP high-dose population through 12 weeks of treatment compared with the control arm, as measured by depth of ulcer using covariate analysis.

The wound healing rates for patients on NorLeu³-A(1-7) were remarkable with the ITT analysis showing a 21% increase and the PP analysis showing a 27% increase over the placebo arm.

REFERENCES

1. American Diabetes Association, Consensus development conference on diabetic foot wound care. *Diabetes Care* 1999; 22:1354-1360.
2. Pecoraro R E, Reiber G E, Burgess E M, Pathways to diabetic limb amputation: basis for prevention. *Diabetes Care* 1990; 13:513-521.
3. Rodgers K E, Roda N, Felix J C, Espinoza T, Maldonado S, diZerega G S. Histological evaluation of the effects of angiotensin peptides on wound repair in diabetic mice. *Experimental Dermatology* 2003; 12(6):784-790.
4. Rodgers K, Xiong S, Felix J, Roda N, Espinoza T, Maldonado S, diZerega G S. Development of angiotensin (1-7) as an agent to accelerate dermal repair. *Wound Repair Regen* 2001; 9:238-250.
5. Rodgers K E, Espinoza T, Felix J, Roda N, Maldonado S, diZerega G S. Acceleration of healing, reduction of fibrotic scar, and normalization of tissue architecture by an angiotensin analogue, Norleu³-A (1-7). *Plast Reconstr Surg* 2003; 111:1195-1206.
6. Wagner, F J. A classification and treatment program for diabetic, neuropathic, and dysvascular foot problems. *Am Acad of Orthopaedic Surgeons. Instructional Course Lecture* 1979; 28:143-165.

7. Foster A V, Eaton C, McConville D O, Edmonds M E. Application of OpSite film: a new and effective treatment of painful diabetic neuropathy. *Diabetes Med* 1994; 11(8): 768-772.
8. Sheehan P, Jones P, Caselli A, Giurini J M, Veves A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. *Diabetes Care* 2003; 26:1879-1882.
9. Rodgers, K E, Abiko M, Girgis W, St. Amand K M, Campeau J D, diZerega G S. Acceleration of dermal tissue repair by Angiotensin II. *Wound Repair Regen* 1997; 5:175-183.
10. Rodgers, K E, DeCherney A H, St. Amand K M, Dougherty W R, Felix J C, Girgis W, diZerega G S. Histologic alterations in dermal repair after thermal injury: effects of topical angiotensin II. *Burn Care and Rehabilitation* 1997; 18:381-388.
11. Okuyama N, Roda N, Guerrero A, Dougherty W, Nguyen T, diZerega G S, Rodgers K E. Effect of angiotensin II on the viability, vascularity of random flaps in a rat model. *Annals Plastic Surgery Res* 1999; 68:913-918.
12. Rodgers K E, Ellefson D D, Espinoza T, Roda N, Maldonado S, diZerega G S. Effect of NorLeu³-A (1-7) on scar formation over time after full thickness incision injury in the rat. *Wound Repair Regen* 2005; 13:309-317.
13. Santos R A, Brosnihan K B, Chappell M C, Pesquero J, Chernicky C L, Greene L J, and Ferrario C M. Converting enzyme activity and angiotensin metabolism in the dog brainstem. *Hypertension* 1988; 11 (suppl 4):53-1-57.
14. Santos R A S, Brosnihan K B, Jacobsen D W, DiCorleto P, and Ferrario C M. Production of Ang-(1-7) by human vascular endothelium. *Hypertension* 1992; 19 (suppl II): II-56-II-61.
15. Santos et al. Characterization of a new angiotensin antagonist selective for angiotensin-(1-7): evidence that the actions of angiotensin-(1-7) are mediated by specific angiotensin receptors. *Brain Res Bull* 1994; 35:293-298.
16. Yamamoto K, Chappell M C, Brosnihan K B, Ferrario C M. In vivo metabolism of angiotensin I by neutral endopeptidase (EC 3.4.24.11) in spontaneously hypertensive rats. *Hypertension* 1992; 19:692-696.
17. Chappell M C, Tallant E A, Brosnihan K B, Ferrario C M. Processing of angiotensin peptides by NG108-15 neuroblastoma X glioma hybrid cell line. *Peptides* 1990; 22:375-380.
18. Chappell M C, Jacobsen D W, Tallant E A. Characterization of angiotensin II receptor subtypes in pancreatic acinar AR42J cells. *Peptides* 1995; 16:741-747.

19. Chappell M C, Tallant E A, Brosnihan K B, Ferrario C M. Conversion of angiotensin I to angiotensin-(1-7) by thimet oligopeptidase (E.C.3.4.24.15) in vascular smooth muscle cells. *J Vasc Biol Med* 1995; 5:129-137.
20. Daemen M J A P, Lombardi D M, Bosman F T, Schwartz S M. Angiotensin II induces smooth muscle cell proliferation in the normal and injured rat arterial wall. *Circ Res* 1991; 68:450-56.
21. Dzau V E, Pratt R, Gibbons G, Schunkert H, Lorell B, Ingelfinger J. Molecular mechanism of angiotensin in the regulation of vascular and cardiac growth. *J Mol Cell Cardiol* 1989; 21 [Suppl III]:S7.
22. Naftilan A J, Pratt R E, Dzau V J. Induction of platelet-derived growth factor A-chain and c-myc gene expression by Angiotensin II in culture rat vascular smooth muscle cells. *J Clin Invest* 1989; 83:1419-24.
23. Stouffer G A, Owens G K. Angiotensin II induced mitogenesis of spontaneously hypertensive rat derived cultured smooth muscle cells is dependent on autocrine production of transforming growth factor- β . *Circ Res* 1992; 70:820-28.
24. Koibuchi Y, Lee W S, Gibbons G H, Pratt R E. Role of transforming growth factor 13-1 in the cellular growth response to Angiotensin II. *Hypertension* 1993; 21:1046-50.
25. Kawahara Y, Sunako M, Tsuda T, Fukazaki H, Fukumoto Y, Takai Y. Angiotensin II induces expression of the c-fos gene through protein kinase C activation and calcium ion mobilization in cultured vascular smooth muscle cells. *BBRC* 1988; 150:52-9.
26. Mangiarua E I, Palmer V L, Lloyd L L, McCumbee W D. Platelet-derived growth factor mediates angiotensin II-induced DNA synthesis in vascular smooth muscle cells. *Arch Physiol Biochem* 1997; 105(2):151-7.
27. Su E J, Lombardi D M, Wiener J, Daemen M J, Reidy M A, and Schwartz M A. Mitogenic effect of angiotensin II on the rat carotid arteries and type II or III mesenteric microvessels but not type I mesenteric microvessels is mediated by endogenous basic fibroblast growth factor. *Circ Res* 1998; 82:321.
28. Rodgers L C, Driver V R, Armstrong D G. Assessment of the diabetic foot. In Krasner D L, Rodeheaver G T, Sibbald R G eds. *Chronic Wound Care: A Clinical Source Book for Healthcare Professionals*. 4th ed. Malvern Pa.: HMP Communications, 2007: 549-556.
29. Bolton L, McNees P, van Rijswijk L et al. Wound healing outcomes using standardized care. *JWOCN* 2004; 31:65-71.

SEQUENCE LISTING

```

<160> NUMBER OF SEQ ID NOS: 3

<210> SEQ ID NO 1
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa is Nle

<400> SEQUENCE: 1

```

-continued

Asp Arg Xaa Tyr Ile His Pro
1 5

<210> SEQ ID NO 2
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa is Nle

<400> SEQUENCE: 2

Asp Arg Xaa Tyr Ile
1 5

<210> SEQ ID NO 3
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa is Nle

<400> SEQUENCE: 3

Asp Arg Xaa Tyr Ile His
1 5

We claim:

1. A pharmaceutical formulation, comprising:
 - (a) 0.5% to 4% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis; and
 - (b) a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or a salt thereof;
 wherein the peptide, or salt thereof is present at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
2. The pharmaceutical formulation of claim 1, wherein the peptide, or salt thereof comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1) or a salt thereof.
3. The pharmaceutical formulation of claim 1, wherein the peptide, or salt thereof consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1) or a salt thereof.
4. The pharmaceutical formulation of claim 1, comprising 1% to 3% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
5. The pharmaceutical formulation of claim 1 comprising 2% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
6. The pharmaceutical formulation of claim 1, wherein the formulation comprises a hydrogel formulation.
7. The pharmaceutical formulation of claim 1, wherein the peptide, or salt thereof, is present at a concentration of about 0.03% to about 0.5% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
8. The pharmaceutical formulation of claim 1, wherein the formulation is selected from the group consisting of a cream, an ointment, a paste, and a lotion.
9. The pharmaceutical formulation of claim 1, wherein the formulation further comprises one or more of (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and (g) a buffer.
10. The pharmaceutical formulation of claim 1, further comprising one or more other active agents suitable for treating diabetic ulcers.
11. The pharmaceutical formulation of claim 4, wherein the peptide, or salt thereof consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or a salt thereof.
12. The pharmaceutical formulation of claim 5, wherein the peptide, or salt thereof consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or a salt thereof.
13. The pharmaceutical formulation of claim 6, wherein the peptide, or salt thereof consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or a salt thereof.
14. The pharmaceutical formulation of claim 7, wherein the peptide, or salt thereof consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or a salt thereof.
15. The pharmaceutical formulation of claim 8, wherein the peptide, or salt thereof consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or a salt thereof.
16. The pharmaceutical formulation of claim 9, wherein the peptide, or salt thereof consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or a salt thereof.
17. The pharmaceutical formulation of claim 10, wherein the peptide, or salt thereof consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or a salt thereof.
18. A method for treating diabetic foot ulcers, comprising administering to a human patient suffering from a diabetic foot ulcer an amount of a pharmaceutical formulation according to claim 1 effective to treat the diabetic foot ulcer.
19. The method of claim 18, wherein the peptide, or salt thereof, comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1).
20. The method of claim 18, wherein the peptide, or salt thereof, consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1).

21. The method of claim 18, wherein the diabetic foot ulcer is one caused, at least in part, by neuropathy and resulting pressure.

22. The method of claim 18, wherein the diabetic foot ulcer comprises one or more calluses. 5

23. The method of claim 18, wherein the diabetic foot ulcer is a chronic ulcer.

24. The method of claim 23, wherein the chronic foot ulcer has not responded to any other treatment.

25. The method of claim 18, wherein the peptide, or salt 10 thereof, is administered topically.

26. The method of claim 25, wherein the peptide, or salt thereof, is administered as a topical formulation forming a continuous film covering the entire area of the diabetic ulcer.

27. The method of claim 25, wherein the peptide, or salt 15 thereof, is administered in a hydrogel formulation.

* * * * *



US008207233B1

(12) **United States Patent**
Rodgers et al.

(10) **Patent No.:** **US 8,207,233 B1**
(45) **Date of Patent:** **Jun. 26, 2012**

(54) **METHODS FOR TREATING DIABETIC FOOT ULCERS**

(75) **Inventors:** **Kathleen E. Rodgers**, Long Beach, CA (US); **Gere S. diZerega**, San Luis Obispo, CA (US)

(73) **Assignee:** **University of Southern California**, Los Angeles, CA (US)

(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(h) by 0 days.

(21) **Appl. No.:** **13/364,061**

(22) **Filed:** **Feb. 1, 2012**

Related U.S. Application Data

(60) **Provisional application No.** **61/438,780**, filed on Feb. 2, 2011.

(51) **Int. Cl.**
A61K 38/08 (2006.01)
A61K 47/38 (2006.01)
C07K 7/14 (2006.01)
C07K 17/04 (2006.01)
C07K 17/12 (2006.01)

(52) **U.S. Cl.** **514/928; 424/488; 530/316; 930/40**

(58) **Field of Classification Search** **None**
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,015,629 A 5/1991 DiZerega et al.
5,629,292 A 5/1997 DiZerega et al.
5,716,935 A 2/1998 DiZerega et al.
5,834,432 A 11/1998 DiZerega et al.
5,955,430 A 9/1999 DiZerega et al.
6,096,709 A 8/2000 DiZerega et al.
6,110,895 A 8/2000 DiZerega et al.
6,165,978 A 12/2000 DiZerega et al.
6,177,407 B1 1/2001 Rodgers et al.
6,248,587 B1 6/2001 Rodgers et al.
6,258,778 B1 7/2001 Rodgers et al.
6,335,195 B1 1/2002 Rodgers et al.
6,444,646 B1 9/2002 DiZerega et al.
6,455,500 B1 9/2002 Rodgers et al.
6,455,501 B1 9/2002 DiZerega et al.
6,475,988 B1 11/2002 Rodgers et al.
6,482,800 B1 11/2002 Rodgers et al.
6,498,138 B1 12/2002 Rodgers et al.
6,566,335 B1 5/2003 Rodgers et al.
6,730,775 B1 5/2004 DiZerega et al.
6,762,167 B1 7/2004 Rodgers et al.
6,821,953 B1 11/2004 DiZerega et al.
6,916,783 B2 7/2005 Rodgers et al.
7,022,675 B2 4/2006 DiZerega et al.
7,118,748 B1 10/2006 Rodgers et al.
7,173,011 B2 2/2007 Rodgers et al.
7,176,183 B2 2/2007 DiZerega et al.
7,338,938 B2 3/2008 Rodgers et al.
7,744,927 B2 6/2010 Rodgers et al.
7,776,828 B2 8/2010 Rodgers et al.
7,786,085 B2 8/2010 Rodgers et al.
2009/0227507 A1 9/2009 Rodgers et al.

FOREIGN PATENT DOCUMENTS

WO 98/33813 8/1998
WO 99/31125 6/1999
WO 99/40107 8/1999
WO 01/44270 6/2001
WO 2011/120032 3/2011
WO 2012/021578 2/2012

OTHER PUBLICATIONS

American Diabetes Association, Consensus development conference on diabetic foot wound care. *Diabetes Care* 1999; 22:1354-1360.
Pecoraro RE, Reiber GE, Burgess EM, Pathways to diabetic limb amputation: basis for prevention. *Diabetes Care* 1990; 13:513-521.
Rodgers KE, Roda N, Felix JC, Espinoza T, Maldonado S, diZerega GS. Histological evaluation of the effects of angiotensin peptides on wound repair in diabetic mice. *Experimental Dermatology* 2003; 12(6):784-790.
Rodgers K, Xiong S, Felix J, Roda N, Espinoza T, Maldonado S, diZerega GS. Development of angiotensin (1-7) as an agent to accelerate dermal repair. *Wound Repair Regen* 2001; 9:238-250.
Rodgers KE, Espinoza T, Felix J, Roda N, Maldonado S, diZerega GS. Acceleration of healing, reduction of fibrotic scar, and normalization of tissue architecture by an angiotensin analogue, Norleu3-A (1-7). *Plast Reconstr Surg* 2003; 111:1195-1206.
Wagner, FJ. A classification and treatment program for diabetic, neuropathic, and dysvascular foot problems. *Am Acad of Orthopaedic Surgeons. Instructional Course Lecture* 1979; 28:143-165.
Foster AV, Eaton C, McConville DO, Edmonds ME. Application of OpSite film: a new and effective treatment of painful diabetic neuropathy. *Diabetes Med* 1994; 11(8):768-772.
Sheehan P, Jones P, Caselli A, Giurini JM, Veves A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. *Diabetes Care* 2003; 26:1879-1882.
Rodgers, KE, Abiko M, Girgis W, St. Amand KM, Campeau JD, diZerega GS. Acceleration of dermal tissue repair by Angiotensin II. *Wound Repair Regen* 1997; 5:175-183.
Rodgers, KE, DeCherney AH, St. Amand KM, Dougherty WR, Felix JC, Girgis W, diZerega GS. Histologic alterations in dermal repair after thermal injury: effects of topical angiotensin II. *Burn Care and Rehabilitation* 1997; 18:381-388.
Okuyama N, Roda N, Guerrero A, Dougherty W, Nguyen T, diZerega GS, Rodgers KE. Effect of angiotensin II on the viability, vascularity of random flaps in a rat model. *Annals Plastic Surgery Res* 1999; 68:913-918.
Rodgers KE, Ellefson DD, Espinoza T, Roda N, Maldonado S, diZerega GS. Effect of NorLeu3-A (1-7) on scar formation over time after full thickness incision injury in the rat. *Wound Repair Regen* 2005; 13:309-317.
Santos RS, Brosnihan KB, Chappell MC, Pesquero J, Chernicky CL, Greene LJ, and Ferrario CM. Converting enzyme activity and angiotensin metabolism in the dog brainstem. *Hypertension* 1988; 11(suppl I):I-53-I-57.
Santos RAS, Brosnihan KB, Jacobsen DW, DiCorleto P, and Ferrario CM. Production of Ang-(1-7) by human vascular endothelium. *Hypertension* 1992; 19(suppl II):II-56-II-61.

(Continued)

Primary Examiner — David Romeo

(74) **Attorney, Agent, or Firm** — McDonnell Boehnen Hulbert & Berghoff LLP

(57) **ABSTRACT**

The present invention provides methods and pharmaceutical formulations for treating diabetic foot ulcers.

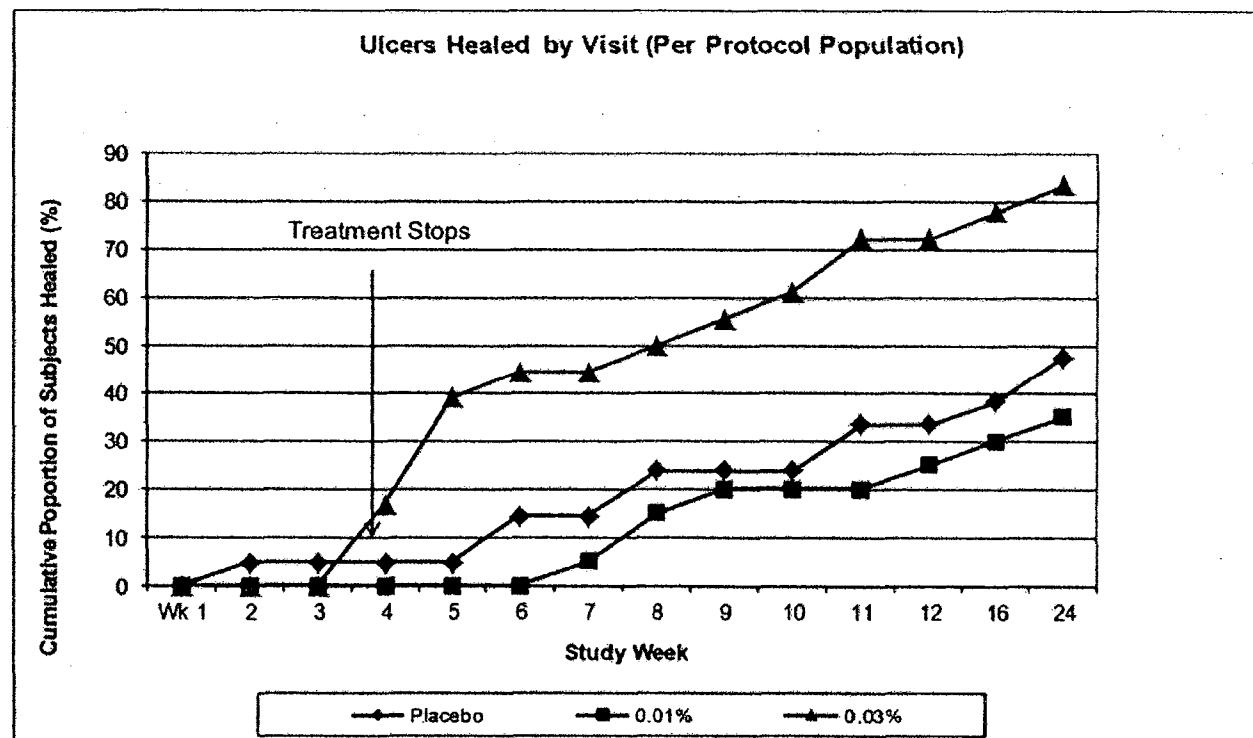
24 Claims, 3 Drawing Sheets

22

OTHER PUBLICATIONS

- Santos et al. Characterization of a new angiotensin antagonist selective for angiotensin-(1-7): evidence that the actions of angiotensin-(1-7) are mediated by specific angiotensin receptors. *Brain Res Bull* 1994; 35:293-298.
- Yamamoto K, Chappell MC, Brosnihan KB, Ferrario CM. In vivo metabolism of angiotensin I by neutral endopeptidase (EC 3.4.24.11) in spontaneously hypertensive rats. *Hypertension* 1992; 19:692-696.
- Chappell MC, Tallant EA, Brosnihan KB, Ferrario CM. Processing of angiotensin peptides by NG108-15 neuroblastoma X glioma hybrid cell line. *Peptides* 1990; 22:375-380.
- Chappell MC, Jacobsen DW, Tallant EA. Characterization of angiotensin II receptor subtypes in pancreatic acinar AR42J cells. *Peptides* 1995; 16:741-747.
- Chappell MC, Tallant EA, Brosnihan KB, Ferrario CM. Conversion of angiotensin I to angiotensin-(1-7) by thimet oligopeptidase (E.C. 3.4.24.15) in vascular smooth muscle cells. *J Vasc Biol Med* 1995; 5:129-137.
- Daemen MJAP, Lombardi DM, Bosman FT, Schwartz SM. Angiotensin II induces smooth muscle cell proliferation in the normal and injured rat arterial wall. *Circ Res* 1991; 68:450-56.
- Dzau VJ, Pratt R, Gibbons G, Schunkert H, Lorell B, Ingelfinger J. Molecular mechanism of angiotensin in the regulation of vascular and cardiac growth. *J Mol Cell Cardiol* 1989; 21 [Suppl III]:S7.
- Naftilan AJ, Pratt RE, Dzau VJ. Induction of platelet-derived growth factor A-chain and c-myc gene expression by Angiotensin II in culture rat vascular smooth muscle cells. *J Clin Invest* 1989; 83:1419-24.
- Kawahara Y, Sunako M, Tsuda T, Fukazaki H, Fukumoto Y, Takai Y. Angiotensin II induces expression of the c-fos gene through protein kinase C activation and calcium ion mobilization in cultured vascular smooth muscle cells. *BBRC* 1988; 150:52-9.
- Mangiarua EI, Palmer VL, Lloyd LL, McCumbee WD. Platelet-derived growth factor mediates angiotensin II-induced DNA synthesis in vascular smooth muscle cells. *Arch Physiol Biochem* 1997; 105(2):151-7.
- Su EJ, Lombardi DM, Wiener J, Daemen MJ, Reidy MA, and Schwartz MA. Mitogenic effect of angiotensin II on the rat carotid arteries and type II or III mesenteric microvessels but not type I mesenteric microvessels is mediated by endogenous basic fibroblast growth factor. *Circ Res* 1998; 82:321.
- Rodgers LC, Driver VR, Armstrong DG. Assessment of the diabetic foot. In Krasner DL, Rodeheaver GT, Sibbald RG eds. *Chronic Wound Care: A Clinical Source Book for Healthcare Professionals*. 4th ed. Malvern PA: HMP Communications, 2007: 549-556.
- Bolton L, McNees P, van Rijswijk L et al. Wound healing outcomes using standardized care. *JWOCN* 2004; 31:65-71.
- Stouffer GA, Owens GK. Angiotensin II induced mitogenesis of spontaneously hypertensive rat derived cultured smooth muscle cells is dependent on autocrine production of transforming growth factor- β . *Circ Res* 1992; 70:820-28.
- Koibuchi Y, Lee WS, Gibbons GH, Pratt RE. Role of transforming growth factor β -1 in the cellular growth response to Angiotensin II. *Hypertension* 1993; 21:1046-50.
- Rodgers, et al., (2006) *J. Peptide Res.*, 66(suppl. 1):41-47.

Figure 1

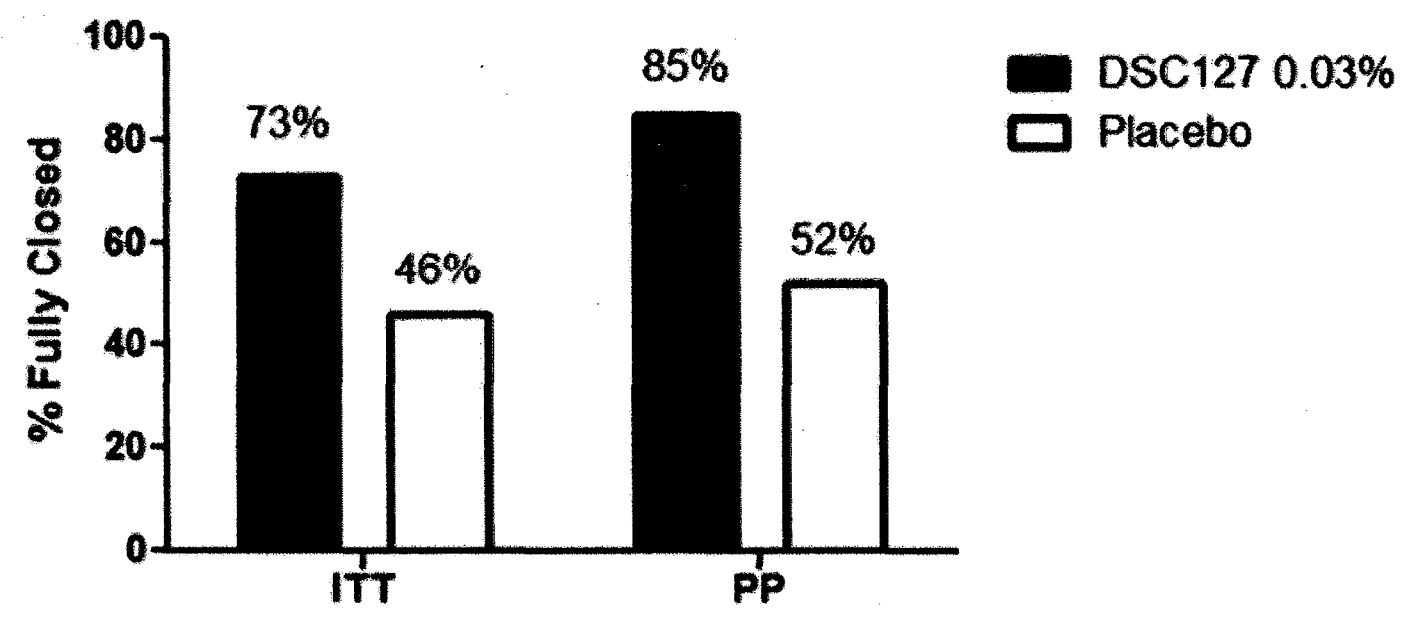


24

24

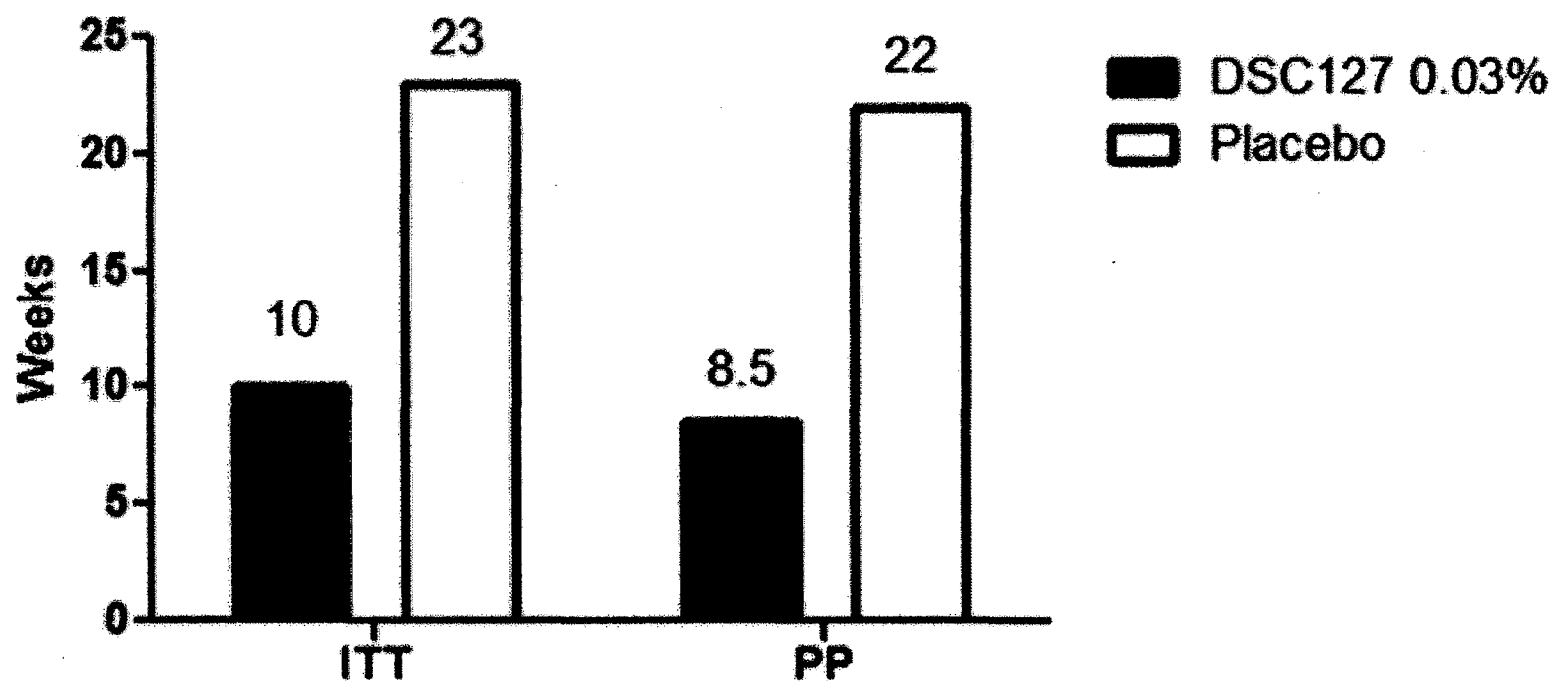
25

Figure 2



25

Figure 3



METHODS FOR TREATING DIABETIC FOOT
ULCERS

CROSS REFERENCE

This application claims priority to U.S. Provisional Patent Application Ser. No. 61/438,780 filed Feb. 2, 2011, incorporated by reference herein in its entirety.

BACKGROUND

Diabetes is common, disabling and deadly. In the U.S., diabetes has reached epidemic proportions. According to the American Diabetes Association, about 24 million people (8% of the total U.S. population) have diabetes, and nearly two million new cases are diagnosed in people aged 20 years or older each year. If current trends continue, 1 in 3 Americans will develop diabetes at some point in their lifetime, and those with diabetes will lose, on average, 10-15 years of life expectancy. Importantly, up to 25% of people with diabetes will develop a diabetic foot ulcer, resulting in 3 million diabetic foot ulcers annually in the U.S. alone. More than half of all foot ulcers will become infected, thus requiring hospitalization, and 1 in 5 will require an amputation that carries a high risk of mortality.

Without question, diabetes puts tremendous economic pressure on the U.S. healthcare system. Total costs (direct and indirect) of diabetes have reached \$174 billion annually, and people with diagnosed diabetes have medical expenditures that are over two times higher than medical expenditures for people without diabetes. Hospitalization costs alone are \$16,000 to \$20,000 for a patient with a diabetic foot ulcer, and direct and indirect costs of an amputation range from \$20,000 to \$60,000 per patient. A recent study by researchers at the University of Chicago suggested that treatment costs for diabetes in the United States would reach \$336 billion by the year 2034. Advanced, cost-effective treatment modalities for diabetes and its co-morbidities, including diabetic foot ulcers, are in great need, yet in short supply, globally. According to the American Diabetes Association, by the year 2025 the prevalence of diabetes is expected to rise by 72% to 324 million people worldwide.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides methods for treating diabetic foot ulcers, comprising administering to a human patient suffering from a diabetic foot ulcer an amount of a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof, effective to treat the diabetic foot ulcer. In one embodiment, the peptide comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In another embodiment, the peptide consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In various embodiments, the diabetic foot ulcer is one caused, at least in part, by neuropathy and resulting pressure; the diabetic foot ulcer comprises one or more calluses; and the diabetic foot ulcer is a chronic ulcer. In a further embodiment, the chronic foot ulcer has not responded to any other treatment.

In another embodiment, the peptide is administered topically. In a further embodiment, the peptide is administered as a topical formulation forming a continuous film covering the entire area of the diabetic ulcer. In a still further embodiment, the peptide is administered in a hydrogel formulation. In another embodiment, the peptide is administered at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In a further

embodiment, the peptide is administered in a gel formulation that about 0.5% to about 4% hydroxyethyl cellulose (HEC) on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

All embodiments of the first aspect of the invention can be combined unless the context dictates otherwise.

In a second aspect, the present invention provides pharmaceutical formulations, comprising:

(a) about 0.5% to about 4% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis; and

(b) a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof;

wherein the peptide is present at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

In one embodiment, the peptide comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In another embodiment, the peptide consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In a further embodiment, the pharmaceutical formulation comprises about 1% to about 3% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In a still further embodiment, the pharmaceutical formulation comprises about 2% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In another embodiment, the formulation comprises a hydrogel formulation. All embodiments of the second aspect of the invention can be combined unless the context dictates otherwise.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a graph showing the ulcers healed by visit in the Per Protocol (PP) patient population taking NorLeu³-A(1-7).

FIG. 2 is a graph showing the percentage of fully healed ulcers (100% wound closure) in the patient population taking NorLeu³-A(1-7) (also referred to as "DSC127").

FIG. 3 is a graph showing the median time to fully healed ulcers (100% wound closure) in the patient population taking NorLeu³-A(1-7) (also referred to as "DSC127").

DETAILED DESCRIPTION OF THE INVENTION

All references cited are herein incorporated by reference in their entirety. Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991, Academic Press, San Diego, Calif.), "Guide to Protein Purification" in *Methods in Enzymology* (M. P. Deutscher, ed., (1990) Academic Press, Inc.); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990, Academic Press, San Diego, Calif.), *Culture of Animal Cells: A Manual of Basic Technique*, 2nd Ed. (R. I. Freshney. 1987, Liss, Inc. New York, N.Y.), *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E. J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, Tex.).

As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. "And" as used herein is interchangeably used with "or" unless expressly stated otherwise.

All embodiments of any aspect of the invention can be used in combination, unless the context clearly dictates otherwise.

In a first aspect, the present invention provides methods for treating diabetic foot ulcers, comprising administering to a human patient suffering from a diabetic foot ulcer an amount

of a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof, effective to treat the diabetic foot ulcer.

As demonstrated in the examples that follow, the inventors have demonstrated that the methods of the invention can be used to treat diabetic foot ulcers, and provide substantial improvement over standard therapies.

Nle3A(1-7) (or NorLeu³-A(1-7); also referred to as "DSC127") is a peptide consisting of the amino acid sequence Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1). In various embodiments, the peptide administered to the human patient may comprise or consist of Asp-Arg-Nle-Tyr-Ile (SEQ ID NO:2), Asp-Arg-Nle-Tyr-Ile-His (SEQ ID NO:3), or most preferably Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salts thereof. Nle3A(1-7) or salts thereof may be chemically synthesized or recombinantly expressed, each of which can be accomplished using standard methods in the art.

In one embodiment, the peptide, or salt thereof, is administered at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In various further embodiments, the peptide, or salt thereof, is administered at a concentration of about 0.03% to about 0.75%; about 0.03% to about 0.5%; about 0.03% to about 0.25%; about 0.03% to about 0.1%; about 0.03% to about 0.075%; about 0.03% to about 0.05%; and about 0.03%; all on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

The human patient may be suffering from Type I diabetes or Type II diabetes, and has a foot ulcer, defined as an open wound anywhere on the foot (heel, mid-foot, and forefoot).

As used herein, "treating" a diabetic foot ulcer includes

- (a) limiting the progression in size, area, and/or depth of the foot ulcer;
- (b) reducing size, area, and/or depth of the foot ulcer;
- (c) increasing rate of healing and/or reducing time to healing;
- (d) healing of the foot ulcer (100% epithelialization with no drainage); and
- (e) decreased incidence of amputation or slowing in time to amputation.

The foot ulcer may be caused by any underlying pathology, including but not limited to neuropathy, trauma, deformity, high plantar pressures, callus formation, edema, and peripheral arterial disease. In preferred embodiments, the human diabetic foot ulcer is one caused, at least in part, by neuropathy and resulting pressure (weight bearing on the extremity due to lack of feeling in the foot). As is known to those of skill in the art, human diabetic foot ulcers tend to be due to neuropathy and pressure, which differs significantly from, for example, murine acute wounds. In a further preferred embodiment, the diabetic foot ulcer comprises one or more calluses.

In a further embodiment, the diabetic foot ulcer is a chronic ulcer. As used herein, a "chronic" foot ulcer is one that has been present for at least 7 days with no reduction in size; preferably at least 14 days; even more preferably, present at least 21 or 28 days with no reduction in size. In a further preferred embodiment that can be combined with any of these embodiments, the chronic foot ulcer has not responded (ie: no reduction in size, area, and/or depth of the foot ulcer; no healing of the foot ulcer) to any other treatment.

The peptide or salt thereof may be administered by any suitable route, preferably via topical administration. In one embodiment, the methods of the invention can comprise administering a topical formulation as often as deemed appropriate, ie: once per day, twice per day, etc. The methods may further comprise administration of the peptide, or salt thereof for as longed as deemed desirable by an attending

physician, for example, until healing of the ulcer. For administration, it is preferred that the topical formulation form a continuous film covering the entire area of the ulcer, including the margins. In a preferred embodiment, the topical formulation is applied with a thickness of approximately 0.25 to 2 mm; preferably 0.5 to 1.5 mm; preferably about 1 mm in thickness.

In one embodiment, the topical administration comprises administration in a formulation selected from the group consisting of hydrogels, creams, ointments, pastes, and lotions. The formulations may be applied in any suitable manner, which may include any wound dressings to seal in the formulation deemed appropriate by the human patient or caregiver. Exemplary such dressings, include, but are not limited to, semipermeable films, foams, hydrocolloids, and calcium alginate swabs.

The methods may further comprise debridement in and around the wound in combination with administration of the peptide and formulations thereof. Debridement of all necrotic, callus, and fibrous tissue is typically carried for treatment of diabetic foot ulcers. Unhealthy tissue is sharply debrided back to bleeding tissue to allow full visualization of the extent of the ulcer and to detect underlying abscesses or sinuses. Any suitable debridement technique can be used, as determined by an attending physician. The wound can then be thoroughly flushed with sterile saline or a non-cytotoxic cleanser following debridement.

In another embodiment, the topical formulation comprises about 0.5% to about 4% hydroxyethyl cellulose (HEC) on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In various further embodiments, the topical formulation may comprise about 1% to about 3% HEC, or about 2% HEC, on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. These formulations comprising low percentage HEC (ie: 2%) matrices provided a 10-fold increase in peptide release over a 24 hour period from formulations such as those comprising 10% carboxymethylcellulose (CMC), a result that would be unexpected to those of skill in the art. Furthermore, the data show that the HEC matrices are more biocompatible than HPMC and CMC formulations tested.

The peptides, or salt thereof may be administered together with one or more (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer. In some embodiments, the buffer in the pharmaceutical composition is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The peptides may be administered with a lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain embodiments, the peptides may be administered with a preservative e.g. benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the peptides may be administered with a bulking agent, like glycine. In yet other embodiments, the peptides may be administered with a surfactant e.g., polysorbate-20, polysorbate-40, polysorbate-60, polysorbate-65, polysorbate-80 polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleate, or a combination thereof. The peptides may be administered with a tonicity adjusting agent, e.g., a compound that renders the formulation substantially isotonic or isoosmotic with human blood. Exemplary tonicity adjusting agents include sucrose, sorbitol, glycine, methionine, mannitol, dextrose, inositol, sodium chloride, arginine and arginine hydrochloride. In other

embodiments, the peptides may be administered with a stabilizer, e.g., a molecule which, when combined with the peptide substantially prevents or reduces chemical and/or physical instability of the protein of interest in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride, paraben, and combinations of methyl paraben and propyl paraben.

In all aspects and embodiments of the invention, suitable acids which are capable of forming salts with the peptides include, but are not limited to, inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid and the like; and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid and the like. Suitable bases capable of forming salts with the peptides include, but are not limited to, inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide and the like; and organic bases such as mono-, di- and tri-alkyl and aryl amines (e.g., triethylamine, diisopropyl amine, methyl amine, dimethyl amine and the like) and optionally substituted ethanol-amines (e.g., ethanolamine, diethanolamine and the like).

The peptides or salts thereof can further be derivatized to provide enhanced half-life, for example, by linking to polyethylene glycol. The peptides or salts thereof may comprise L-amino acids, D-amino acids (which are resistant to L-amino acid-specific proteases *in vivo*), a combination of D- and L-amino acids, and various "designer" amino acids (e.g., β -methyl amino acids, C α -methyl amino acids, and N α -methyl amino acids, etc.) to convey special properties.

The polypeptides may be the sole active agent in the pharmaceutical composition, or the composition may further comprise one or more other active agents suitable treating diabetic foot ulcers, such as antibiotics. The methods may be used in conjunction with other foot ulcer therapies, including but not limited to negative pressure wound therapy, total contact casts, removable cast walkers, half-shoes, becaplermin, infection control, and hyperbaric oxygen therapy.

The methods may include any other embodiments as disclosed in the example that follows. Such embodiments may be used in any combination in the methods of the invention, unless the context clearly dictates otherwise.

In a second aspect, the present invention provides pharmaceutical formulations, comprising:

(a) about 0.5% to about 4% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis; and

(b) a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof;

wherein the peptide is present at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

The pharmaceutical formulations are demonstrated herein to be particularly effective for treating diabetic foot ulcers, such as chronic diabetic foot ulcers that are not effectively treated using standard therapies.

In one embodiment, the peptide comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In another embodiment, the peptide consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof.

In one embodiment, the peptide, or salt thereof, is present in the formulation at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In various further embodiments, the pep-

tide, or salt thereof, is present in the formulation at a concentration of about 0.03% to about 0.75%; about 0.03% to about 0.5%;

about 0.03% to about 0.25%; about 0.03% to about 0.1%; about 0.03% to about 0.075%; about 0.03% to about 0.05%; and about 0.03%; all on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

In a further embodiment, the pharmaceutical formulation comprises about 1% to about 3% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. These formulations comprising low percentage HEC (ie: 2%) matrices provided a 10-fold increase in peptide release over a 24 hour period from formulations such as those comprising 10% carboxymethylcellulose (CMC), a result that would be unexpected to those of skill in the art. Furthermore, the data show that the HEC matrices are more biocompatible than HPMC and CMC formulations tested. In a still further embodiment, the pharmaceutical formulation comprises about 2% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In another embodiment, the formulation comprises a hydrogel formulation.

In one embodiment, the formulation is topical gel-based formulation selected from the group consisting of hydrogels, creams, ointments, pastes, and lotions. The formulation is administered so as to form a continuous film covering the entire area of the ulcer, including the margins, without running off. In a preferred embodiment, the topical formulation is applied with a thickness of approximately 0.25 to 2 mm; preferably 0.5 to 1.5 mm; preferably about 1 mm in thickness. In another non-limiting embodiment the formulation is applied at approximately 0.075 mL per cm² surface area.

In all aspects and embodiments of the invention, suitable acids which are capable of forming salts with the peptides include, but are not limited to, inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid and the like; and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid and the like. Suitable bases capable of forming salts with the peptides include, but are not limited to, inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide and the like; and organic bases such as mono-, di- and tri-alkyl and aryl amines (e.g., triethylamine, diisopropyl amine, methyl amine, dimethyl amine and the like) and optionally substituted ethanol-amines (e.g., ethanolamine, diethanolamine and the like).

The pharmaceutical formulations may further comprise (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer. In some embodiments, the buffer in the pharmaceutical formulations is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The pharmaceutical formulations may also include a lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain embodiments, the pharmaceutical formulations includes a preservative e.g. benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the pharmaceutical formulations includes a bulking agent, like glycine. In yet other embodiments, the pharmaceutical formulations includes a surfactant e.g., polysorbate-20, polysorbate-40, polysorbate-60, polysorbate-65, polysorbate-80, polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbi-

tan monopalmitate, sorbitan monostearate, sorbitan monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleate, or a combination thereof. The pharmaceutical formulations may also include a tonicity adjusting agent, e.g., a compound that renders the formulation substantially isotonic or isoosmotic with human blood. Exemplary tonicity adjusting agents include sucrose, sorbitol, glycine, methionine, mannitol, dextrose, inositol, sodium chloride, arginine and arginine hydrochloride. In other embodiments, the pharmaceutical formulations additionally includes a stabilizer, e.g., a molecule which, when combined with a protein of interest substantially prevents or reduces chemical and/or physical instability of the protein of interest in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride.

The peptides or salts thereof can further be derivatized to provide enhanced half-life, for example, by linking to polyethylene glycol. The peptides or salts thereof may comprise L-amino acids, D-amino acids (which are resistant to L-amino acid-specific proteases *in vivo*), a combination of D- and L-amino acids, and various "designer" amino acids (e.g., β -methyl amino acids, α -methyl amino acids, and $N\alpha$ -methyl amino acids, etc.) to convey special properties.

The peptide may be the sole active agent in the pharmaceutical composition, or the composition may further comprise one or more other active agents suitable for treating diabetic ulcers.

The formulations may include any other embodiments as disclosed in the example that follows. Such embodiments may be used in any combination in the formulations of the invention, unless the context clearly dictates otherwise.

Example Randomized, Parallel-Group,
Double-Blind, Placebo-Controlled Phase 2 Clinical
Trial to Evaluate the Safety and Effectiveness of
NorLeu³-A(1-7) in Treating Subjects with Diabetic
Ulcers

The study was designed as a randomized, parallel-group, double-blind, placebo-controlled, multi-center trial. After 14-days of best-of-care to evaluate ulcer healing and ensure the wounds were chronic, four-weeks of active treatment were then followed by eight weeks of observation and assessment. The study compared the effects of two concentrations of NorLeu³-A(1-7) and placebo, measuring both clinical efficacy and safety. Sustained tissue integrity was evaluated for all subjects during a follow-up period lasting 12 weeks after complete wound closure.

Subjects were randomized in a 1:1:1 ratio to one of the three treatment groups:

Group 1: Placebo Vehicle Control without NorLeu³-A(1-7) (also referred to as "DSC127") (2% Hydroxyethyl Cellulose (HEC) with 0.1% methyl paraben, 0.02% propyl paraben)

Group 2: 0.03% NorLeu³-A(1-7) in Vehicle

Group 3: 0.01% NorLeu³-A(1-7) in Vehicle

The four-week treatment period required daily application of the treatment to the wound site. The first application each week was at the clinic and for the remainder of the week the patient self-administered the treatment.

If wound healing occurred during the treatment or assessment periods a final assessment visit was conducted and the integrity was assessed 4 and 12 weeks later (usually weeks 16 and 24 of the study).

Inclusion Criteria

The following patients were considered eligible for participation in the study:

- 1) Male or female ambulatory subjects who were at least 18 years of age at screening
- 2) At start of Screening Period and upon enrollment into the study, had at least one chronic non-healing Wagner Grade 1 or Grade 2 (ulcers of partial or full thickness and not involving bone, tendon or capsule (probing to tendon or capsule), and that have no sign of infection or myelitis) plantar neuropathic diabetic ulcer between 1.0-6.0 cm² on the midfoot or forefoot, including the toes but excluding the heel. Non-healing is defined as present for a minimum of one month but not longer than ten months with less than 30% reduction in size in response to treatment (non-study treatment, but including off-loading) during the Screening Period. If more than one ulcer is present that meets the inclusion criteria, the larger was studied and treated according to the protocol. Non-study ulcers were treated according to institutional best practice, using per protocol offloading.
- 3) Have an Ankle Brachial Index (ABI) greater than 0.7 for neuroischemic or greater than 0.8 for neuropathic DFU.
- 4) Have a Tissue Oxygen Pressure (TcPO₂) greater than 40 mm Hg or great toe systolic pressure greater than 50 mmHg to ensure healing potential.
- 5) Have Type I or Type II diabetes under metabolic control as confirmed by a glycosylated hemoglobin (HbA_{1c}) of less than or equal to 12%, and a serum creatinine level of no greater than 3 mg/dL obtained within 3 months of study enrollment.
- 6) Assess the baseline level of neuropathy of the foot using Semmes-Weinstein filaments. Patients were considered to have site specific neuropathy sufficient for loss of protective sensation (LOPS) if they were unable to feel a #5.07 monofilament applied to at least 5 of the following 7 sites (28) on the study foot:
plantar to toes and metatarsals 1, 3 and 5 (3 sites)
plantar to midfoot medial and lateral (2 sites)
plantar heel (1 site)
dorsal distal first interspace (1 site)
- 7) Female subjects of child-bearing potential must have negative pregnancy test at the time of initiation of study therapy.
- 8) Female subjects of child-bearing potential must have been willing to use a medically acceptable method of birth control, such as Essure®, hormonal contraception (oral pills, implantable device or skin patch), intrauterine device, tubal ligation or double barrier, during the treatment and assessment period of study participation.
- 9) Ability and willingness to understand and comply with study procedures and to give written informed consent prior to enrollment in the study or initiation of study procedures.

Exclusion Criteria

If a subject met any of the following criteria, he or she was excluded from the study:

- 1) Has a known hypersensitivity to any of the study medication components.
- 2) Exposure to any investigational agent within 30 days of entry into study.
- 3) Females who are pregnant or nursing.
- 4) Females unwilling to use a medically acceptable method of birth control, such as Essure®, hormonal contraception (oral pills, implantable device or skin patch), intrauterine device, tubal ligation or double barrier, during the treatment and assessment period of study participation.

- 5) Active malignant disease of any kind A subject, who has had a malignant disease in the past, was treated and is currently disease-free, may be considered for study entry.
- 6) Chronic renal insufficiency (serum creatinine during screening is greater than 3.0 g/dL obtained within 3 months of study enrollment).
- 7) Chronic liver dysfunction evidenced by transaminase levels greater than twice normal.
- 8) Receiving hemodialysis or chronic ambulatory peritoneal dialysis (CAPD) therapy.
- 9) Resting blood pressure (at the time of the initial visit of the Screening Period) which exceeds 160 systolic and/or 90 diastolic mmHg on 3 consecutive readings at least 15 minutes apart.
- 10) Prior radiation therapy of the foot with the ulcer under study.
- 11) Current use of corticosteroids (within past 8 weeks), immunosuppressants (within past 8 weeks).
- 12) Known to be HIV positive.
- 13) Subjects whose ulcer was primarily ischemic in etiology as diagnosed by an ABI of <0.7 or great toe systolic pressure <40 mmHg or TcPO₂<40 mmHg in the supine position and <40 mmHg while sitting, measured on the forefoot with electrode set at 44 C.
- 14) Sickle-cell anemia, Raynaud's or other peripheral vascular disease.
- 15) Current history of drug abuse.
- 16) Subjects receiving a biologic agent to include growth factors and skin equivalents (Regranex™, Apligraf™, or Dermagraft™) in the past 30 days.
- 17) Subjects with uncontrolled diabetes defined as a glycosylated hemoglobin (HbA_{1c})>12%, or a serum creatinine level of greater than 3 g/dL obtained within 3 months of study enrollment determined on two separate occasions at least 3 weeks apart.
- 18) Subject with an ulcer which is determined to be clinically infected and requires topical antimicrobials or agents known to affect wound healing or has been taking systemic antibiotics for more than 7 days for any reason.
- 19) Subject with a Wagner Grade 3 or higher DFU, deep abscess or infection of the joint or tendon, or gangrene or osteomyelitis.
- 20) An EKG with a marked baseline prolongation of QT/QTc interval (e.g., repeated demonstration of QTc interval >450 milliseconds (ms))

A total of 80 subjects were enrolled in the study; 27 were randomized to the 0.03% dose of NorLeu³-A(1-7), 25 were randomized to placebo and 28 were randomized to the 0.01% dose. All subjects also received best standard of care, which included debridement, wound cleansing, application of an occlusive dressing and standardized proper off-loading.

Results

Recent results from a Phase 2 clinical trial with NorLeu³-A(1-7) in patients with diabetic foot ulcers showed that the proportion of study ulcers healed by 12 weeks as defined by 100% epithelialized with no drainage, as well as all secondary endpoints. The double-blind, placebo-controlled, multi-center clinical trial randomized 80 subjects to receive one of two doses of NorLeu³-A(1-7) (0.03% and 0.01%) or vehicle placebo (2% hydroxyethyl cellulose in phosphate buffer with 0.1% methyl paraben and 0.02% propyl paraben) control, in addition to best standard of care (which included debridement). The drug was well-tolerated and there were no significant adverse events associated with NorLeu³-A(1-7) treatment.

In the Intent-to-Treat (ITT) population (all subjects receiving any study medication and from whom any post-baseline data are available): Results show that 54% of the diabetic wounds treated with 0.03% (high dose) of NorLeu³-A(1-7) achieved 100% closure in 12 weeks or less, compared with 33% of patients receiving placebo control, and 30% of patients receiving the 0.01% dose (low dose) of NorLeu³-A(1-7). Based on odds ratio analysis, patients treated with NorLeu³-A(1-7) 0.03% were 2.3 times more likely to have their wounds heal completely as compared to patients treated with placebo/standard of care.

In the Per Protocol (PP) population (all patients that did not have a major protocol violation affecting efficacy): Results show that 65% of the diabetic wounds treated with 0.03% dose of NorLeu³-A(1-7) achieved 100% closure in 12 weeks or less, compared with 38% of patients receiving placebo control, and 28% of patients receiving the 0.01% dose of NorLeu³-A(1-7). See FIGS. 1-3. Based on odds ratio analysis, patients treated with NorLeu³-A(1-7) 0.03% were 3.0 times more likely to have their wounds heal completely as compared to patients treated with placebo/standard of care.

The high dose of NorLeu³-A(1-7) well exceeded the trial's primary endpoint measurement target of an improvement of 15 percentage points in complete healing of wounds within the 12-week duration of the study for both the ITT (21% increase) and PP (27% increase) populations compared with placebo.

The trial was not powered for statistical significance, however there was a statistically significant (p=0.049) 50% improvement in the rate of healing in the PP high-dose population through 12 weeks of treatment compared with the control arm, as measured by depth of ulcer using covariate analysis.

The wound healing rates for patients on NorLeu³-A(1-7) were remarkable with the ITT analysis showing a 21% increase and the PP analysis showing a 27% increase over the placebo arm.

REFERENCES

1. American Diabetes Association, Consensus development conference on diabetic foot wound care. *Diabetes Care* 1999; 22:1354-1360.
2. Pecoraro R E, Reiber G E, Burgess E M, Pathways to diabetic limb amputation: basis for prevention. *Diabetes Care* 1990; 13:513-521.
3. Rodgers K E, Roda N, Felix J C, Espinoza T, Maldonado S, diZerega G S. Histological evaluation of the effects of angiotensin peptides on wound repair in diabetic mice. *Experimental Dermatology* 2003; 12(6):784-790.
4. Rodgers K, Xiong S, Felix J, Roda N, Espinoza T, Maldonado S, diZerega G S. Development of angiotensin (1-7) as an agent to accelerate dermal repair. *Wound Repair Regen* 2001; 9:238-250.
5. Rodgers K E, Espinoza T, Felix J, Roda N, Maldonado S, diZerega G S. Acceleration of healing, reduction of fibrotic scar, and normalization of tissue architecture by an angiotensin analogue, Norleu³-A (1-7). *Plast Reconstr Surg* 2003; 111:1195-1206.
6. Wagner, F J. A classification and treatment program for diabetic, neuropathic, and dysvascular foot problems. *Am Acad of Orthopaedic Surgeons. Instructional Course Lecture* 1979; 28:143-165.
7. Foster A V, Eaton C, McConville D O, Edmonds M E. Application of OpSite film: a new and effective treatment of painful diabetic neuropathy. *Diabetes Med* 1994; 11(8): 768-772.

8. Sheehan P, Jones P, Caselli A, Giurini J M, Veves A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. *Diabetes Care* 2003; 26:1879-1882.
9. Rodgers, K E, Abiko M, Girgis W, St. Amand K M, Campeau J D, diZerega G S. Acceleration of dermal tissue repair by Angiotensin II. *Wound Repair Regen* 1997; 5:175-183.
10. Rodgers, K E, DeCherney A H, St. Amand K M, Dougherty W R, Felix J C, Girgis W, diZerega G S. Histologic alterations in dermal repair after thermal injury: effects of topical angiotensin II. *Burn Care and Rehabilitation* 1997; 18:381-388.
11. Okuyama N, Roda N, Guerrero A, Dougherty W, Nguyen T, diZerega G S, Rodgers K E. Effect of angiotensin II on the viability, vascularity of random flaps in a rat model. *Annals Plastic Surgery Res* 1999; 68:913-918.
12. Rodgers K E, Ellefson D D, Espinoza T, Roda N, Maldonado S, diZerega G S. Effect of NorLeu³-A (1-7) on scar formation over time after full thickness incision injury in the rat. *Wound Repair Regen* 2005; 13:309-317.
13. Santos R A, Brosnihan K B, Chappell M C, Pesquero J, Chemicky C L, Greene L J, and Ferrario C M. Converting enzyme activity and angiotensin metabolism in the dog brainstem. *Hypertension* 1988; 11(suppl I):I-53-I-57.
14. Santos R A S, Brosnihan K B, Jacobsen D W, DiCorleto P, and Ferrario C M. Production of Ang-(1-7) by human vascular endothelium. *Hypertension* 1992; 19(suppl II):II-56-II-61.
15. Santos et al. Characterization of a new angiotensin antagonist selective for angiotensin-(1-7): evidence that the actions of angiotensin-(1-7) are mediated by specific angiotensin receptors. *Brain Res Bull* 1994; 35:293-298.
16. Yamamoto K, Chappell M C, Brosnihan K B, Ferrario C M. In vivo metabolism of angiotensin I by neutral endopeptidase (EC 3.4.24.11) in spontaneously hypertensive rats. *Hypertension* 1992; 19:692-696.
17. Chappell M C, Tallant E A, Brosnihan K B, Ferrario C M. Processing of angiotensin peptides by NG108-15 neuroblastoma X glioma hybrid cell line. *Peptides* 1990; 22:375-380.
18. Chappell M C, Jacobsen D W, Tallant E A. Characterization of angiotensin II receptor subtypes in pancreatic acinar AR42J cells. *Peptides* 1995; 16:741-747.
19. Chappell M C, Tallant E A, Brosnihan K B, Ferrario C M. Conversion of angiotensin I to angiotensin-(1-7) by thimet

- oligopeptidase (E.C.3.4.24.15) in vascular smooth muscle cells. *J Vasc Biol Med* 1995; 5:129-137.
20. Daemen MJAP, Lombardi D M, Bosman F T, Schwartz S M. Angiotensin II induces smooth muscle cell proliferation in the normal and injured rat arterial wall. *Circ Res* 1991; 68:450-56.
21. Dzau V E, Pratt R, Gibbons G, Schunkert H, Lorell B, Ingelfinger J. Molecular mechanism of angiotensin in the regulation of vascular and cardiac growth. *J Mol Cell Cardiol* 1989; 21 [Suppl III]:57.
22. Naftilan A J, Pratt R E, Dzau V J. Induction of platelet-derived growth factor A-chain and c-myc gene expression by Angiotensin II in culture rat vascular smooth muscle cells. *J Clin Invest* 1989; 83:1419-24.
23. Stouffer G A, Owens G K. Angiotensin II induced mitogenesis of spontaneously hypertensive rat derived cultured smooth muscle cells is dependent on autocrine production of transforming growth factor- β . *Circ Res* 1992; 70:820-28.
24. Koibuchi Y, Lee W S, Gibbons G H, Pratt R E. Role of transforming growth factor β -1 in the cellular growth response to Angiotensin II. *Hypertension* 1993; 21:1046-50.
25. Kawahara Y, Sunako M, Tsuda T, Fukazaki H, Fukumoto Y, Takai Y. Angiotensin II induces expression of the c-fos gene through protein kinase C activation and calcium ion mobilization in cultured vascular smooth muscle cells. *BBRC* 1988; 150:52-9.
26. Mangiarua E I, Palmer V L, Lloyd L L, McCumbee W D. Platelet-derived growth factor mediates angiotensin II-induced DNA synthesis in vascular smooth muscle cells. *Arch Physiol Biochem* 1997; 105(2):151-7.
27. Su E J, Lombardi D M, Wiener J, Daemen M J, Reidy M A, and Schwartz M A. Mitogenic effect of angiotensin II on the rat carotid arteries and type II or III mesenteric microvessels but not type I mesenteric microvessels is mediated by endogenous basic fibroblast growth factor. *Circ Res* 1998; 82:321.
28. Rodgers L C, Driver V R, Armstrong D G. Assessment of the diabetic foot. In Krasner D L, Rodeheaver G T, Sibbald R G eds. *Chronic Wound Care: A Clinical Source Book for Healthcare Professionals*. 4th ed. Malvern PA: HMP Communications, 2007: 549-556.
29. Bolton L, McNees P, van Rijswijk L et al. Wound healing outcomes using standardized care. *JWOCN* 2004; 31:65-71.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 3

<210> SEQ ID NO 1
 <211> LENGTH: 7
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (3)..(3)
 <223> OTHER INFORMATION: Xaa is Nle

<400> SEQUENCE: 1

Asp Arg Xaa Tyr Ile His Pro
 1 5

-continued

<210> SEQ ID NO 2
 <211> LENGTH: 5
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (3)..(3)
 <223> OTHER INFORMATION: Xaa is Nle

<400> SEQUENCE: 2

Asp Arg Xaa Tyr Ile
 1 5

<210> SEQ ID NO 3
 <211> LENGTH: 6
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (3)..(3)
 <223> OTHER INFORMATION: Xaa is Nle

<400> SEQUENCE: 3

Asp Arg Xaa Tyr Ile His
 1 5

We claim:

1. A method for treating diabetic foot ulcers, comprising administering to a human patient suffering from a diabetic foot ulcer an amount of a peptide of at least 5 contiguous amino acids of Nle3 A(1-7) effective to treat the diabetic foot ulcer, wherein the peptide is administered topically in a gel formulation that comprises about 0.5% to about 4% hydroxyethyl cellulose (HEC) on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

2. The method of claim 1, wherein the peptide comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO: 1).

3. The method of claim 1, wherein the peptide consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO: 1).

4. The method of claim 1, wherein the diabetic foot ulcer is one caused, at least in part, by neuropathy and resulting pressure.

5. The method of claim 1, wherein the diabetic foot ulcer comprises one or more calluses.

6. The method of claim 1, wherein the diabetic foot ulcer is a chronic ulcer.

7. The method of claim 6, wherein the chronic foot ulcer has not responded to any other treatment.

8. The method of claim 1, wherein the peptide is administered as a topical formulation forming a continuous film covering the entire area of the diabetic ulcer.

9. The method of claim 1, wherein the peptide is administered in a hydrogel formulation.

10. The method of claim 1, wherein the peptide is administered at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

11. The method of claim 2, wherein the diabetic foot ulcer is one caused, at least in part, by neuropathy and resulting pressure.

12. The method of claim 2, wherein the diabetic foot ulcer comprises one or more calluses.

13. The method of claim 2, wherein the diabetic foot ulcer is a chronic ulcer.

14. The method of claim 13, wherein the chronic foot ulcer has not responded to any other treatment.

15. The method of claim 2, wherein the peptide is administered as a topical formulation forming a continuous film covering the entire area of the diabetic ulcer.

16. The method of claim 2, wherein the peptide is administered in a hydrogel formulation.

17. The method of claim 2, wherein the peptide is administered at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

18. The method of claim 3, wherein the diabetic foot ulcer is one caused, at least in part, by neuropathy and resulting pressure.

19. The method of claim 3, wherein the diabetic foot ulcer comprises one or more calluses.

20. The method of claim 3, wherein the diabetic foot ulcer is a chronic ulcer.

21. The method of claim 20, wherein the chronic foot ulcer has not responded to any other treatment.

22. The method of claim 3, wherein the peptide is administered as a topical formulation forming a continuous film covering the entire area of the diabetic ulcer.

23. The method of claim 3, wherein the peptide is administered in a hydrogel formulation.

24. The method of claim 3, wherein the peptide is administered at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

* * * * *

33



US008207234B1

(12) **United States Patent**
Rodgers et al.

(10) **Patent No.:** **US 8,207,234 B1**
(45) **Date of Patent:** **Jun. 26, 2012**

(54) **METHODS FOR TREATING DIABETIC FOOT ULCERS**

7,776,828 B2 8/2010 Rodgers et al.
7,786,085 B2 8/2010 Rodgers et al.
2009/0227507 A1 9/2009 Rodgers et al.

(75) Inventors: **Kathleen E. Rodgers**, Long Beach, CA (US); **Gere S. diZerega**, San Luis Obispo, CA (US)

FOREIGN PATENT DOCUMENTS

WO 98/33813 8/1998
WO 99/31125 6/1999
WO 99/40107 8/1999
WO 01/44270 6/2001
WO 2011/120032 3/2011
WO 2012/021578 2/2012

(73) Assignee: **University of Southern California**, Los Angeles, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

OTHER PUBLICATIONS

(21) Appl. No.: **13/441,653**

(22) Filed: **Apr. 6, 2012**

Related U.S. Application Data

(62) Division of application No. 13/364,061, filed on Feb. 1, 2012.

(60) Provisional application No. 61/438,780, filed on Feb. 2, 2011.

(51) Int. Cl.
A61K 38/08 (2006.01)
A61K 47/38 (2006.01)
C07K 7/14 (2006.01)
C07K 17/04 (2006.01)
C07K 17/12 (2006.01)

(52) U.S. Cl. **514/928; 424/488; 530/316; 930/40**

(58) Field of Classification Search None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,015,629 A 5/1991 DiZerega et al.
5,629,292 A 5/1997 DiZerega et al.
5,716,935 A 2/1998 DiZerega et al.
5,834,432 A 11/1998 DiZerega et al.
5,955,430 A 9/1999 DiZerega et al.
6,096,709 A 8/2000 DiZerega et al.
6,110,895 A 8/2000 DiZerega et al.
6,165,978 A 12/2000 DiZerega et al.
6,177,407 B1 1/2001 Rodgers et al.
6,248,587 B1 6/2001 Rodgers et al.
6,258,778 B1 7/2001 Rodgers et al.
6,335,195 B1 1/2002 Rodgers et al.
6,444,646 B1 9/2002 DiZerega et al.
6,455,500 B1 9/2002 Rodgers et al.
6,455,501 B1 9/2002 DiZerega et al.
6,475,988 B1 11/2002 Rodgers et al.
6,482,800 B1 11/2002 Rodgers et al.
6,498,138 B1 12/2002 Rodgers et al.
6,566,335 B1 5/2003 Rodgers et al.
6,730,775 B1 5/2004 DiZerega et al.
6,762,167 B1 7/2004 Rodgers et al.
6,821,953 B1 11/2004 DiZerega et al.
6,916,783 B2 7/2005 Rodgers et al.
7,022,675 B2 4/2006 DiZerega et al.
7,118,748 B1 10/2006 Rodgers et al.
7,173,011 B2 2/2007 Rodgers et al.
7,176,183 B2 2/2007 DiZerega et al.
7,338,938 B2 3/2008 Rodgers et al.
7,744,927 B2 6/2010 Rodgers et al.

American Diabetes Association, Consensus development conference on diabetic foot wound care. Diabetes Care 1999; 22:1354-1360.
Pecoraro RE, Reiber GE, Burgess EM, Pathways to diabetic limb amputation: basis for prevention. Diabetes Care 1990; 13:513-521.
Rodgers KE, Roda N, Felix JC, Espinoza T, Maldonado S, diZerega GS. Histological evaluation of the effects of angiotensin peptides on wound repair in diabetic mice. Experimental Dermatology 2003; 12(6):784-790.
Rodgers K, Xiong S, Felix J, Roda N, Espinoza T, Maldonado S, diZerega GS. Development of angiotensin (1-7) as an agent to accelerate dermal repair. Wound Repair Regen 2001; 9:238-250.
Rodgers KE, Espinoza T, Felix J, Roda N, Maldonado S, diZerega GS. Acceleration of healing, reduction of fibrotic scar, and normalization of tissue architecture by an angiotensin analogue, Norleu3-A (1-7). Plast Reconstr Surg 2003; 111:1195-1206.
Wagner, FJ. A classification and treatment program for diabetic, neuropathic, and dysvascular foot problems. Am Acad of Orthopaedic Surgeons. Instructional Course Lecture 1979; 28:143-165.
Foster AV, Eaton C, McConville DO, Edmonds ME. Application of OpSite film: a new and effective treatment of painful diabetic neuropathy. Diabetes Med 1994; 11(8):768-772.
Sheehan P, Jones P, Caselli A, Giurini JM, Veves A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. Diabetes Care 2003; 26:1879-1882.
Rodgers, KE, Abiko M, Girgis W, St. Amand KM, Campeau JD, diZerega GS. Acceleration of dermal tissue repair by Angiotensin II. Wound Repair Regen 1997; 5:175-183.
Rodgers, KE, DeCherney AH, St. Amand KM, Dougherty WR, Felix JC, Girgis W, diZerega GS. Histologic alterations in dermal repair after thermal injury: effects of topical angiotensin II. Burn Care and Rehabilitation 1997; 18:381-388.
Okuyama N, Roda N, Guerrero A, Dougherty W, Nguyen T, diZerega GS, Rodgers KE. Effect of angiotensin II on the viability, vascularity of random flaps in a rat model. Annals Plastic Surgery Res 1999; 68:913-918.
Rodgers KE, Ellefson DD, Espinoza T, Roda N, Maldonado S, diZerega GS. Effect of NorLeu3-A (1-7) on scar formation over time after full thickness incision injury in the rat. Wound Repair Regen 2005; 13:309-317.
Santos RA, Brosnihan KB, Chappell MC, Pesquero J, Chernicky CL, Greene LJ, and Ferrario CM. Converting enzyme activity and angiotensin metabolism in the dog brainstem. Hypertension 1988; 11(suppl I):I-53-I-57.

(Continued)

Primary Examiner — David Romeo

(74) Attorney, Agent, or Firm — McDonnell Boehnen Hulbert & Berghoff LLP

(57) **ABSTRACT**

The present invention provides methods and pharmaceutical formulations for treating diabetic foot ulcers.

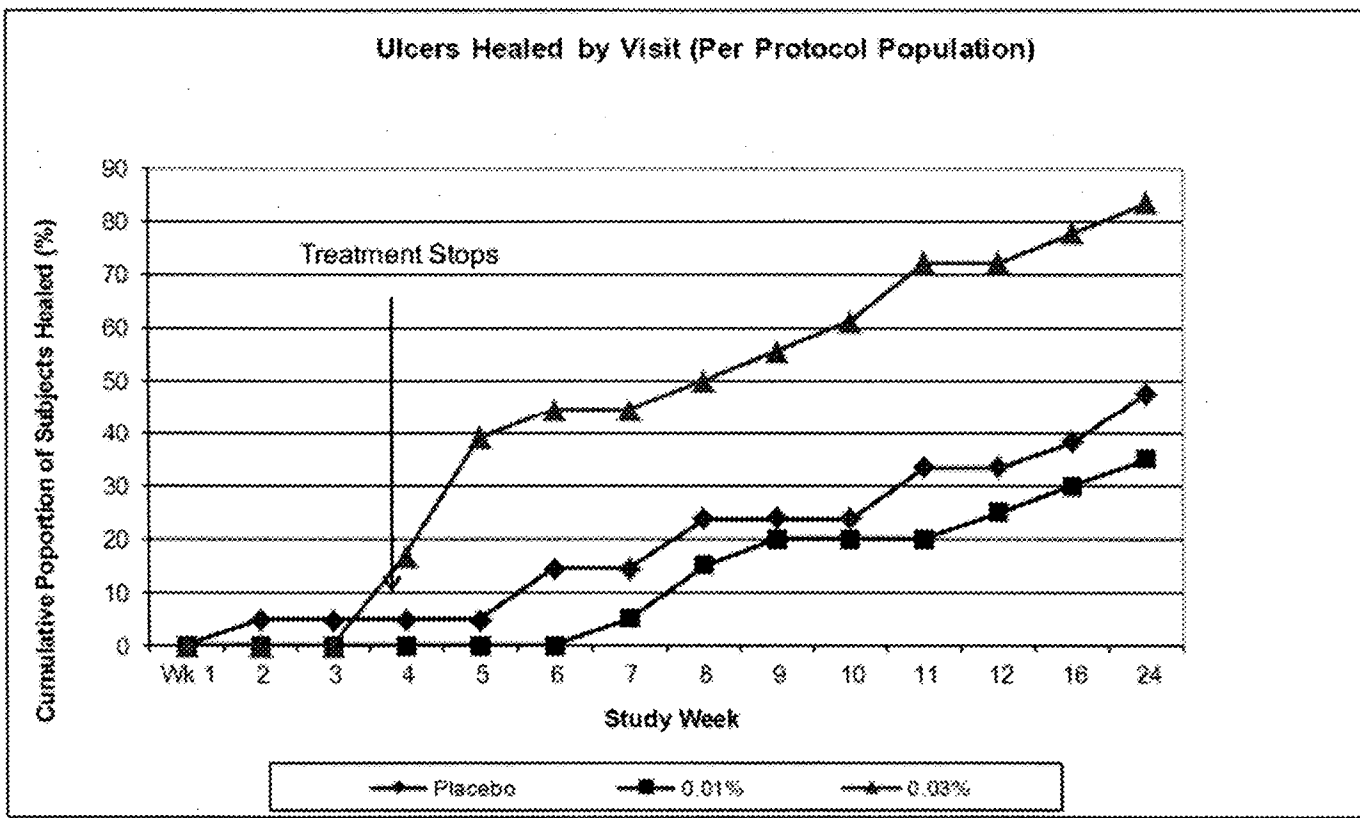
12 Claims, 3 Drawing Sheets

34

OTHER PUBLICATIONS

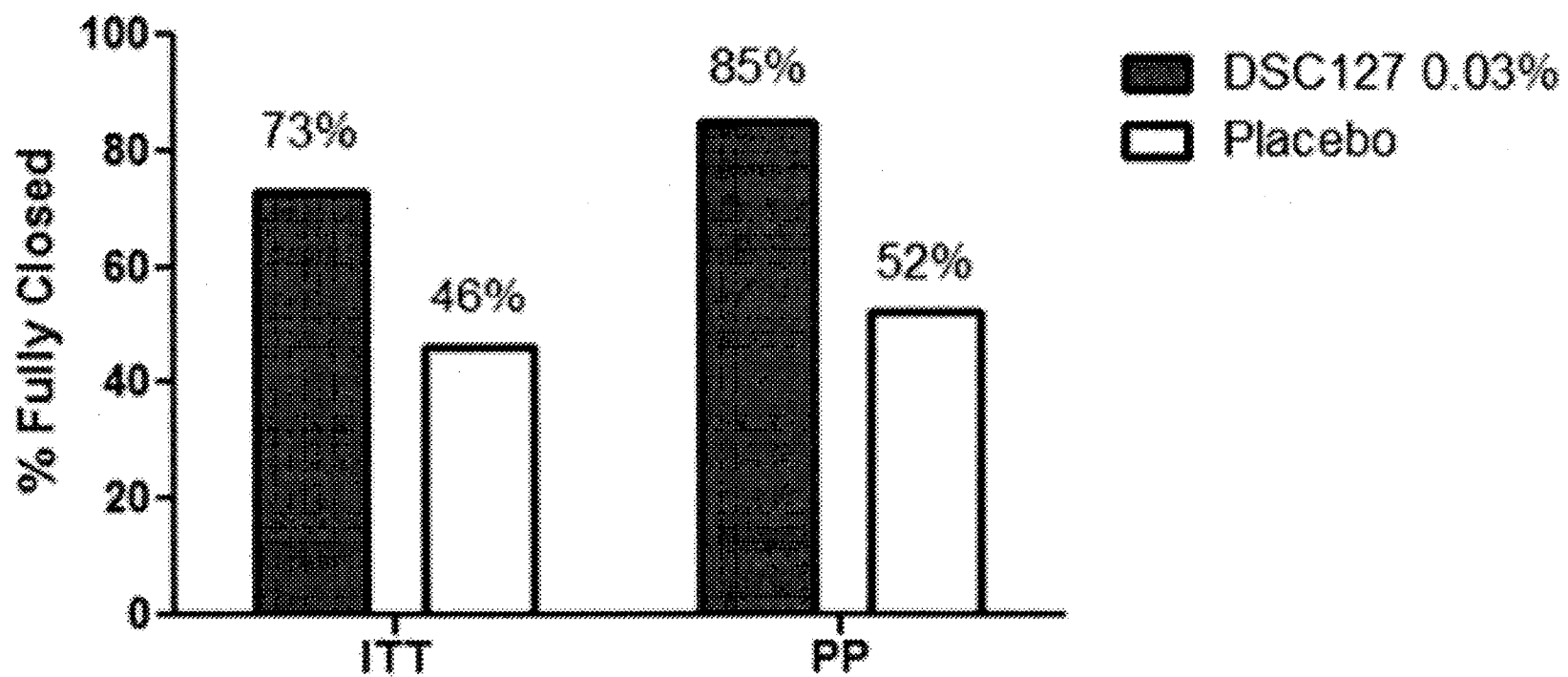
- Santos RAS, Brosnihan KB, Jacobsen DW DiCorleto P, and Ferrario CM. Production of Ang-(1-7) by human vascular endothelium. *Hypertension* 1992; 19(suppl II):II-56-II-61.
- Santos et al. Characterization of a new angiotensin antagonist selective for angiotensin-(1-7): evidence that the actions of angiotensin-(1-7) are mediated by specific angiotensin receptors. *Brain Res Bull* 1994; 35:293-298.
- Yamamoto K, Chappell MC Brosnihan KB, Ferrario CM. In vivo metabolism of angiotensin I by neutral endopeptidase (EC 3.4.24.11) in spontaneously hypertensive rats. *Hypertension* 1992; 19:692-696.
- Chappell MC, Tallant EA, Brosnihan KB, Ferrario CM. Processing of angiotensin peptides by NG108-15 neuroblastoma X glioma hybrid cell line. *Peptides* 1990; 22:375-380.
- Chappell MC, Jacobsen DW, Tallant EA. Characterization of angiotensin II receptor subtypes in pancreatic acinar AR42J cells. *Peptides* 1995; 16:741-747.
- Chappell MC, Tallant EA, Brosnihan KB, Ferrario CM. Conversion of angiotensin I to angiotensin-(1-7) by thimet oligopeptidase (E.C. 3.4.24.15) in vascular smooth muscle cells. *J Vasc Biol Med* 1995; 5:129-137.
- Daemen MJAP, Lombardi DM, Bosman FT, Schwartz SM. Angiotensin II induces smooth muscle cell proliferation in the normal and injured rat arterial wall. *Circ Res* 1991; 68:450-56.
- Dzau VE, Pratt R, Gibbons G, Schunkert H, Lorell B, Ingelfinger J. Molecular mechanism of angiotensin in the regulation of vascular and cardiac growth. *J Mol Cell Cardiol* 1989; 21 [Suppl III]:S7.
- Naftilan AJ, Pratt RE, Dzau VJ. Induction of platelet-derived growth factor A-chain and c-myc gene expression by Angiotensin II in culture rat vascular smooth muscle cells. *J Clin Invest* 1989; 83:1419-24.
- Kawahara Y, Sunako M, Tsuda T, Fukazaki H, Fukumoto Y, Takai Y. Angiotensin II induces expression of the c-fos gene through protein kinase C activation and calcium ion mobilization in cultured vascular smooth muscle cells. *BBRC* 1988; 150:52-9.
- Mangiarua EI, Palmer VL, Lloyd LL, McCumbee WD. Platelet-derived growth factor mediates angiotensin II-induced DNA synthesis in vascular smooth muscle cells. *Arch Physiol Biochem* 1997; 105(2):151-7.
- Su EJ, Lombardi DM, Wiener J, Daemen MJ, Reidy MA, and Schwartz MA. Mitogenic effect of angiotensin II on the rat carotid arteries and type II or III mesenteric microvessels but not type I mesenteric microvessels is mediated by endogenous basic fibroblast growth factor. *Circ Res* 1998; 82:321.
- Rodgers LC, Driver VR, Armstrong DG. Assessment of the diabetic foot. In Krasner DL, Rodeheaver GT, Sibbald RG eds. *Chronic Wound Care: A Clinical Source Book for Healthcare Professionals*. 4th ed. Malvern PA: HMP Communications, 2007: 549-556.
- Bolton L, McNees P, van Rijswijk L et al. Wound healing outcomes using standardized care. *JWOCN* 2004; 31:65-71.
- Stouffer GA, Owens GK. Angiotensin II induced mitogenesis of spontaneously hypertensive rat derived cultured smooth muscle cells is dependent on autocrine production of transforming growth factor-B. *Circ Res* 1992; 70:820-28.
- Koibuchi Y, Lee WS, Gibbons GH, Pratt RE. Role of transforming growth factor B-1 in the cellular growth response to Angiotensin II. *Hypertension* 1993; 21:1046-50.
- Rodgers, et al., (2006) *J. Peptide Res.*, 66(suppl. 1):41-47.

Figure 1



27

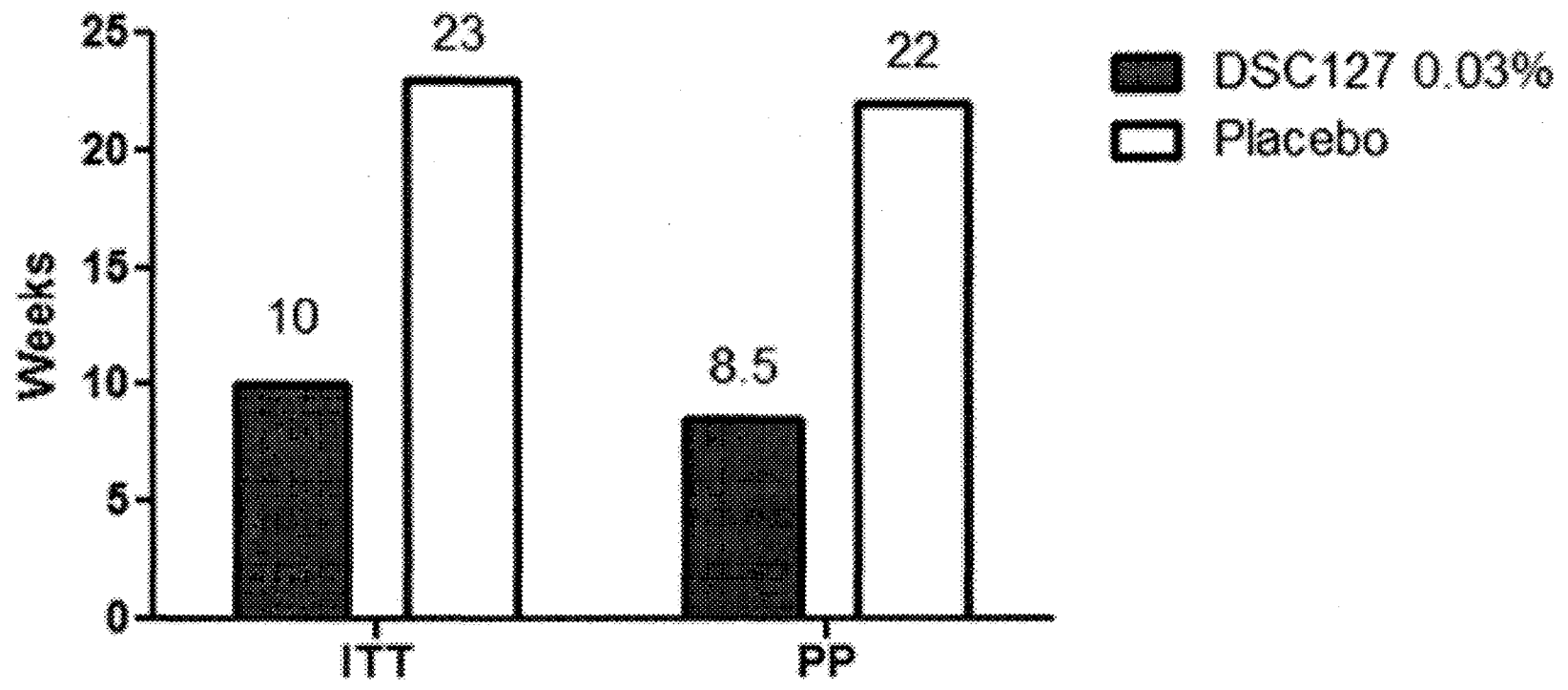
Figure 2



27

27

Figure 3



28

1

METHODS FOR TREATING DIABETIC FOOT ULCERS

CROSS REFERENCE

This application is a divisional of U.S. application Ser. No. 13/364,061 filed Feb. 1, 2012, and claims priority to U.S. Provisional Patent Application Ser. No. 61/438,780 filed Feb. 2, 2011, incorporated by reference herein in its entirety.

BACKGROUND

Diabetes is common, disabling and deadly. In the U.S., diabetes has reached epidemic proportions. According to the American Diabetes Association, about 24 million people (8% of the total U.S. population) have diabetes, and nearly two million new cases are diagnosed in people aged 20 years or older each year. If current trends continue, 1 in 3 Americans will develop diabetes at some point in their lifetime, and those with diabetes will lose, on average, 10-15 years of life expectancy. Importantly, up to 25% of people with diabetes will develop a diabetic foot ulcer, resulting in 3 million diabetic foot ulcers annually in the U.S. alone. More than half of all foot ulcers will become infected, thus requiring hospitalization, and 1 in 5 will require an amputation that carries a high risk of mortality.

Without question, diabetes puts tremendous economic pressure on the U.S. healthcare system. Total costs (direct and indirect) of diabetes have reached \$174 billion annually, and people with diagnosed diabetes have medical expenditures that are over two times higher than medical expenditures for people without diabetes. Hospitalization costs alone are \$16,000 to \$20,000 for a patient with a diabetic foot ulcer, and direct and indirect costs of an amputation range from \$20,000 to \$60,000 per patient. A recent study by researchers at the University of Chicago suggested that treatment costs for diabetes in the United States would reach \$336 billion by the year 2034. Advanced, cost-effective treatment modalities for diabetes and its co-morbidities, including diabetic foot ulcers, are in great need, yet in short supply, globally. According to the American Diabetes Association, by the year 2025 the prevalence of diabetes is expected to rise by 72% to 324 million people worldwide.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides methods for treating diabetic foot ulcers, comprising administering to a human patient suffering from a diabetic foot ulcer an amount of a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof, effective to treat the diabetic foot ulcer. In one embodiment, the peptide comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In another embodiment, the peptide consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In various embodiments, the diabetic foot ulcer is one caused, at least in part, by neuropathy and resulting pressure; the diabetic foot ulcer comprises one or more calluses; and the diabetic foot ulcer is a chronic ulcer. In a further embodiment, the chronic foot ulcer has not responded to any other treatment.

In another embodiment, the peptide is administered topically. In a further embodiment, the peptide is administered as a topical formulation forming a continuous film covering the entire area of the diabetic ulcer. In a still further embodiment, the peptide is administered in a hydrogel formulation. In another embodiment, the peptide is administered at a concentration of about 0.03% to about 1% on a weight (mg)/volume

(ml) basis, or on a weight/weight (mg) basis. In a further embodiment, the peptide is administered in a gel formulation that about 0.5% to about 4% hydroxyethyl cellulose (HEC) on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

All embodiments of the first aspect of the invention can be combined unless the context dictates otherwise.

In a second aspect, the present invention provides pharmaceutical formulations, comprising:

(a) about 0.5% to about 4% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis; and

(b) a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof;

wherein the peptide is present at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

In one embodiment, the peptide comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In another embodiment, the peptide consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In a further embodiment, the pharmaceutical formulation comprises about 1% to about 3% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In a still further embodiment, the pharmaceutical formulation comprises about 2% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In another embodiment, the formulation comprises a hydrogel formulation. All embodiments of the second aspect of the invention can be combined unless the context dictates otherwise.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a graph showing the ulcers healed by visit in the Per Protocol (PP) patient population taking NorLeu³-A(1-7).

FIG. 2 is a graph showing the percentage of fully healed ulcers (100% wound closure) in the patient population taking NorLeu³-A(1-7) (also referred to as "DSC127").

FIG. 3 is a graph showing the median time to fully healed ulcers (100% wound closure) in the patient population taking NorLeu³-A(1-7) (also referred to as "DSC127").

DETAILED DESCRIPTION OF THE INVENTION

All references cited are herein incorporated by reference in their entirety. Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991, Academic Press, San Diego, Calif.), "Guide to Protein Purification" in *Methods in Enzymology* (M. P. Deutscher, ed., (1990) Academic Press, Inc.); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990, Academic Press, San Diego, Calif.), *Culture of Animal Cells: A Manual of Basic Technique*, 2nd Ed. (R. I. Freshney. 1987, Liss, Inc. New York, N.Y.), *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E. J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, Tex.).

As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. "And" as used herein is interchangeably used with "or" unless expressly stated otherwise.

All embodiments of any aspect of the invention can be used in combination, unless the context clearly dictates otherwise.

In a first aspect, the present invention provides methods for treating diabetic foot ulcers, comprising administering to a

human patient suffering from a diabetic foot ulcer an amount of a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof, effective to treat the diabetic foot ulcer.

As demonstrated in the examples that follow, the inventors have demonstrated that the methods of the invention can be used to treat diabetic foot ulcers, and provide substantial improvement over standard therapies.

Nle3A(1-7) (or NorLeu³-A(1-7); also referred to as "DSC127") is a peptide consisting of the amino acid sequence Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1). In various embodiments, the peptide administered to the human patient may comprise or consist of Asp-Arg-Nle-Tyr-Ile (SEQ ID NO:2), Asp-Arg-Nle-Tyr-Ile-His (SEQ ID NO:3), or most preferably Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salts thereof. Nle3A(1-7) or salts thereof may be chemically synthesized or recombinantly expressed, each of which can be accomplished using standard methods in the art.

In one embodiment, the peptide, or salt thereof, is administered at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In various further embodiments, the peptide, or salt thereof, is administered at a concentration of about 0.03% to about 0.75%; about 0.03% to about 0.5%; about 0.03% to about 0.25%; about 0.03% to about 0.1%; about 0.03% to about 0.075%; about 0.03% to about 0.05%; and about 0.03%; all on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

The human patient may be suffering from Type I diabetes or Type II diabetes, and has a foot ulcer, defined as an open wound anywhere on the foot (heel, mid-foot, and forefoot).

As used herein, "treating" a diabetic foot ulcer includes

- (a) limiting the progression in size, area, and/or depth of the foot ulcer;
- (b) reducing size, area, and/or depth of the foot ulcer;
- (c) increasing rate of healing and/or reducing time to healing;
- (d) healing of the foot ulcer (100% epithelialization with no drainage); and
- (e) decreased incidence of amputation or slowing in time to amputation.

The foot ulcer may be caused by any underlying pathology, including but not limited to neuropathy, trauma, deformity, high plantar pressures, callus formation, edema, and peripheral arterial disease. In preferred embodiments, the human diabetic foot ulcer is one caused, at least in part, by neuropathy and resulting pressure (weight bearing on the extremity due to lack of feeling in the foot). As is known to those of skill in the art, human diabetic foot ulcers tend to be due to neuropathy and pressure, which differs significantly from, for example, murine acute wounds. In a further preferred embodiment, the diabetic foot ulcer comprises one or more calluses.

In a further embodiment, the diabetic foot ulcer is a chronic ulcer. As used herein, a "chronic" foot ulcer is one that has been present for at least 7 days with no reduction in size; preferably at least 14 days; even more preferably, present at least 21 or 28 days with no reduction in size. In a further preferred embodiment that can be combined with any of these embodiments, the chronic foot ulcer has not responded (ie: no reduction in size, area, and/or depth of the foot ulcer; no healing of the foot ulcer) to any other treatment.

The peptide or salt thereof may be administered by any suitable route, preferably via topical administration. In one embodiment, the methods of the invention can comprise administering a topical formulation as often as deemed appropriate, ie: once per day, twice per day, etc. The methods may further comprise administration of the peptide, or salt

thereof for as longed as deemed desirable by an attending physician, for example, until healing of the ulcer. For administration, it is preferred that the topical formulation form a continuous film covering the entire area of the ulcer, including the margins. In a preferred embodiment, the topical formulation is applied with a thickness of approximately 0.25 to 2 mm; preferably 0.5 to 1.5 mm; preferably about 1 mm in thickness.

In one embodiment, the topical administration comprises administration in a formulation selected from the group consisting of hydrogels, creams, ointments, pastes, and lotions. The formulations may be applied in any suitable manner, which may include any wound dressings to seal in the formulation deemed appropriate by the human patient or caregiver. Exemplary such dressings, include, but are not limited to, semipermeable films, foams, hydrocolloids, and calcium alginate swabs.

The methods may further comprise debridement in and around the wound in combination with administration of the peptide and formulations thereof. Debridement of all necrotic, callus, and fibrous tissue is typically carried for treatment of diabetic foot ulcers. Unhealthy tissue is sharply debrided back to bleeding tissue to allow full visualization of the extent of the ulcer and to detect underlying abscesses or sinuses. Any suitable debridement technique can be used, as determined by an attending physician. The wound can then be thoroughly flushed with sterile saline or a non-cytotoxic cleanser following debridement.

In another embodiment, the topical formulation comprises about 0.5% to about 4% hydroxyethyl cellulose (HEC) on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In various further embodiments, the topical formulation may comprise about 1% to about 3% HEC, or about 2% HEC, on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. These formulations comprising low percentage HEC (ie: 2%) matrices provided a 10-fold increase in peptide release over a 24 hour period from formulations such as those comprising 10% carboxymethylcellulose (CMC), a result that would be unexpected to those of skill in the art. Furthermore, the data show that the HEC matrices are more biocompatible than HPMC and CMC formulations tested.

The peptides, or salt thereof may be administered together with one or more (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer. In some embodiments, the buffer in the pharmaceutical composition is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The peptides may be administered with a lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain embodiments, the peptides may be administered with a preservative e.g. benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the peptides may be administered with a bulking agent, like glycine. In yet other embodiments, the peptides may be administered with a surfactant e.g., polysorbate-20, polysorbate-40, polysorbate-60, polysorbate-65, polysorbate-80 polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleate, or a combination thereof. The peptides may be administered with a tonicity adjusting agent, e.g., a compound that renders the formulation substantially isotonic or isoosmotic with human blood. Exemplary tonicity adjusting agents include sucrose, sorbitol, glycine, methionine, mannitol, dextrose, inositol, sodium

chloride, arginine and arginine hydrochloride. In other embodiments, the peptides may be administered with a stabilizer, e.g., a molecule which, when combined with the peptide substantially prevents or reduces chemical and/or physical instability of the protein of interest in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride, paraben, and combinations of methyl paraben and propyl paraben.

In all aspects and embodiments of the invention, suitable acids which are capable of forming salts with the peptides include, but are not limited to, inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid and the like; and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid and the like. Suitable bases capable of forming salts with the peptides include, but are not limited to, inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide and the like; and organic bases such as mono-, di- and tri-alkyl and aryl amines (e.g., triethylamine, diisopropyl amine, methyl amine, dimethyl amine and the like) and optionally substituted ethanol-amines (e.g., ethanolamine, diethanolamine and the like).

The peptides or salts thereof can further be derivatized to provide enhanced half-life, for example, by linking to polyethylene glycol. The peptides or salts thereof may comprise L-amino acids, D-amino acids (which are resistant to L-amino acid-specific proteases *in vivo*), a combination of D- and L-amino acids, and various "designer" amino acids (e.g., β -methyl amino acids, α -methyl amino acids, and $N\alpha$ -methyl amino acids, etc.) to convey special properties.

The polypeptides may be the sole active agent in the pharmaceutical composition, or the composition may further comprise one or more other active agents suitable treating diabetic foot ulcers, such as antibiotics. The methods may be used in conjunction with other foot ulcer therapies, including but not limited to negative pressure wound therapy, total contact casts, removable cast walkers, half-shoes, becaplermin, infection control, and hyperbaric oxygen therapy.

The methods may include any other embodiments as disclosed in the example that follows. Such embodiments may be used in any combination in the methods of the invention, unless the context clearly dictates otherwise.

In a second aspect, the present invention provides pharmaceutical formulations, comprising:

(a) about 0.5% to about 4% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis; and

(b) a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof;

wherein the peptide is present at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

The pharmaceutical formulations are demonstrated herein to be particularly effective for treating diabetic foot ulcers, such as chronic diabetic foot ulcers that are not effectively treated using standard therapies.

In one embodiment, the peptide comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof. In another embodiment, the peptide consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof.

In one embodiment, the peptide, or salt thereof, is present in the formulation at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In various further embodiments, the pep-

tide, or salt thereof, is present in the formulation at a concentration of about 0.03% to about 0.75%; about 0.03% to about 0.5%; about 0.03% to about 0.25%; about 0.03% to about 0.1%; about 0.03% to about 0.075%; about 0.03% to about 0.05%; and about 0.03%; all on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.

In a further embodiment, the pharmaceutical formulation comprises about 1% to about 3% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. These formulations comprising low percentage HEC (ie: 2%) matrices provided a 10-fold increase in peptide release over a 24 hour period from formulations such as those comprising 10% carboxymethylcellulose (CMC), a result that would be unexpected to those of skill in the art. Furthermore, the data show that the HEC matrices are more biocompatible than HPMC and CMC formulations tested. In a still further embodiment, the pharmaceutical formulation comprises about 2% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis. In another embodiment, the formulation comprises a hydrogel formulation.

In one embodiment, the formulation is topical gel-based formulation selected from the group consisting of hydrogels, creams, ointments, pastes, and lotions. The formulation is administered so as to form a continuous film covering the entire area of the ulcer, including the margins, without running off. In a preferred embodiment, the topical formulation is applied with a thickness of approximately 0.25 to 2 mm; preferably 0.5 to 1.5 mm; preferably about 1 mm in thickness. In another non-limiting embodiment the formulation is applied at approximately 0.075 mL per cm² surface area.

In all aspects and embodiments of the invention, suitable acids which are capable of forming salts with the peptides include, but are not limited to, inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid and the like; and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid and the like. Suitable bases capable of forming salts with the peptides include, but are not limited to, inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide and the like; and organic bases such as mono-, di- and tri-alkyl and aryl amines (e.g., triethylamine, diisopropyl amine, methyl amine, dimethyl amine and the like) and optionally substituted ethanol-amines (e.g., ethanolamine, diethanolamine and the like).

The pharmaceutical formulations may further comprise (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer. In some embodiments, the buffer in the pharmaceutical formulations is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The pharmaceutical formulations may also include a lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain embodiments, the pharmaceutical formulations includes a preservative e.g. benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the pharmaceutical formulations includes a bulking agent, like glycine. In yet other embodiments, the pharmaceutical formulations includes a surfactant e.g., polysorbate-20, polysorbate-40, polysorbate-60, polysorbate-65, polysorbate-80, polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan

monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleate, or a combination thereof. The pharmaceutical formulations may also include a tonicity adjusting agent, e.g., a compound that renders the formulation substantially isotonic or isoosmotic with human blood. Exemplary tonicity adjusting agents include sucrose, sorbitol, glycine, methionine, mannitol, dextrose, inositol, sodium chloride, arginine and arginine hydrochloride. In other embodiments, the pharmaceutical formulations additionally includes a stabilizer, e.g., a molecule which, when combined with a protein of interest substantially prevents or reduces chemical and/or physical instability of the protein of interest in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride.

The peptides or salts thereof can further be derivatized to provide enhanced half-life, for example, by linking to polyethylene glycol. The peptides or salts thereof may comprise L-amino acids, D-amino acids (which are resistant to L-amino acid-specific proteases *in vivo*), a combination of D- and L-amino acids, and various "designer" amino acids (e.g., β -methyl amino acids, C α -methyl amino acids, and N α -methyl amino acids, etc.) to convey special properties.

The peptide may be the sole active agent in the pharmaceutical composition, or the composition may further comprise one or more other active agents suitable for treating diabetic ulcers.

The formulations may include any other embodiments as disclosed in the example that follows. Such embodiments may be used in any combination in the formulations of the invention, unless the context clearly dictates otherwise.

Example

Randomized, Parallel-Group, Double-Blind,
Placebo-Controlled Phase 2 Clinical Trial to
Evaluate the Safety and Effectiveness of
NorLeu³-A(1-7) in Treating Subjects with Diabetic
Ulcers

The study was designed as a randomized, parallel-group, double-blind, placebo-controlled, multi-center trial. After 14-days of best-of-care to evaluate ulcer healing and ensure the wounds were chronic, four-weeks of active treatment were then followed by eight weeks of observation and assessment. The study compared the effects of two concentrations of NorLeu³-A(1-7) and placebo, measuring both clinical efficacy and safety. Sustained tissue integrity was evaluated for all subjects during a follow-up period lasting 12 weeks after complete wound closure.

Subjects were randomized in a 1:1:1 ratio to one of the three treatment groups:

Group 1: Placebo Vehicle Control without NorLeu³-A(1-7) (also referred to as "DSC127") (2% Hydroxyethyl Cellulose (HEC) with 0.1% methyl paraben, 0.02% propyl paraben)

Group 2: 0.03% NorLeu³-A(1-7) in Vehicle

Group 3: 0.01% NorLeu³-A(1-7) in Vehicle

The four-week treatment period required daily application of the treatment to the wound site. The first application each week was at the clinic and for the remainder of the week the patient self-administered the treatment.

If wound healing occurred during the treatment or assessment periods a final assessment visit was conducted and the integrity was assessed 4 and 12 weeks later (usually weeks 16 and 24 of the study).

Inclusion Criteria

The following patients were considered eligible for participation in the study:

- 1) Male or female ambulatory subjects who were at least 18 years of age at screening
- 2) At start of Screening Period and upon enrollment into the study, had at least one chronic non-healing Wagner Grade 1 or Grade 2 (ulcers of partial or full thickness and not involving bone, tendon or capsule (probing to tendon or capsule), and that have no sign of infection or myelitis) plantar neuropathic diabetic ulcer between 1.0-6.0 cm² on the midfoot or forefoot, including the toes but excluding the heel. Non-healing is defined as present for a minimum of one month but not longer than ten months with less than 30% reduction in size in response to treatment (non-study treatment, but including off-loading) during the Screening Period. If more than one ulcer is present that meets the inclusion criteria, the larger was studied and treated according to the protocol. Non-study ulcers were treated according to institutional best practice, using per protocol offloading.
- 3) Have an Ankle Brachial Index (ABI) greater than 0.7 for neuroischemic or greater than 0.8 for neuropathic DFU.
- 4) Have a Tissue Oxygen Pressure (TcPO₂) greater than 40 mm Hg or great toe systolic pressure greater than 50 mmHg to ensure healing potential.
- 5) Have Type I or Type II diabetes under metabolic control as confirmed by a glycosylated hemoglobin (HbA1c) of less than or equal to 12%, and a serum creatinine level of no greater than 3 mg/dL obtained within 3 months of study enrollment.
- 6) Assess the baseline level of neuropathy of the foot using Semmes-Weinstein filaments. Patients were considered to have site specific neuropathy sufficient for loss of protective sensation (LOPS) if they were unable to feel a #5.07 monofilament applied to at least 5 of the following 7 sites (28) on the study foot:
plantar to toes and metatarsals 1, 3 and 5 (3 sites)
plantar to midfoot medial and lateral (2 sites)
plantar heel (1 site)
dorsal distal first interspace (1 site)
- 7) Female subjects of child-bearing potential must have negative pregnancy test at the time of initiation of study therapy.
- 8) Female subjects of child-bearing potential must have been willing to use a medically acceptable method of birth control, such as Essure®, hormonal contraception (oral pills, implantable device or skin patch), intrauterine device, tubal ligation or double barrier, during the treatment and assessment period of study participation.
- 9) Ability and willingness to understand and comply with study procedures and to give written informed consent prior to enrollment in the study or initiation of study procedures.

Exclusion Criteria

If a subject met any of the following criteria, he or she was excluded from the study:

- 1) Has a known hypersensitivity to any of the study medication components.
- 2) Exposure to any investigational agent within 30 days of entry into study.
- 3) Females who are pregnant or nursing.
- 4) Females unwilling to use a medically acceptable method of birth control, such as Essure®, hormonal contraception (oral pills, implantable device or skin patch), intrauterine device, tubal ligation or double barrier, during the treatment and assessment period of study participation.

42

- 5) Active malignant disease of any kind A subject, who has had a malignant disease in the past, was treated and is currently disease-free, may be considered for study entry.
- 6) Chronic renal insufficiency (serum creatinine during screening is greater than 3.0 g/dL obtained within 3 months of study enrollment).
- 7) Chronic liver dysfunction evidenced by transaminase levels greater than twice normal.
- 8) Receiving hemodialysis or chronic ambulatory peritoneal dialysis (CAPD) therapy.
- 9) Resting blood pressure (at the time of the initial visit of the Screening Period) which exceeds 160 systolic and/or 90 diastolic mmHg on 3 consecutive readings at least 15 minutes apart.
- 10) Prior radiation therapy of the foot with the ulcer under study.
- 11) Current use of corticosteroids (within past 8 weeks), immunosuppressants (within past 8 weeks).
- 12) Known to be HIV positive.
- 13) Subjects whose ulcer was primarily ischemic in etiology as diagnosed by an ABI of <0.7 or great toe systolic pressure <40 mmHg or TcPO₂ <40 mmHg in the supine position and <40 mmHg while sitting, measured on the forefoot with electrode set at 44 C.
- 14) Sickle-cell anemia, Raynaud's or other peripheral vascular disease.
- 15) Current history of drug abuse.
- 16) Subjects receiving a biologic agent to include growth factors and skin equivalents (Regranex™, Apligraf™, or Dermagraft™) in the past 30 days.
- 17) Subjects with uncontrolled diabetes defined as a glycosylated hemoglobin (HbA_{1c}) >12%, or a serum creatinine level of greater than 3 g/dL obtained within 3 months of study enrollment determined on two separate occasions at least 3 weeks apart.
- 18) Subject with an ulcer which is determined to be clinically infected and requires topical antimicrobials or agents known to affect wound healing or has been taking systemic antibiotics for more than 7 days for any reason.
- 19) Subject with a Wagner Grade 3 or higher DFU, deep abscess or infection of the joint or tendon, or gangrene or osteomyelitis.
- 20) An EKG with a marked baseline prolongation of QT/QTc interval (e.g., repeated demonstration of QTc interval >450 milliseconds (ms))

A total of 80 subjects were enrolled in the study; 27 were randomized to the 0.03% dose of NorLeu³-A(1-7), 25 were randomized to placebo and 28 were randomized to the 0.01% dose. All subjects also received best standard of care, which included debridement, wound cleansing, application of an occlusive dressing and standardized proper off-loading.

Recent results from a Phase 2 clinical trial with NorLeu³-A(1-7) in patients with diabetic foot ulcers showed that the proportion of study ulcers healed by 12 weeks as defined by 100% epithelialized with no drainage, as well as all secondary endpoints. The double-blind, placebo-controlled, multi-center clinical trial randomized 80 subjects to receive one of two doses of NorLeu³-A(1-7) (0.03% and 0.01%) or vehicle placebo (2% hydroxyethyl cellulose in phosphate buffer with 0.1% methyl paraben and 0.02% propyl paraben) control, in addition to best standard of care (which included debridement). The drug was well-tolerated and there were no significant adverse events associated with NorLeu³-A(1-7) treatment.

In the Intent-to-Treat (ITT) population (all subjects receiving any study medication and from whom any post-baseline data are available): Results show that 54% of the diabetic wounds treated with 0.03% (high dose) of NorLeu³-A(1-7) achieved 100% closure in 12 weeks or less, compared with 33% of patients receiving placebo control, and 30% of patients receiving the 0.01% dose (low dose) of NorLeu³-A(1-7). Based on odds ratio analysis, patients treated with NorLeu³-A(1-7) 0.03% were 2.3 times more likely to have their wounds heal completely as compared to patients treated with placebo/standard of care.

In the Per Protocol (PP) population (all patients that did not have a major protocol violation affecting efficacy): Results show that 65% of the diabetic wounds treated with 0.03% dose of NorLeu³-A(1-7) achieved 100% closure in 12 weeks or less, compared with 38% of patients receiving placebo control, and 28% of patients receiving the 0.01% dose of NorLeu³-A(1-7). See FIGS. 1-3. Based on odds ratio analysis, patients treated with NorLeu³-A(1-7) 0.03% were 3.0 times more likely to have their wounds heal completely as compared to patients treated with placebo/standard of care.

The high dose of NorLeu³-A(1-7) well exceeded the trial's primary endpoint measurement target of an improvement of 15 percentage points in complete healing of wounds within the 12-week duration of the study for both the ITT (21% increase) and PP (27% increase) populations compared with placebo.

The trial was not powered for statistical significance, however there was a statistically significant ($p=0.049$) 50% improvement in the rate of healing in the PP high-dose population through 12 weeks of treatment compared with the control arm, as measured by depth of ulcer using covariate analysis.

The wound healing rates for patients on NorLeu³-A(1-7) were remarkable with the ITT analysis showing a 21% increase and the PP analysis showing a 27% increase over the placebo arm.

REFERENCES

1. American Diabetes Association, Consensus development conference on diabetic foot wound care. *Diabetes Care* 1999; 22:1354-1360.
2. Pecoraro R E, Reiber G E, Burgess E M, Pathways to diabetic limb amputation: basis for prevention. *Diabetes Care* 1990; 13:513-521.
3. Rodgers K E, Roda N, Felix J C, Espinoza T, Maldonado S, diZerega G S. Histological evaluation of the effects of angiotensin peptides on wound repair in diabetic mice. *Experimental Dermatology* 2003; 12(6):784-790.
4. Rodgers K, Xiong S, Felix J, Roda N, Espinoza T, Maldonado S, diZerega G S. Development of angiotensin (1-7) as an agent to accelerate dermal repair. *Wound Repair Regen* 2001; 9:238-250.
5. Rodgers K E, Espinoza T, Felix J, Roda N, Maldonado S, diZerega G S. Acceleration of healing, reduction of fibrotic scar, and normalization of tissue architecture by an angiotensin analogue, Norleu³-A (1-7). *Plast Reconstr Surg* 2003; 111:1195-1206.
6. Wagner, F J. A classification and treatment program for diabetic, neuropathic, and dysvascular foot problems. *Am Acad of Orthopaedic Surgeons. Instructional Course Lecture* 1979; 28:143-165.
7. Foster A V, Eaton C, McConville D O, Edmonds M E. Application of OpSite film: a new and effective treatment of painful diabetic neuropathy. *Diabetes Med* 1994; 11(8):768-772.

8. Sheehan P, Jones P, Caselli A, Giurini J M, Veves A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. *Diabetes Care* 2003; 26:1879-1882.
9. Rodgers, K E, Abiko M, Girgis W, St. Amand K M, Campeau J D, diZerega G S. Acceleration of dermal tissue repair by Angiotensin II. *Wound Repair Regen* 1997; 5:175-183.
10. Rodgers, K E, DeCherney A H, St. Amand K M, Dougherty W R, Felix J C, Girgis W, diZerega G S. Histologic alterations in dermal repair after thermal injury: effects of topical angiotensin II. *Burn Care and Rehabilitation* 1997; 18:381-388.
11. Okuyama N, Roda N, Guerrero A, Dougherty W, Nguyen T, diZerega G S, Rodgers K E. Effect of angiotensin II on the viability, vascularity of random flaps in a rat model. *Annals Plastic Surgery Res* 1999; 68:913-918.
12. Rodgers K E, Ellefson D D, Espinoza T, Roda N, Maldonado S, diZerega G S. Effect of NorLeu³-A (1-7) on scar formation over time after full thickness incision injury in the rat. *Wound Repair Regen* 2005; 13:309-317.
13. Santos R A, Brosnihan K B, Chappell M C, Pesquero J, Chemicky C L, Greene L J, and Ferrario C M. Converting enzyme activity and angiotensin metabolism in the dog brainstem. *Hypertension* 1988; 11(suppl I):1-53-1-57.
14. Santos R A S, Brosnihan K B, Jacobsen D W, DiCorleto P, and Ferrario C M. Production of Ang-(1-7) by human vascular endothelium. *Hypertension* 1992; 19(suppl II):II-56-II-61.
15. Santos et al. Characterization of a new angiotensin antagonist selective for angiotensin-(1-7): evidence that the actions of angiotensin-(1-7) are mediated by specific angiotensin receptors. *Brain Res Bull* 1994; 35:293-298.
16. Yamamoto K, Chappell M C, Brosnihan K B, Ferrario C M. In vivo metabolism of angiotensin I by neutral endopeptidase (EC 3.4.24.11) in spontaneously hypertensive rats. *Hypertension* 1992; 19:692-696.
17. Chappell M C, Tallant E A, Brosnihan K B, Ferrario C M. Processing of angiotensin peptides by NG108-15 neuroblastoma X glioma hybrid cell line. *Peptides* 1990; 22:375-380.
18. Chappell M C, Jacobsen D W, Tallant E A. Characterization of angiotensin II receptor subtypes in pancreatic acinar AR42J cells. *Peptides* 1995; 16:741-747.
19. Chappell M C, Tallant E A, Brosnihan K B, Ferrario C M. Conversion of angiotensin I to angiotensin-(1-7) by thimet

- oligopeptidase (E.C.3.4.24.15) in vascular smooth muscle cells. *J Vasc Biol Med* 1995; 5:129-137.
20. Daemen M J A P, Lombardi D M, Bosman F T, Schwartz S M. Angiotensin II induces smooth muscle cell proliferation in the normal and injured rat arterial wall. *Circ Res* 1991; 68:450-56.
21. Dzau V E, Pratt R, Gibbons G, Schunkert H, Lorell B, Ingelfinger J. Molecular mechanism of angiotensin in the regulation of vascular and cardiac growth. *J Mol Cell Cardiol* 1989; 21 [Suppl III]:57.
22. Naftilan A J, Pratt R E, Dzau V J. Induction of platelet-derived growth factor A-chain and c-myc gene expression by Angiotensin II in culture rat vascular smooth muscle cells. *J Clin Invest* 1989; 83:1419-24.
23. Stouffer G A, Owens G K. Angiotensin II induced mitogenesis of spontaneously hypertensive rat derived cultured smooth muscle cells is dependent on autocrine production of transforming growth factor- β . *Circ Res* 1992; 70:820-28.
24. Koibuchi Y, Lee W S, Gibbons G H, Pratt R E. Role of transforming growth factor β -1 in the cellular growth response to Angiotensin II. *Hypertension* 1993; 21:1046-50.
25. Kawahara Y, Sunako M, Tsuda T, Fukazaki H, Fukumoto Y, Takai Y. Angiotensin II induces expression of the c-fos gene through protein kinase C activation and calcium ion mobilization in cultured vascular smooth muscle cells. *BBRC* 1988; 150:52-9.
26. Mangiarua E I, Palmer V L, Lloyd L L, McCumbee W D. Platelet-derived growth factor mediates angiotensin II-induced DNA synthesis in vascular smooth muscle cells. *Arch Physiol Biochem* 1997; 105(2):151-7.
27. Su E J, Lombardi D M, Wiener J, Daemen M J, Reidy M A, and Schwartz M A. Mitogenic effect of angiotensin II on the rat carotid arteries and type II or III mesenteric microvessels but not type I mesenteric microvessels is mediated by endogenous basic fibroblast growth factor. *Circ Res* 1998; 82:321.
28. Rodgers L C, Driver V R, Armstrong D G. Assessment of the diabetic foot. In Krasner D L, Rodeheaver G T, Sibbald R G eds. *Chronic Wound Care: A Clinical Source Book for Healthcare Professionals*. 4th ed. Malvern Pa.: HMP Communications, 2007: 549-556.
29. Bolton L, McNees P, van Rijswijk L et al. Wound healing outcomes using standardized care. *JWOCN* 2004; 31:65-71.

SEQUENCE LISTING

```

<160> NUMBER OF SEQ ID NOS: 3

<210> SEQ ID NO 1
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa is Nle

```

```

<400> SEQUENCE: 1

```

```

Asp Arg Xaa Tyr Ile His Pro
1                5

```

-continued

<210> SEQ ID NO 2
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa is Nle

<400> SEQUENCE: 2

Asp Arg Xaa Tyr Ile
1 5

<210> SEQ ID NO 3
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa is Nle

<400> SEQUENCE: 3

Asp Arg Xaa Tyr Ile His
1 5

We claim:

1. A pharmaceutical formulation, comprising:
 - (a) about 0.5% to about 4% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis; and
 - (b) a peptide of at least 5 contiguous amino acids of Nle3 A(1-7);wherein the peptide is present at a concentration of about 0.03% to about 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
2. The pharmaceutical formulation of claim 1, wherein the peptide comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1).
3. The pharmaceutical formulation of claim 1, wherein the peptide consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1).
4. The pharmaceutical formulation of claim 1, comprising about 1% to about 3% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
5. The pharmaceutical formulation of claim 1, comprising about 2% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
6. The pharmaceutical formulation of claim 1, wherein the formulation comprises a hydrogel formulation.
7. The pharmaceutical formulation of claim 2, comprising about 1% to about 3% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
8. The pharmaceutical formulation of claim 2, comprising about 2% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
9. The pharmaceutical formulation of claim 2, wherein the formulation comprises a hydrogel formulation.
10. The pharmaceutical formulation of claim 3, comprising about 1% to about 3% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
11. The pharmaceutical formulation of claim 3, comprising about 2% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
12. The pharmaceutical formulation of claim 3, wherein the formulation comprises a hydrogel formulation.

* * * * *

45



European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact



Ettmayr, Andreas
KEHL, ASCHERL, LIEBHOFF & ETTMAYR
Patentanwälte - Partnerschaft
Emil-Riedel-Strasse 18
80538 München
ALLEMAGNE

Date
03.04.14

Reference 2045-1-EP	Application No./Patent No. 12703408.0 - 1456 / 2536422
Applicant/Proprietor The University Of Southern California	

Decision to grant a European patent pursuant to Article 97(1) EPC

Following examination of European patent application No. 12703408.0 a European patent with the title and the supporting documents indicated in the communication pursuant to Rule 71(3) EPC dated 28.11.13 is hereby granted in respect of the designated Contracting States.

Patent No. : 2536422
Date of filing : 01.02.12
Priority claimed : 02.02.11/USP201161438780

Designated Contracting States
and Proprietor(s) : AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI
LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR
The University Of Southern California
3740 McClintock Avenue
Los Angeles, CA 90089-2561/US

This decision will take effect on the date on which the European Patent Bulletin mentions the grant (Art. 97(3) EPC).

The mention of the grant will be published in European Patent Bulletin 14/18 of 30.04.14.

Examining Division

Orlando M

Boehmerova E

Weisser D



46

ANMERKUNG ZUR ENTSCHEIDUNG ÜBER DIE ERTEILUNG
EINES EUROPÄISCHEN PATENTS (EPA Form 2006)

1. **EPA Informationsbroschüre "Nationales Recht zum EPÜ"**

Diese Broschüre enthält nützliche Informationen zu den formalen Erfordernissen und den Handlungen, die vor den Patentbehörden der Vertragsstaaten vorzunehmen sind, um Rechte in diesen Staaten zu erlangen. Da diese Handlungen einem ständigen Wandel unterworfen sind, sollte immer nur die neueste Ausgabe der Broschüre benutzt werden. Nachträgliche Informationen werden im Amtsblatt veröffentlicht.

2. **Übersetzung der europäischen Patentschrift nach Artikel 65 (1) des Europäischen Patentübereinkommens**

Sie werden erneut darauf hingewiesen, dass bestimmte Vertragsstaaten nach Artikel 65 (1) EPÜ eine Übersetzung der europäischen Patentschrift verlangen; hierauf wird in der Mitteilung gemäß Regel 71 (5) EPÜ verwiesen. Die Nichteinreichung dieser Übersetzung kann zur Folge haben, dass das Patent in dem betreffenden Staat/in den betreffenden Staaten als von Anfang an nicht eingetreten gilt. Weitere Einzelheiten entnehmen Sie bitte der oben genannten Broschüre.

3. **Zahlung von Jahresgebühren für europäische Patente**

Nach Artikel 141 EPÜ können "nationale" Jahresgebühren für das europäische Patent für die Jahre erhoben werden, die an das Jahr anschließen, in dem der Hinweis auf die Erteilung des europäischen Patents im "Europäischen Patentblatt" bekanntgemacht wird. Weitere Einzelheiten entnehmen Sie bitte der oben genannten Broschüre.

NOTE RELATING TO THE DECISION TO GRANT A
EUROPEAN PATENT (EPO Form 2006)

1. **EPO Information Brochure "National law relating to the EPC"**

This brochure provides useful information regarding formal requirements and the steps to be taken before the patent authorities of the Contracting States in order to acquire rights in those states. Since the necessary steps are subject to change the latest edition of the brochure should always be used. Subsequent information is published in the Official Journal.

2. **Translation of the European patent application under Article 65(1) of the European Patent Convention**

Your attention is again drawn to the requirements regarding translation of the European patent specification laid down by a number of Contracting States under Article 65(1) EPC, to which reference is made in the communication under Rule 71(5) EPC. Failure to supply such translation(s) may result in the patent being deemed to be void "ab initio" in the State(s) in question. For further details you are recommended to consult the above-mentioned brochure.

3. **Payment of renewal fees for European patents**

Under Article 141 EPC "national" renewal fees in respect of a European patent may be imposed for the years which follow that in which the mention of the grant of the European patent is published in the "European Patent Bulletin". For further details you are recommended to consult the above-mentioned brochure.

REMARQUE RELATIVE A LA DECISION DE DELIVRANCE
D'UN BREVET EUROPEEN (OEB Form 2006)

1. **Brochure d'information de l'OEB "Droit national relatif à la CBE"**

Cette brochure fournit d'utiles renseignements sur les conditions de forme requises et sur les actes à accomplir auprès des offices de brevet des Etats contractants aux fins d'obtenir des droits dans les Etats contractants. Etant donné que les actes indispensables sont susceptibles de modifications, il serait bon de toujours consulter la dernière édition de la brochure. Toute information ultérieure est publiée au Journal Officiel.

2. **Traduction du fascicule du brevet européen en vertu de l'article 65(1) de la Convention sur le brevet européen**

Votre attention est de nouveau attirée sur l'obligation faite par certains Etats contractants, en vertu de l'article 65(1) CBE, de fournir une traduction du fascicule du brevet européen, à laquelle il est fait référence dans la notification établie conformément à la règle 71(5) CBE. Si la(les) traduction(s) n'est(ne sont) pas fournie(s), le brevet européen peut, dès l'origine, être réputé sans effet dans cet(ces) Etat(s). Pour plus de détails, nous vous renvoyons à la brochure susmentionnée.

3. **Paiement des taxes annuelles pour le brevet européen**

Conformément à l'article 141 CBE des taxes annuelles "nationales" dues au titre du brevet européen peuvent être perçues pour les années suivant celle au cours de laquelle la mention de la délivrance du brevet européen est publiée au "Bulletin européen des brevets". Pour plus de détails, nous vous renvoyons à la brochure susmentionnée.

EP 12703408.0

NEW CLAIMS

1. A peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof, for the use of treating diabetic foot ulcers in a human patient, wherein the peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof, is administered in an amount effective to treat the diabetic foot ulcer, and wherein the peptide is administered topically in a gel formulation that comprises 0.5% to 4% hydroxyethyl cellulose (HEC) on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
2. The peptide for use according to claim 1, wherein the peptide comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof,.
3. The peptide for use according to claim 1, wherein the peptide consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof,.
4. The peptide for use according to any one of claims 1-3, wherein the diabetic foot ulcer is one caused, at least in part, by neuropathy and resulting pressure.
5. The peptide for use according to any one of claims 1-4, wherein the diabetic foot ulcer comprises one or more calluses.
6. The peptide for use according to any one of claims 1-5, wherein the diabetic foot ulcer is a chronic ulcer, and wherein the chronic foot ulcer preferably has not responded to any other treatment.
7. The peptide for use according to any of claims 1-6, wherein the peptide is administered as a topical formulation forming a continuous film covering the entire area of the diabetic ulcer.
8. The peptide for use according to claim 7, wherein the peptide is administered in a hydrogel formulation.

48

9. The peptide for use according to any one of claims 1-8, wherein the peptide is administered at a concentration of 0.03 % to 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
10. A pharmaceutical formulation, comprising:
 - (a) 0.5% to 4% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis; and
 - (b) a peptide of at least 5 contiguous amino acids of Nle3 A(1-7), or salt thereof,; wherein the peptide, or salt thereof, is present at a concentration of 0.03% to 1% on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
11. The pharmaceutical formulation of claim 10, wherein the peptide comprises Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof,.
12. The pharmaceutical formulation of claim 10, wherein the peptide consists of Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:1), or salt thereof,.
13. The pharmaceutical formulation of any one of claims 10-12, comprising 1% to 3% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
14. The pharmaceutical formulation of any one of claims 10-12, comprising 2% HEC on a weight (mg)/volume (ml) basis, or on a weight/weight (mg) basis.
15. The pharmaceutical formulation of any one of claims 10-14, wherein the formulation comprises a hydrogel formulation.

49