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(54) **COMPOSITIONS CAPABLE OF REDUCING ELEVATED BLOOD UREA CONCENTRATION**

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C07K 2/00 (2006.01)
(52) **U.S. Cl.** **530/300; 530/330; 514/2**
(58) **Field of Classification Search** None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,977,244 A 12/1990 Muchmore et al.
5,010,175 A 4/1991 Rutter et al.
5,308,834 A 5/1994 Scott et al.
5,380,668 A 1/1995 Herron
5,436,270 A 7/1995 Wang
5,650,489 A 7/1997 Lam et al.
5,677,275 A 10/1997 Lunardi-Iskandar et al.
5,801,193 A 9/1998 Ojo-Amaize et al.
5,851,997 A 12/1998 Harris
5,854,004 A 12/1998 Czernilofsky et al.
5,877,148 A 3/1999 Lunardi-Iskandar et al.
5,942,494 A 8/1999 Ginsberg et al.
5,958,413 A 9/1999 Anagnostopoulos et al.
5,968,513 A 10/1999 Gallo et al.
5,997,871 A 12/1999 Gallo et al.
6,022,696 A 2/2000 Harding et al.
6,278,794 B1 8/2001 Parekh et al.
6,319,504 B1 11/2001 Gallo et al.
6,361,992 B1 3/2002 Szkudlinski et al.
6,489,296 B1 12/2002 Grinnell et al.
6,507,788 B1 1/2003 Camara y Ferrer et al.

6,583,109 B1 6/2003 Gallo et al.
6,586,403 B1 7/2003 Mathison et al.
6,596,688 B1 7/2003 Gallo et al.
6,620,416 B1 9/2003 Gallo et al.
6,642,201 B1 11/2003 Khavinson et al.
6,727,227 B1 4/2004 Khavinson
6,844,315 B2 1/2005 Khan et al.
6,921,751 B1 7/2005 Khan et al.
7,175,679 B2 2/2007 Khan et al.
7,358,330 B2 4/2008 Khan et al.
7,365,155 B2 4/2008 Khan et al.
7,402,322 B2 7/2008 Khan et al.
7,501,391 B2 3/2009 Khan et al.
7,517,529 B2 4/2009 Khan et al.
7,524,820 B1 4/2009 Khan et al.
7,560,433 B2 7/2009 Khan et al.
7,662,776 B2 2/2010 Khan et al.
7,786,084 B2 8/2010 Benner et al.
7,795,226 B2 9/2010 Benner et al.
2002/0041871 A1 4/2002 Brudnak
2002/0064501 A1 5/2002 Khan et al.
2002/0147306 A1 10/2002 Lin et al.
2002/0155106 A1 10/2002 Hammond
2003/0049273 A1 3/2003 Gallo et al.
2003/0113733 A1 6/2003 Khan et al.

(Continued)

FOREIGN PATENT DOCUMENTS

DE 3715662 11/1987
DE 19953339 5/2001
EP 0 572 688 5/1997
EP 1 138 692 A1 10/2001
EP 1 300 418 4/2003
EP 1 224 212 B1 7/2003
FR 2 706 772 12/1994
GB 2 194 886 A 3/1988
JP 09-176187 A 7/1997
WO WO 92/20795 A1 11/1992

(Continued)

OTHER PUBLICATIONS

Adib-Conquy et al., "NF-κB Expression in Mononuclear Cells in Patients with Sepsis Resembles That Observed in Lipopolysaccharide Tolerance," Am. J. Respir. Crit. Care Med., 2000, pp. 1877-1883, vol. 162.
Agrawal et al., Acute Renal Failure, American Family Physician, 2000, pp. 2077-2088, vol. 61, corresponding to web version of p. 1-12.
Arima et al., "IL-2-Induced Growth of CD8+ T Cell Prolymphocytic Leukemia Cells Mediated by NF-kappaB Induction and IL-2 Receptor alpha Expression," Leukemia Research, 1998, pp. 265-273, vol. 22, No. 3.

(Continued)

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(57) **ABSTRACT**

The invention includes a method of reducing urea concentration in a subject's serum. Such a method comprises administering to the subject (e.g., a mammal such as a human) a composition comprising an oligopeptide (or oligopeptides) having activity in reducing urea concentration in the subject's serum as determined by a mouse renal reperfusion test, wherein the oligopeptide comprises the sequence AQG or MTRV (SEQ ID NO:1), AQGV (SEQ ID NO:2) or LAGV (SEQ ID NO:4).

4 Claims, 3 Drawing Sheets

U.S. PATENT DOCUMENTS

2003/0119720	A1	6/2003	Khan et al.
2003/0148955	A1	8/2003	Pluenneke
2003/0166556	A1	9/2003	Khan et al.
2003/0186244	A1	10/2003	Margus et al.
2003/0212253	A1	11/2003	Hammond et al.
2003/0215434	A1	11/2003	Khan et al.
2003/0219425	A1	11/2003	Khan et al.
2003/0220257	A1	11/2003	Benner et al.
2003/0220258	A1	11/2003	Benner et al.
2003/0220259	A1	11/2003	Benner et al.
2003/0220260	A1	11/2003	Khan et al.
2003/0220261	A1	11/2003	Khan et al.
2003/0224995	A1	12/2003	Khan et al.
2004/0013661	A1	1/2004	Wensvoort et al.
2004/0208885	A1	10/2004	Khan et al.
2005/0037430	A1	2/2005	Khan et al.
2005/0107314	A1	5/2005	Gorczyński et al.
2005/0119184	A1	6/2005	Khan et al.
2005/0214943	A1	9/2005	Khan et al.
2005/0227925	A1	10/2005	Benner et al.
2006/0111292	A1	5/2006	Khan et al.
2006/0142205	A1	6/2006	Benner et al.
2006/0173162	A1	8/2006	Djurup et al.
2006/0275255	A1	12/2006	Gudkov
2007/0111948	A1	5/2007	Turdiev

FOREIGN PATENT DOCUMENTS

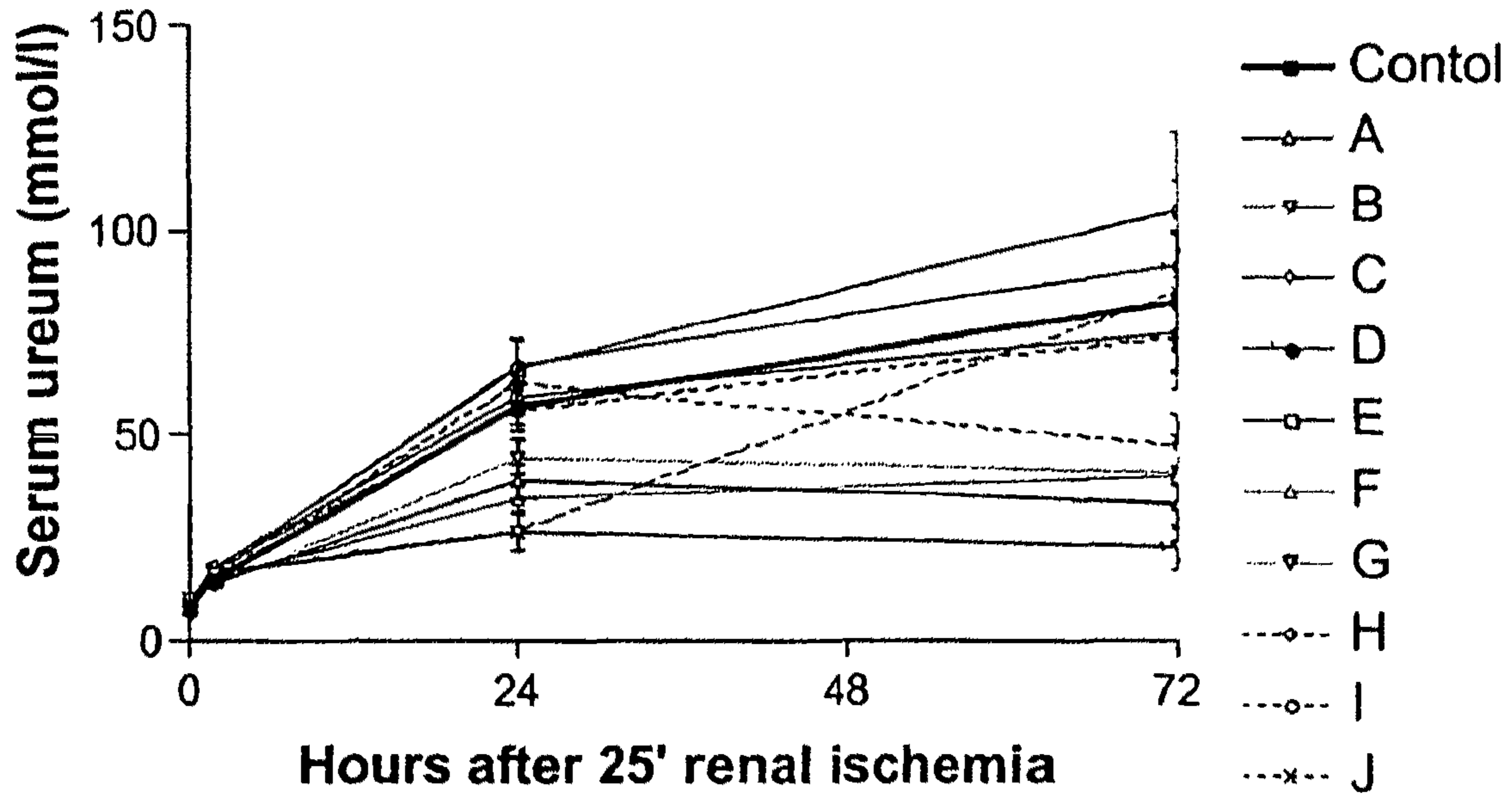
WO	96/04008	2/1996
WO	WO 96/33218	10/1996
WO	97/49373	12/1997
WO	97/49418	12/1997
WO	97/49432	12/1997
WO	WO 97/49721	12/1997
WO	WO 98/06742	2/1998
WO	WO 98/34631	A1 8/1998
WO	WO 98/35691	8/1998
WO	WO 99/31227	6/1999
WO	WO 99/59617	11/1999
WO	WO 00/17348	3/2000
WO	WO 01/10907	A2 2/2001
WO	WO 01/11048	A2 2/2001
WO	WO 01/10457	A2 2/2001
WO	WO 01/29067	4/2001
WO	WO 01/29069	A1 4/2001
WO	WO 01/32196	A1 5/2001
WO	WO 01/36454	A1 5/2001
WO	WO 01/51508	A1 7/2001
WO	WO 01/68113	A1 9/2001
WO	WO 01/72831	10/2001
WO	WO 01/83554	A2 11/2001
WO	WO 02/085117	10/2002
WO	WO 03/029292	A2 4/2003
WO	WO 2004/093897	11/2004
WO	WO 2005/097163	10/2005
WO	WO 2006/069198	A1 6/2006

OTHER PUBLICATIONS

Babu, V. V. Suresh (Synthetic Communcaitons 29 (1)m 79-91, 1999.
 Baeuerle et al., "Function and Activation of NF- κ B in the Immune System," *Annu. Rev. Immunol.*, 1994, pp. 141-179, vol. 12.
 Barton et al., Protective Role of Interleukin 6 in the Lipopolysaccharide-Galactosamine Septic Shock Model, *Infection and Immunity*, Apr. 1993, pp. 1496-1499, vol. 61, No. 4.
 Baud et al., Signaling by proinflammatory cytokines: oligomerization of TRAF2 and TRAF6 is sufficient for JNK and IKK activation and target gene induction via an amino-terminal effector domain, *Genes & Development*, May 1999, pp. 1297-1308, vol. 13.
 Brown et al., "Two Forms of NF-kappaB1 (p105/p50) in Murine Macrophages: Differential Regulation by Lipopolysaccharide, Interleukin-2, and Interferon γ ," *Journal of Interferon and Cytokine Research*, 1997, pp. 295-306, vol. 17.
 Capizzi, *Investigational New Drugs*, 1996, 14:249-256.
 Cillari et al., (*Infection and Immunity*, vol. 62, No. 6, p. 2649-2652, Jun. 1994).

Cui et al., *Am. J. Physiol. Integr. Comp. Physiol.*, 2004, pp. R699-R709, vol. 286.
 Daemen et al., Ischemia-reperfusion-induced IFN-gamma up-regulation: involvement of IL-12 and IL-13, *The Journal of Immunology*, 1999, pp. 5506-5510, vol. 162.
 De Saizieu et al., *Journal of Bacteriology*, vol. 182, No. 17, pp. 4696-4703, Sep. 2000.
 Dechend et al., *Oncogene*, vol. 18, pp. 3316-3323, 1999.
 Donnahoo et al., Early kidney TNF-alpha expression mediates neutrophil infiltration and injury after renal ischemia-reperfusion, *American Journal of Physiology*, Sep. 1999, pp. R922-R929, vol. 277, No. 3, Pt. 2.
 Dwinnell et al., *Atlas of Diseases of the Kidney*, Blackwell Sciences, 1999, pp. 12.1-12.12, Ch. 12.
 Eckardt et al., Hypoxia-induced accumulation of erythropoietin mRNA in isolated hepatocytes is inhibited by protein kinase C, *Pflugers Archiv.*, 1994, pp. 21-30, vol. 426.
 GenBank Accession No. NP_000728, GI: 4502789, publicly available Apr. 2007.
 Hierholzer et al., Essential role of induced nitric oxide in the initiation of the inflammatory response after hemorrhagic shock, *J. Exp. Med.*, Mar. 1998, pp. 917-928, vol. 187, No. 6.
 Huang et al., Ischemia-reperfusion and immediate T cell responses, *Cellular Immunology*, 2007, pp. 4-11, vol. 248.
 Husek et al., Rapid screening of urinary proline-hydroxyproline dipeptide in bone turnover studies, Abstract, *J. Chromatogr B Analyt Technol Biomed Life Sci.*, Feb. 5, 2002, pp. 169-174, vol. 767, No. 1.
 Kalns et al., *Biochem. Biophys. Res. Comm.*, 2002, pp. 41-44, vol. 292.
 Kalns et al., *Biochem. Biophys. Res. Comm.*, 2002, pp. 506-509, vol. 297.
 Keeton and Gould, *Biological Science*, 5th Ed., New York, W.W. Norton & Company, Inc. 1993, p. 4.
 Lin et al., *The Journal of Biological Chemistry*, vol. 270, No. 24, pp. 14255-14258, Jun. 1995.
 Lunardi-Iskandar et al., "Effects of a urinary factor from women in early pregnancy on HIV-a, SIV and associated disease," *Nature Medicine*, pril 1998, pp. 428-434, vol. 4, No. 4.
 Merck Index, 17th ed. 1999, pp. 1145-1146, 1841-1848, 2539, 2551.
 Moayeri et al., *Journal of Clinical Investigation*, Sep. 2003, pp. 670-682, vol. 112, No. 5.
 Ohlsson et al., Interleukin-1 Receptor Antagonist Reduces Mortality from Endotoxin Shock, *Nature*, Dec. 6, 1990, pp. 550-552, vol. 348.
 Oka et al., Immunosuppression in organ transplantation, *Japanese Journal of Pharmacology*, vol. 71, No. 2, pp. 89-100, Jun. 1996.
 Olszyna et al., Levels of Inhibitors of Tumor Necrosis Factor Alpha and Interleukin 1b in Urine and Sera of Patients with Urosepsis, *Infection and Immunity*, Aug. 1998, pp. 3527-3534.
 Partial European Search Report for 02 763 111.8 dated Nov. 23, 2007.
 Riera et al., Neutrophils accentuate renal cold ischemia-reperfusion injury. Dose-dependent protective effect of platelet-activating factor receptor antagonist, *The Journal of Pharmacology and Experimental Therapeutics*, 1997, pp. 786-794, vol. 280, No. 2.
 Roice, M. (*Tetrahedron* 56 (23), 3725-3734, 2000.
 Van Holde, *Physical Biochemistry*, pp. 3947, Prentice-Hall, 1971.
 Wallraff et al., Urinary Excretion of Amino Acids in Pregnancy, *J. Clin. Invest.*, 1950, pp. 1542-1544, vol. 29.
 Werner et al., *Experientia* vol. 42, p. 521-531, 1986).
 Wu et al., Gonadotropin-Releasing Hormone (GNRH) Cleavage Products are Involved in the Regulation of GNRH Gene Expression in the GT1-7 Neuronal Cell Line, *Society for Neuroscience Abstracts*, Nov. 4, 2000, pp. 7.8, XP009091566, vol. 26, No. 1-2.
 U.S. Appl. No. 11/593,329, filed Nov. 6, 2006, Treatment of Ischemic Events.
 U.S. Appl. No. 11/600,294, filed Nov. 15, 2006, Oligopeptide Acetate and Formulations Thereof.
 U.S. Appl. No. 11/975,284, filed Oct. 17, 2007, Treatment for Tumors.
 U.S. Appl. No. 11/981,491, filed Oct. 30, 2007, Treatment of Iatrogenic Disease.
 U.S. Appl. No. 11/982,292, filed Oct. 31, 2007, Treatment of Neurological Disorders.

- U.S. Appl. No. 11/986,043, filed Oct. 30, 2007, Peptide Compositions.
- U.S. Appl. No. 12/001,035, filed Dec. 6, 2007, Gene Regulator.
- Albini, A., et al., "Old drugs as novel angiogenesis inhibitors: Preclinical studies with NAC, hCG, EGCG and somatostatin," 17 *Clinical & Experimental Metastasis* 739 (1999).
- Blackwell, Timothy S., et al., "The Role of Nuclear Factor- κ B in Cytokine Gene Regulation," 17 *Am. J. Respir. Cell Mol. Biol.* 3-9 (1997).
- Christman et al., Nuclear factor kappaB: a pivotal role in the systemic inflammatory response syndrome and new target for therapy, *Intens Care Med*, 1998, pp. 1131-1138, vol. 24.
- Connelly et al., Biphasic Regulation of NF- κ B Activity Underlies the Pro- and Anti-Inflammatory Actions of Nitric Oxide, *The Journal of Immunology*, 2001, pp. 3873-3881, 166, The American Association of Immunologists, USA.
- Friedlander, Tackling anthrax, *Nature*, Nov. 8, 2001, pp. 160-161, vol. 414.
- Iskandar et al., "Effects of a urinary factor from women in early pregnancy on HIV-1. SIV and associated disease", *Nature Medicine*, Apr. 1998, vol. 4, No. 4, pp. 428-434.
- Jyonouchi et al., Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression, *J Neuroim.*, 2001, pp. 170-179, vol. 120.
- Kachra et al., "Low Molecular Weight Components but Not Dimeric HCG Inhibit Growth and Down-Regulate AP-1 Transcription Factor in Kaposi's Sarcoma Cells," *Endocrinology*, 1997, pp. 4038-4041, vol. 138, No. 9.
- Kanungo et al., Advanced Maturation of Heteropneustes Fossilis (Bloch) by Oral Administration of Human Chorionic Gonadotropin, *J. Adv. Zool.*, 1999, pp. 1-5, vol. 20.
- Keller, S., et al., "Human Chorionic Gonadotropin (hCG) Is a Potent Angiogenic Factor for Uterine Endothelial Cells in Vitro," 20(5-6) *Placenta*, p. A37 (Jul. 1999).
- Khan, Nisar A., et al., "Inhibition of Diabetes in NOD Mice by Human Pregnancy Factor," 62(12) *Human Immunology* 1315-1323 (Dec. 2001).
- Khan, Nisar A., et al., "Inhibition of Septic Shock in Mice by an Oligopeptide From the β -Chain of Human Chorionic Gonadotrophin Hormone," 63(1) *Human Immunology* 1-7 (Jan. 2002).
- Lang et al., "Induction of apoptosis in Kaposi's sarcoma spindle cell cultures by the subunits of human chorionic gonadotropin", *AIDS* 1997, vol. 11, No. 11, pp. 133-1340.
- Medzhitov, Toll-like Receptors and Innate Immunity, *Nature Reviews/Immunology*, Nov. 2001, pp. 135-145, vol. 1.
- Muchmore et al., Immunoregulatory Properties of Fractions from Human Pregnancy Urine: Evidence that Human Chorionic Gonadotropin is not Responsible, *The Journal of Immunology*, Mar. 1997, pp. 881-886, vol. 118, No. 3.
- Muchmore et al., Purification and Characterization of a Mannose-Containing Disaccharide Obtained from Human Pregnancy Urine, *Journal of Experimental Medicine*, Dec. 1984, pp. 1672-1685, vol. 160.
- Patil, A., et al., "The Study of the Effect of Human Chorionic Gonadotrophic (HCG) Hormone on the Survival of Adrenal Medulla Transplant in Brain. Preliminary Study," 87 *Acta Neurochir (WIEN)* 76-78 (1987).
- Rohrig et al., Growth-stimulating Influence of Human Chorionic Gonadotropin (hCG) on *Plasmodium falciparum* in vitro, *Zentralblatt Bakt*, 1999, pp. 89-99, vol. 289.
- Slater, Lewis M., et al., "Decreased Mortality of Murine Graft-Versus-Host Disease by Human Chorionic gonadotropin," 23(1) *Transplantation* 103-104 (Jan. 1977).
- Tak et al., NF-kappaB: a key role in inflammatory diseases, *J Clin Invest.*, 2001, pp. 7-11, vol. 107.
- Tan et al., The role of activation of nuclear factor-kappa B of rat brain in the pathogenesis of experimental allergic encephalomyelitis, *Acta Physiol Sinica*, 2003, pp. 58-64, vol. 55.
- Tovey et al., Mucosal Cytokine Therapy: Marked Antiviral and Antitumor Activity, *J. Interferon Cytokine Res.*, 1999, pp. 911-921, vol. 19.
- Wulczyn, F. Gregory, et al., "The NF-kB/Rel and I κ B gene families: mediators of immune response and inflammation," 74(12) *J. Mol. Med.* 749-769 (1996).
- Yamamoto, Y., et al., "Role of the NF-kB Pathway in the Pathogenesis of Human Disease States," 1(3) *Current Molecular Medicine* 287-296 (Jul. 2001).
- Ivanov et al., "Hemoglobin as a Source of Endogenous Bioactive Peptides: The Concept of Tissue-Specific Peptide Pool," *Biopolymers*, 1997, pp. 171-188, vol. 39.
- Khavinson et al, Gerontological Aspects of Genome Peptide Regulation, 2005, S. Karger AG, Basel, Switzerland.
- Khavinson et al., "Effects of Livagen Peptide on Chromatin Activation in Lymphocytes from Old People," *Bulletin of Experimental Biology and Medicine*, Oct. 2002, pp. 389-392, vol. 134, No. 4.
- Khavinson et al., "Effects of Short Peptides on Lymphocyte Chromatin in Senile Subjects," *Bulletin of Experimental Biology and Medicine*, Jan. 2004, pp. 78-81, vol. 137, No. 1.
- Khavinson et al., "Epithalon Peptide Induces Telomerase Activity and Telomere Elongation in Human Somatic Cells," *Bulletin of Experimental Biology and Medicine*, Jun. 2003, pp. 590-592, vol. 135, No. 6.
- Khavinson et al., "Inductive Activity of Retinal Peptides," *Bulletin of Experimental Biology and Medicine*, Nov. 2002, pp. 482-484, vol. 134, No. 5.
- Khavinson et al., "Mechanisms Underlying Geroprotective Effects of Peptides," *Bulletin of Experimental Biology and Medicine*, Jan. 2002, pp. 1-5, vol. 133, No. 1.
- Khavinson et al., "Peptide Promotes Overcoming of the Division Limit in Human Somatic Cell," *Bulletin of Experimental Biology and Medicine*, May 2004, pp. 503-506, vol. 137, No. 5.
- Morozov et al., "Natural and Synthetic Thymic Peptides as Therapeutics for Immune Dysfunction," *Int. J. Immunopharmac.*, 1997, pp. 501-505, vol. 19, No. 9/10.



Op 24 uur post-reperfusie:

(C-term: CARBOXYL; N-term: FREE)

- A p=0.0491 NMPF-47 LAGV
- B p=0.0008 NMPF-46 AQGV
- C p=0.9248 NMPF-44 LAG
- D p=0.4043 NMPF-43 AQG
- E p=0.1848 NMPF-12 MTR
- F p=0.0106 NMPF-11 MTRV
- G p=0.1389 NMPF-7 VLPALPQ
- H p=0.5613 NMPF-6 VLPALP
- I p=0.9301 NMPF-4 LQGV
- J p=0.0030 NMPF-3 LQG

Op 72 uur post-reperfusie:

(C-term: CARBOXYL; N-term: FREE)

- A p=0.0017 NMPF-47 LAGV
- B p<0.0001 NMPF-46 AQGV
- C p=0.8186 NMPF-44 LAG
- D p=0.2297 NMPF-43 AQG
- E p=0.0242 NMPF-12 MTR
- F p=0.0021 NMPF-11 MTRV
- G p=0.0049 NMPF-7 VLPALPQ
- H p=0.3297 NMPF-6 VLPALP
- I p=0.8328 NMPF-4 LQGV
- J p=0.9445 NMPF-3 LQG

FIG. 1

Serum urea values following 25 minutes of renal ischemia
(Mean \pm SEM)

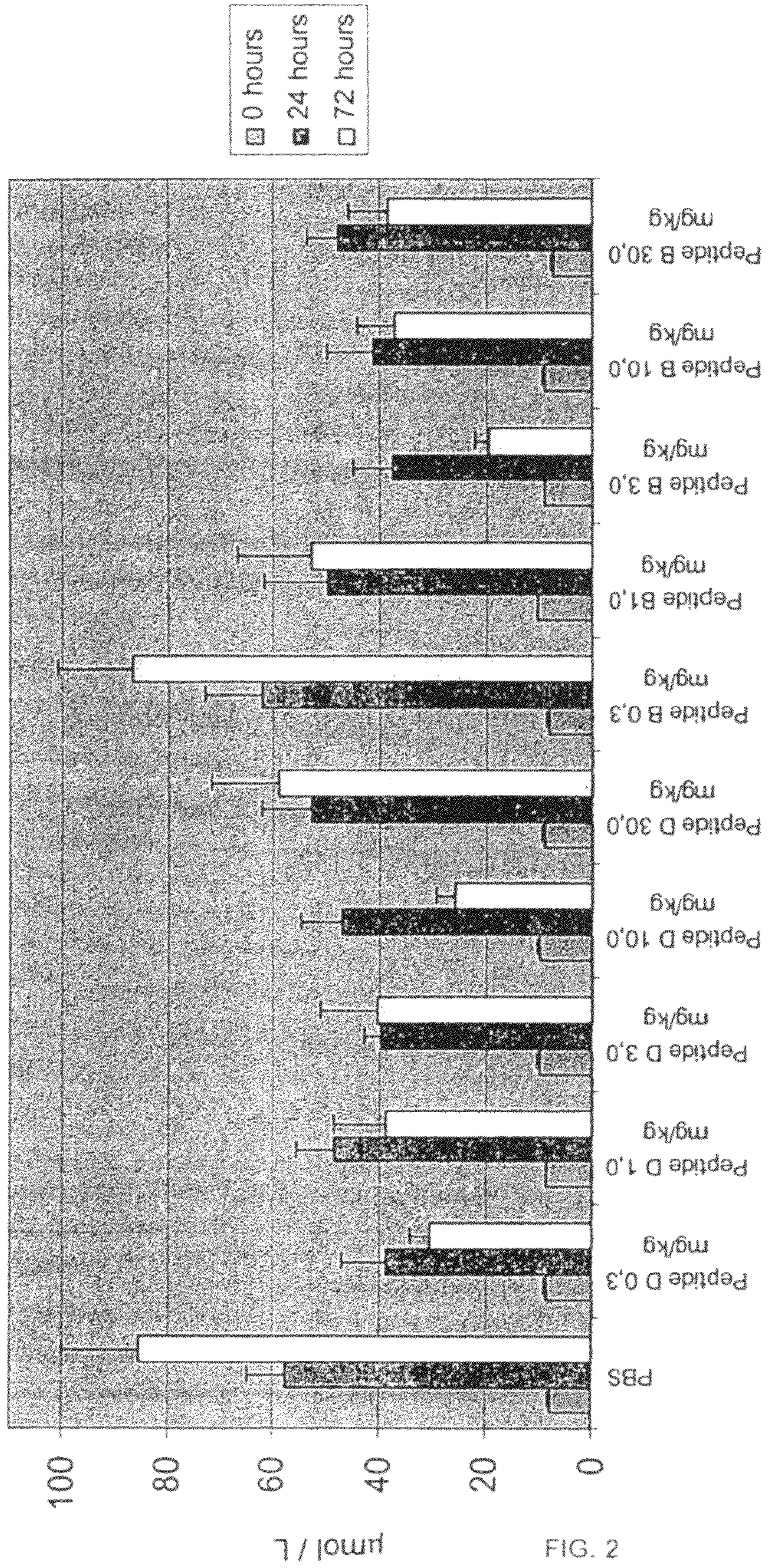


FIG. 2

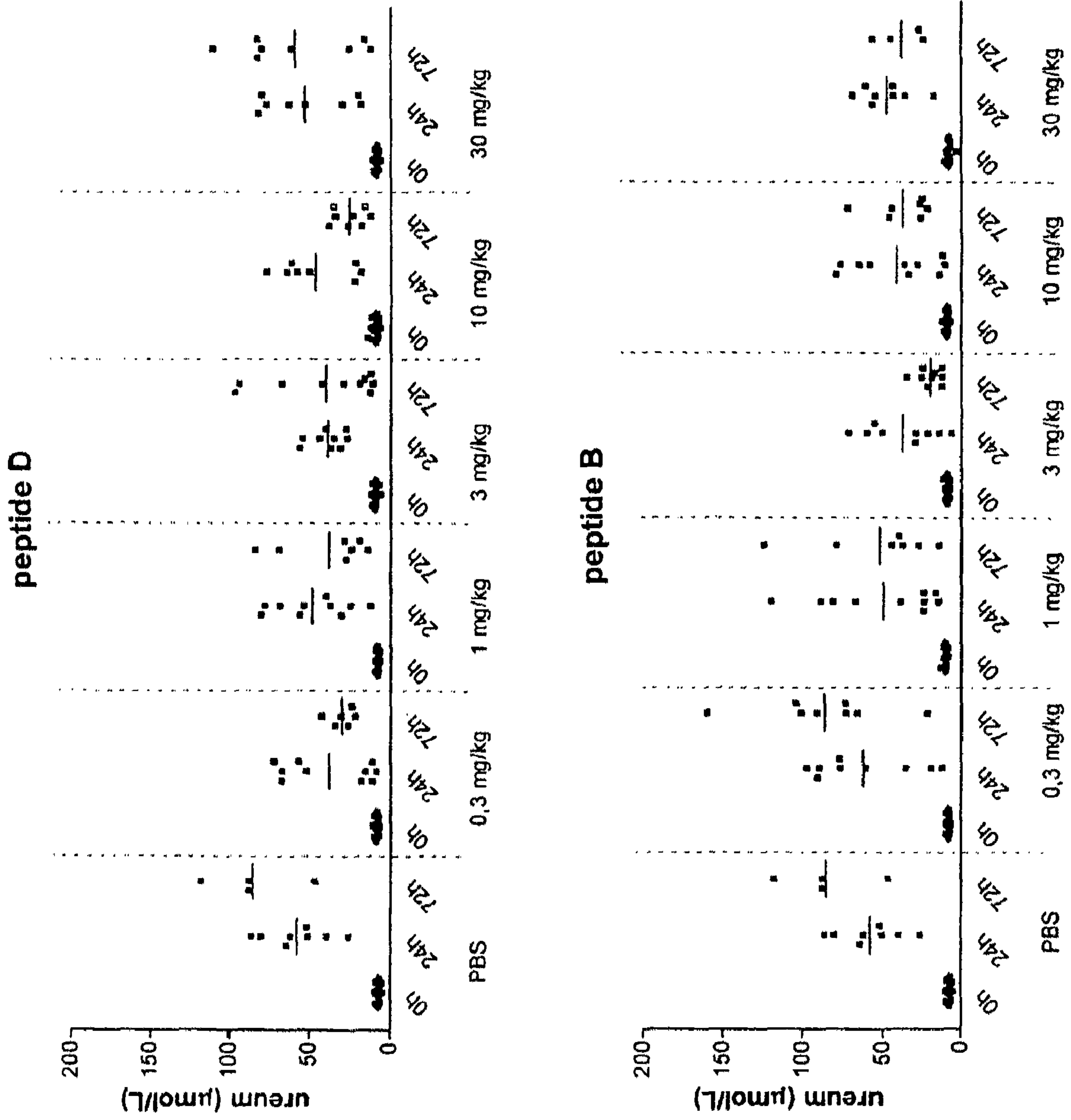


FIG. 3

**COMPOSITIONS CAPABLE OF REDUCING
ELEVATED BLOOD UREA
CONCENTRATION**

Matter enclosed in heavy brackets [] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

CROSS-REFERENCE TO RELATED
APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 11/249,541, filed on Oct. 13, 2005 now abandoned, which is a continuation-in-part of International Application No. PCT/EP2005/003707, filed on Apr. 8, 2005, designating the United States of America, U.S. patent application Ser. No. 10/821,256, filed on Apr. 8, 2004 now abandoned, and U.S. patent application Ser. No. 10/262,522, filed on Sep. 30, 2002 now U.S. Pat. No. 7,365,155, which itself is a continuation of International Application No. PCT/NL01/00259, (International Publication No. WO 01/72831 A2) filed [Mar. 3, 2001] *Mar. 29, 2001*, designating the United States of America, the contents of the entirety of all of which are incorporated by this reference.

TECHNICAL FIELD

The invention relates generally to biotechnology, and more specifically to compositions having immunoregulatory activity, which compounds include particular oligopeptides derived from human chorionic gonadotropin ("hCG").

BACKGROUND

U.S. Pat. No. 5,380,668 to Herron (Jan. 10, 1995), the contents of the entirety of which are incorporated by this reference, discloses, among other things, various compounds having the antigenic binding activity of hCG. The oligopeptides disclosed therein are disclosed generally for use in diagnostic methods.

Various patents and patent applications to Gallo et al. (e.g., U.S. Pat. No. 5,677,275 (corresponding to WO 96/04008 A1), U.S. Pat. No. 5,877,148 (also corresponding to WO 96/04008 A1), WO 97/49721 A1, U.S. Pat. No. 6,319,504 (corresponding to WO 97/49373), U.S. Patent Application 2003/0049273 A1 (also corresponding to WO 97/49373), U.S. Pat. No. 5,968,513 (corresponding to WO 97/49418), U.S. Pat. No. 5,997,871 (corresponding to WO 97/49432), U.S. Pat. Nos. 6,620,416, 6,596,688, WO 01/11048 A2, WO 01/10907 A2., and U.S. Pat. No. 6,583,109) relate to various oligopeptides and their use in, among other things, "inhibiting HIV infection," "treating or preventing HIV infection," "treating or preventing cancer," "treating or preventing a condition characterized by loss of body cell mass," "treating or preventing a condition associated with pathological angiogenesis," "treating or preventing hematopoietic deficiency," "ex vivo gene therapy," "expanding blood cells in vitro," and/or "providing blood cells to a subject."

DISCLOSURE OF THE INVENTION

As described in PCT International Publication No. WO 03/029292 A2 (published Apr. 10, 2003), PCT International Publication No. WO 01/72831 A2 (published Oct. 4, 2001), and U.S. Patent Application Publications 20020064501 A1

(published May 30, 2002), 20030119720 A1 (published Jun. 26, 2003), 20030113733 A1 (published Jun. 19, 2003), and 20030166556 A1 (published Sep. 4, 2003), the contents of all of which are incorporated by this reference, compositions containing some of the oligopeptides described herein have immunoregulatory activity useful in, for example, the treatment of sepsis and other disease states and conditions.

The invention includes a method of reducing blood urea nitrogen (BUN) concentration (herein also called urea concentration) in a subject's serum. Such a method comprises administering to the subject (e.g., a mammal such as a human) a composition comprising an oligopeptide (or oligopeptides) having activity in reducing urea concentration in the subject's serum as determined by a mouse renal reperfusion test, wherein the oligopeptide comprises the sequence AQG or LAGV (SEQ ID NO:4), or AQGV (SEQ ID NO:2) or MTRV (SEQ ID NO:1).

The oligopeptide of the composition will typically be from three (3) to twelve (12) amino acids in length. In the case where the composition includes the oligopeptide AQG or LAGV (SEQ ID NO:4) or AQGV (SEQ ID NO:2), the composition may be administered orally. The oligopeptide will preferably be of synthetic origin (e.g., produced by a Merrifield synthesis). When the composition is administered to the subject parenterally, the composition will typically consist essentially of oligopeptide and PBS (e.g., in an amount of from about 0.25 to about 10 mg/kg body mass of the subject).

The invention is thought to be useful for instances, when, for example, the subject is undergoing acute renal failure, especially when the subject is undergoing persistent oliguria, is not producing more than ½ ml urine per hour per kilogram body mass of the subject, and/or has a serum potassium level greater than 6.5 mmol per liter serum.

In one preferred embodiment, the invention involves administering a purified, synthetic or isolated peptide consisting of AQGV (SEQ ID NO:2), or an acid addition salt thereof. A typical dosage of this peptide will vary from about 0.5 to about 35 mg/kg body weight of the subject

In another preferred embodiment, typically when a relatively low dosage is preferred, the invention involves administering a purified, synthetic or isolated peptide consisting of AQG, or an acid addition salt thereof at, for example, a dosage of the peptide from about 0.1 to about 10 mg/kg body weight of the subject.

The invention also provides use of a composition according to the invention for the preparation of a pharmaceutical composition or medicament for the treatment of a disorder such as acute renal failure. Such a composition is preferably prepared using a purified, synthetic or isolated peptide consisting of AQG or AQGV (SEQ ID NO:2) or LAGV (SEQ ID NO:4) or MTRV (SEQ ID NO:1), or an acid addition salt thereof. Most preferably, AQG (when low dosages are preferred) or AQGV (SEQ ID NO:2) is used.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 graphically depicts the results of Example 1. Shown are the BUN (urea) values at the various points in time after treatment with peptides A to F or without treatment (control).

FIG. 2 graphically depicts the results of Example 4. Shown are the BUN (urea) values at the various points in time after treatment with varying dosages of peptide D or peptide B or without treatment (PBS).

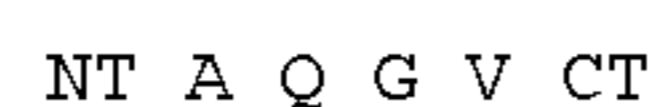
FIG. 3 depicts the blood urea values of Example 4 at 0, 24, and 72 hours post-clamping after administration of "peptide D" AQG and "peptide B" AQGV (SEQ ID NO:2). PBS control compared to peptide administered groups.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, a "purified, synthetic or isolated" peptide is one that has been purified from a natural or biotechnological source, or, more preferably, is synthesized as described herein.

"Composition," as used herein, refers to chemical compounds that contain or consist of the oligopeptide. The oligopeptide is preferably isolated before inclusion within the composition. The oligopeptide most preferably consists of three (3) to six (6) amino acids.

For instance, the previously described preferred compound could, in one embodiment be:



wherein NT at the N-terminus is selected from the group of H—, CH₃—, an acyl group, or a general protective group; and CT at the C-terminus is selected from the group of small (e.g. 1 to 5 amino acids) peptides, —OH, —OR¹, —NH₂, —NHR¹, —NR¹R², or —N(CH₂)₁₋₆NR¹R², wherein R¹R², when present, are independently selected from H, alkyl, aryl, (ar)alkyl, and wherein R¹R², can be cyclically bonded to one another.

"Alkyl" as used herein, is preferably a saturated branched or unbranched hydrocarbon having one to six carbon atoms, for example, methyl, ethyl, and isopentyl.

"Aryl" as used herein, is an aromatic hydrocarbon group, preferably having 6 to 10 carbon atoms, such as phenyl or naphthyl.

"(Ar)alkyl" as used herein, is an arene group (having both aliphatic and aromatic portions), preferably having 7 to 13 carbon atoms such as benzyl, ethylbenzyl, n-propylbenzyl, and isobutylbenzyl.

"Oligopeptide" as used herein, are peptides having from 3 to 12 amino acids joined together by peptide bonds. Equivalent to oligopeptides are compounds having the same or equivalent side chains as the particular amino acids used in an oligopeptide, and arranged sequentially in the same order as the peptides, but joined together by non-peptide bonds, e.g., by isosteric linkages such as the keto isostere, hydroxy isostere, diketo isostere, or the keto-difluoromethylene isostere.

"Composition" also includes, for example, an acceptable salt of the oligopeptide or a labeled oligopeptide. As used herein, "acceptable salt" refers to salts that retain the desired activity of the oligopeptide or equivalent compound, but preferably do not detrimentally affect the activity of the oligopeptide or other component of a system in which uses the oligopeptide. Examples of such salts are acid addition salts formed with inorganic acids, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like. Salts may also be formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, and the like. Salts may be formed with polyvalent metal cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel and the like or with an organic cation formed from N,N'-dibenzylethylenediamine or ethylenediamine, or combinations thereof (e.g., a zinc tannate salt).

The invention also provides use of an oligopeptide having activity in reducing urea concentration in a subject's serum as determined by a mouse renal reperfusion test, the oligopeptide preferably comprising the sequence AQG or MTRV (SEQ ID NO:1) or LAGV (SEQ ID NO:4), in the production of a pharmaceutical composition for reducing urea concentration in a subject's serum, in particular when the subject is undergoing acute renal failure. It is preferred that the oligopeptide to be used in the production of the pharmaceutical composition consists of AQGV (SEQ ID NO:2).

Such a pharmaceutical composition may be administered to the subject parenterally or orally. Such a pharmaceutical composition may consist essentially of oligopeptide and PBS. It is preferred that the oligopeptide is of synthetic origin. Suitable treatment for example entails administering the oligopeptide in the pharmaceutical composition to the patient intravenously in an amount of from about 0.1 to about 35 mg/kg body mass of the subject. It may be useful that the pharmaceutical composition consists essentially of from one to three different oligopeptides.

Such treatment is in particular preferred when the subject is undergoing persistent oliguria, for example when the subject's kidneys are not producing more than 1/2 ml urine per hour per kilogram body mass of the subject, or when the subject has a serum potassium level greater than 6.5 mmol per liter serum.

The thus developed chemical entity can be administered and introduced in-vivo systemically, topically, or locally. The peptide, or its modification or derivative, can be administered as the entity as such or as a pharmaceutically acceptable acid- or base addition salt, formed by reaction with an inorganic acid (such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid); or with an organic acid (such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid); or by reaction with an inorganic base (such as sodium hydroxide, ammonium hydroxide, potassium hydroxide); or with an organic base (such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines). A selected peptide and any of the derived entities may also be conjugated to sugars, lipids, other polypeptides, nucleic acids and PNA; and function in-situ as a conjugate or be released locally after reaching a targeted tissue or organ.

A "substitution" with regard to the various amino acids generally relate to substituting a group such as alkoxy, halogen, hydroxy, nitro, or lower alkyl onto an aromatic ring for hydrogen that would usually be present. Substitutions can also be made on the alkyl chain connecting the aromatic portion to the peptide backbone, with, for instance lower alkyl groups substituting for hydrogen. Still further substitutions can be made at the alpha position of an amino acid, also using an alkyl group.

Preferred substitutions involve the use of fluorine or chlorine as a halogen, and methoxy as an alkoxy group. With regard to alkyl and lower alkyl, generally alkyl groups having fewer (1 to 3) carbon atoms are preferred.

The compounds according to the general formula may be prepared in a manner conventional for such compounds. To that end, suitably N alpha protected (and side-chain protected if reactive side-chains are present) amino acid derivatives or peptides are activated and coupled to suitably carboxyl protected amino acid or peptide derivatives either in solution or on a solid support. Protection of the alpha-amino functions generally takes place by urethane functions such as the acid-labile tertiary-butyloxycarbonyl group ("Boc"), benzyloxycarbonyl ("Z") group and substituted analogs or the base-

labile 9-fluorenyl-methyloxycarbonyl ("Fmoc") group. The Z group can also be removed by catalytic hydrogenation. Other suitable protecting groups include the Nps, Bmv, Bpoc, Aloc, MSC, etc. A good overview of amino protecting groups is given in *The Peptides, Analysis, Synthesis, Biology*, Vol. 3 E. Gross and J. Meienhofer, eds. (Academic Press, New York, 1981). Protection of carboxyl groups can take place by ester formation, for example, base-labile esters like methyl or ethyl, acid labile esters like tert. butyl or, substituted, benzyl esters or hydrogenolytically. Protection of side-chain functions like those of lysine and glutamic or aspartic acid can take place using the aforementioned groups. Protection of thiol, and although not always required, of guanidino, alcohol and imidazole groups can take place using a variety of reagents such as those described in *The Peptides, Analysis, Synthesis, Biology*, id. or in *Pure and Applied Chemistry*, 59(3), 331-344 (1987). Activation of the carboxyl group of the suitably protected amino acids or peptides can take place by the azide, mixed anhydride, active ester, or carbodiimide method especially with the addition of catalytic and racemization-suppressing compounds like 1-N-N-hydroxybenzotriazole, N-hydroxysuccinimide, 3-hydroxy-4-oxo-3,4-dihydro-1,2,3,-benzotriazine, N-hydroxy-5norbornene-2,3-dicarboxyimide. Also the anhydrides of phosphorus based acids can be used. See, e.g., *The Peptides, Analysis, Synthesis, Biology*, supra and *Pure and Applied Chemistry*, 59(3), 331-344 (1987).

It is also possible to prepare the compounds by the solid phase method of Merrifield. Different solid supports and different strategies are known see, e.g. Barany and Merrifield in *The Peptides, Analysis, Synthesis, Biology*, Vol. 2, E. Gross and J. Meienhofer, eds. (Acad. Press, New York, 1980), Kneib-Cordonier and Mullen *Int. J. Peptide Protein Res.*, 30, 705-739 (1987) and Fields and Noble *Int. J. Peptide Protein Res.*, 35, 161-214 (1990). The synthesis of compounds in which a peptide bond is replaced by an isostere, can, in general, be performed using the previously described protecting groups and activation procedures. Procedures to synthesize the modified isosteres are described in the literature e.g. for the $-\text{CH}_2-\text{NH}-$ isostere and for the $-\text{CO}-\text{CH}_2-$ isostere.

Removal of the protecting groups, and, in the case of solid phase peptide synthesis, the cleavage from the solid support, can take place in different ways, depending on the nature of those protecting groups and the type of linker to the solid support. Usually deprotection takes place under acidic conditions and in the presence of scavengers. See, e.g. volumes 3, 5 and 9 of the series on *The Peptides Analysis, Synthesis, Biology*, supra.

Another possibility is the application of enzymes in synthesis of such compounds; for reviews see, e.g., H. D. Jakubke in *The Peptides, Analysis, Synthesis, Biology*, Vol. 9, S. Udenfriend and J. Meienhofer, eds. (Acad. Press, New York, 1987).

Although possibly not desirable from an economic point of view, oligopeptides according to the invention could also be made according to recombinant DNA methods. Such methods involve the preparation of the desired oligopeptide thereof by means of expressing recombinant polynucleotide sequence that codes for one or more of the oligopeptides in question in a suitable microorganism as host. Generally the process involves introducing into a cloning vehicle (e.g., a plasmid, phage DNA, or other DNA sequence able to replicate in a host cell) a DNA sequence coding for the particular oligopeptide or oligopeptides, introducing the cloning vehicle into a suitable eucaryotic or prokaryotic host cell, and

culturing the host cell thus transformed. When a eucaryotic host cell is used, the compound may include a glycoprotein portion.

As used herein, a "functional analogue" or "derivative" of a peptide includes an amino acid sequence, or other sequence monomers, which has been altered such that the functional properties of the sequence are essentially the same in kind, not necessarily in amount. An analogue or derivative can be provided in many ways, for instance, through "conservative amino acid substitution." Also peptidomimetic compounds can be designed that functionally or structurally resemble the original peptide taken as the starting point but that are for example composed of non-naturally occurring amino acids or polyamides. With "conservative amino acid substitution," one amino acid residue is substituted with another residue with generally similar properties (size, hydrophobicity), such that the overall functioning is likely not to be seriously affected. However, it is often much more desirable to improve a specific function. A derivative can also be provided by systematically improving at least one desired property of an amino acid sequence. This can, for instance, be done by an Ala-scan and/or replacement net mapping method. With these methods, many different peptides are generated, based on an original amino acid sequence but each containing a substitution of at least one amino acid residue. The amino acid residue may either be replaced by alanine (Ala-scan) or by any other amino acid residue (replacement net mapping). This way, many positional variants of the original amino acid sequence are synthesized. Every positional variant is screened for a specific activity. The generated data are used to design improved peptide derivatives of a certain amino acid sequence.

A derivative or analogue can also be, for instance, generated by substitution of an L-amino acid residue with a D-amino acid residue. This substitution, leading to a peptide that does not naturally occur in nature, can improve a property of an amino acid sequence. It is, for example, useful to provide a peptide sequence of known activity of all D-amino acids in retro inversion format, thereby allowing for retained activity and increased half-life values. By generating many positional variants of an original amino acid sequence and screening for a specific activity, improved peptide derivatives comprising such D-amino acids can be designed with further improved characteristics.

A person skilled in the art is well able to generate analogous compounds of an amino acid sequence. This can, for instance, be done through screening of a peptide library. Such an analogue has essentially the same functional properties of the sequence in kind, not necessarily in amount. Also, peptides or analogues can be circularized, for example, by providing them with (terminal) cysteines, dimerized or multimerized, for example, by linkage to lysine or cysteine or other compounds with side-chains that allow linkage or multimerization, brought in tandem- or repeat-configuration, conjugated or otherwise linked to carriers known in the art, if only by a labile link that allows dissociation.

Synthetic versions of these oligopeptides as described above, and functional analogues or derivatives or breakdown products, are herein provided to lower BUN concentration be used in methods to the treatment of disease.

The term "pharmaceutical composition" as used herein is intended to cover both the active composition of the invention alone or a composition containing the composition of the invention together with a pharmaceutically acceptable carrier, diluent or excipient. Acceptable diluents of an oligopeptide as described herein in the detailed description are for example physiological salt solutions or phosphate buffered

salt solutions. In one embodiment, an oligopeptide or composition is administered in an effective concentration to an animal or human systemically, for example, by intravenous, intra-muscular or intraperitoneal administration. Another way of administration comprises perfusion of organs or tissue, be it in vivo or ex vivo, with a perfusion fluid comprising an oligopeptide or composition according to the invention. The administration may be done as a single dose, as a discontinuous sequence of various doses, or continuously for a period of time sufficient to permit substantial modulation of gene expression. In the case of a continuous administration, the duration of the administration may vary depending upon a number of factors that would readily be appreciated by those skilled in the art.

The administration dose of the active molecule may be varied over a fairly broad range. The concentrations of an active molecule that can be administered would be limited by efficacy at the lower end and the solubility of the compound at the upper end. The optimal dose or doses for a particular patient should and can be determined by the physician or medical specialist involved, taking into consideration well-known relevant factors such as the condition, weight and age of the patient, etc.

The active molecule may be administered directly in a suitable vehicle, such as, for example, phosphate-buffered saline ("PBS") or solutions in alcohol or DMSO. Pursuant to preferred embodiments of the present invention, however, the active molecule is administered through a single dose delivery using a drug-delivery system. A suitable drug-delivery system would be pharmacologically inactive or at least tolerable. It should preferably not be immunogenic nor cause inflammatory reactions, and should permit release of the active molecule so as to maintain effective levels thereof over the desired time period. Alternatives are known in the art as suitable for purposes of sustained release and are contemplated as within the scope of the present invention. Suitable delivery vehicles include, but are not limited to, the following: microcapsules or microspheres; liposomes and other lipid-based release systems; viscous instillates; absorbable and/or biodegradable mechanical barriers and implants; and polymeric delivery materials, such as polyethylene oxide/polypropylene oxide block copolymers, polyesters, crosslinked polyvinyl alcohols, polyanhydrides, polymethacrylate and polymethacrylamide hydrogels, anionic carbohydrate polymers, etc. Useful delivery systems are well known in the art.

One formulation to achieve the active molecule release comprises injectable microcapsules or microspheres made from a biodegradable polymer, such as poly(dl-lactide), poly(dl-lactide-co-glycolide), polycaprolactone, polyglycolide, polylactic acid-co-glycolide, poly(hydroxybutyric acid), polyesters or polyacetals. Injectable systems comprising microcapsules or microspheres having a diameter of about 50 to about 500 micrometers offer advantages over other delivery systems. For example, they generally use less active molecules and may be administered by paramedical personnel. Moreover, such systems are inherently flexible in the design of the duration and rate of separate drug release by selection of microcapsule or microsphere size, drug loading and dosage administered. Further, they can be successfully sterilized by gamma irradiation.

The design, preparation, and use of microcapsules and microspheres are well within the reach of persons skilled in the art and detailed information concerning these points is available in the literature. Biodegradable polymers (such as

lactide, glycolide and caprolactone polymers) may also be used in formulations other than microcapsules and microspheres; e.g., pre-made films and spray-on films of these polymers containing the active molecule would be suitable for use in accordance with the present invention. Fibers or filaments comprising the active molecule are also contemplated as within the scope of the present invention.

Another highly suitable formulation for a single-dose delivery of the active molecule in accordance with the present invention involves liposomes. The encapsulation of an active molecule in liposomes or multilamellar vesicles is a well-known technique for targeted drug delivery and prolonged drug residence. The preparation and use of drug-loaded liposomes is well within the reach of persons skilled in the art and well documented in the literature.

Yet another suitable approach for single-dose delivery of an active molecule in accordance with the present invention involves the use of viscous instillates. In this technique, high molecular weight carriers are used in admixture with the active molecule, giving rise to a structure that produces a solution with high viscosity. Suitable high molecular weight carriers include, but are not limited to, the following: dextrans and cyclodextrans; hydrogels; (cross-linked) viscous materials, including (cross-linked) viscoelastics; carboxymethylcellulose; hyaluronic acid; and chondroitin sulfate. The preparation and use of drug-loaded viscous instillates is well known to persons skilled in the art.

Pursuant to yet another approach, the active molecule may be administered in combination with absorbable mechanical barriers such as oxidized regenerated cellulose. The active molecule may be covalently or non-covalently (e.g., ionically) bound to such a barrier, or it may simply be dispersed therein.

The invention is further explained with the aid of the following illustrative examples.

EXAMPLES

Example 1

Six oligopeptides (i.e., A: LAGV (SEQ ID NO:4), B: AQGV (SEQ ID NO:2), C: LAG, D: AQG, E: MTR, and F: MTRV (SEQ Ip NO:1)) were tested and compared with PBS (control) in a double blind animal study for each peptide's relative ability to aid recovery in a mouse renal ischemia reperfusion test. In this test, the mice were anesthetized, and one kidney from each mouse was removed. The other kidney was tied off for 25 minutes, and the serum urea levels were allowed to increase. Both before and after tying off, each of the separate peptides was administered to thirty (30) different mice (5 mg oligopeptide/kg body mass intravenously), after which, the mortality of the mice was determined for each oligopeptide as well as was the BUN concentration at two hours, 24 hours and 72 hours. The results are shown in FIG. 1 and (excluding the results of peptide A (LAGV (SEQ ID NO:4)) obtained in example 1) in Table 2 below.

Under inhalation anesthesia, the left kidney with its artery and vein was isolated and occluded for 25 minutes using a microvascular clamp. During surgery animals were placed on a heating path to maintain body temperature at 37° C. Five minutes before placing the clamp, and 5 minutes before releasing the clamp, 5 mg/kg of peptide, dissolved in 0.1 mL of sterile saline, was administered intravenously. After reperfusion of the left kidney the right kidney was removed. Kidney function was assessed by measuring blood urea nitrogen before clamping, and at 2, 24, and 72 hours after reperfusion.

TABLE 1

Results - (mortality at 72 hours post-reperfusion).						
PBS						
	A (LAGV) (SEQ ID NO: 4)	B (AQGV) (SEQ ID NO: 2)	C (LAG)	D (AQG)	E (MTR)	F (MTRV) (SEQ ID NO: 1)
6/10	6/10	0/10	4/10	4/10	4/10	2/10
*P < (vs PBS)	NS	0.01	0.01	0.01	0.01	0.01

*2 x 2 Chi-square test. df = 1

Peptide A (SEQ ID NO:4) was the first peptide administered in the renal ischemia reperfusion test. The personnel who performed the experiments went through a learning curve while working with peptide A (SEQ ID NO:4). During administration of the peptide in the inferior caval vein, some animals experienced moderate blood loss from the site of injection, whereas others did not. Inadvertently the animals were returned to the stable without drinking water present in their cages the first night after surgery. Also, by mistake, the animals that were intended to be sacrificed at 72 h were killed 48 h after reperfusion. None of these or other problems were encountered during the experiments with peptides B-F. (See, Example 2)

As can be seen, mice administered the oligopeptides MTRV (SEQ ID NO:1) and especially AQGV (SEQ ID NO:2) did much better in terms of both survival (a significant reduction in mortality versus the PBS control group) and reduced BUN concentration than the control group (PBS) or the group administered the other oligopeptides, with more

mice surviving and the serum urea levels being much lower than in the other groups. However, the oligopeptides LAG, AQG, and MTR, in this experiment having no reducing effect on BUN concentration, each caused a significant reduction of mortality compared to the PBS control, where MTR did significantly raise BUN levels in the tested mice at 72 hrs.

Example 2

One oligopeptide (A: (LAGV (SEQ ID NO:4))) was retested for its capacity to reduce BUN levels in the mice test for the reasons as described above. The results are shown in Table 2 below. As can be seen, mice administered the oligopeptide LAGV (SEQ ID NO:4) now did much better in terms of both survival (a significant reduction in mortality versus the PBS control group) and reduced BUN concentration than the control group (PBS).

Example 3

Four additional oligopeptides (G (VLPALPQ (SEQ ID NO:5)), H (VLPALP (SEQ ID NO:6)), I (LQGV (SEQ ID NO:3)) and J (LQG)) were tested for their capacity to reduce BUN levels in the mice test as described above. The results are shown in Table 2 below. As can be seen, mice administered the oligopeptide LQG did show reduced BUN concentration early in the experiment (at 24 hours post-reperfusion) and mice administered VLPALPQ (SEQ ID NO:5) did much better in terms of reduced BUN concentration late in the experiment (at 72 hours post-reperfusion) than the control group (PBS) or the group administered the other oligopeptides, with more mice surviving and the serum urea levels being much lower than in the other groups.

TABLE 2

BUN after 25 min renal ischemia tested in mice with peptides A-J							
Peptide		t = 0 hr	2 hr	24 hr	72 hr	C-term: N-term:	CARBOXYL FREE
A	Mean	8.166667	14.03333	38.86364	32.8875	NMPF-47	LAGV (SEQ ID NO: 4)
	sd	1.774658	1.011599	14.54711	14.31228		
	N	18	3	11	8		
B	Mean	9.713333	16.62	26.36	22.31	NMPF-46	AQGV (SEQ ID NO: 2)
	sd	1.882722	2.185203	20.62105	15.96444		
	N	30	10	20	10		
C	Mean	10.15185	18.13333	59.24375	74.4	NMPF-44	LAG
	SD	1.789794	1.88326	16.19662	33.12546		
	N	29	6	16	6		
D	Mean	9.303846	17.7	66.75625	91.18333	NMPF-43	AQG
	SD	1.502127	1.561135	24.50445	51.22154		
	N	26	8	16	6		
E	mean	8.403846	17.13	66.23333	104.0167	NMPF-12	MTR
	SD	1.739076	1.625526	17.55069	48.97193		
	N	26	10	6	6		
F	mean	7.462963	15.08571	34.57368	39.8375	NMPF-11	MTRV(SEQ ID NO: 1)
	SD	1.338526	1.422941	15.18083	21.45973		
	N	30	7	18	8		
G	mean	8.256667	13.58	37.79375	37.6375	NMPF-7	VLPALPQ (SEQ ID NO: 5)
	SD	1.304021	1.927462	18.33007	29.32872		
	N	30	7	18	8		
H	mean	8.423333	16.24	62.4	47.05	NMPF-6	VLPALP (SEQ ID NO: 6)
	SD	1.255521	1.370482	13.33867	20.92728		
	N	30	10	9	7		
I	mean	7.518182	17.53333	56.08333	73.17778	NMPF-4	LQGV (SEQ ID NO: 3)
	SD	1.537356	2.956913	14.53573	23.3083		
	N	22	3	18	9		
J	mean	7.82069	16.75	26.74	83.95714	NMPF-3	LQG
	SD	1.330515	1.44123	15.51796	40.32129		
	N	29	8	9	8		
PBS control	mean	8.172414	15.0875	56.81	82.075		
	SD	1.549169	2.215167	22.4659	34.82713		
	N	29	8	15	4		

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At 24 hour post-reperfusion statistical analyses revealed P-values of:

A	p = 0.0491	NMPF-47	LAGV	(SEQ ID NO: 4)	5
B	p = 0.0008	NMPF-46	AQGV	(SEQ ID NO: 2)	
C	p = 0.9248	NMPF-44	LAG		
D	p = 0.4043	NMPF-43	AQG		
E	p = 0.1848	NMPF-12	MTR		
F	p = 0.0106	NMPF-11	MTRV	(SEQ ID NO: 1)	10
G	p = 0.1389	NMPF-7	VLPALPQ	(SEQ ID NO: 5)	
H	p = 0.5613	NMPF-6	VLPALP	(SEQ ID NO: 6)	
I	p = 0.9301	NMPF-4	LQGV	(SEQ ID NO: 3)	
J	p = 0.0030	NMPF-3	LQG		

At 24 hour post-reperfusion statistical analyses revealed P-values of:

A	p = 0.0017	NMPF-47	LAGV	(SEQ ID NO: 4)	20
B	p < 0.0001	NMPF-46	AQGV	(SEQ ID NO: 2)	
C	p = 0.8186	NMPF-44	LAG		
D	p = 0.2297	NMPF-43	AQG		
E	p = 0.0242	NMPF-12	MTR		
F	p = 0.0021	NMPF-11	MTRV	(SEQ ID NO: 1)	
G	p = 0.0049	NMPF-7	VLPALPQ	(SEQ ID NO: 5)	25
H	p = 0.3297	NMPF-6	VLPALP	(SEQ ID NO: 6)	
I	p = 0.8328	NMPF-4	LQGV	(SEQ ID NO: 3)	
J	p = 0.9445	NMPF-3	LQG		

P values were calculated by Mann Whitney U-test (SPSS for Windows).

Example 4

To determine dose-response relationships, two peptides (D (AQG, having a good effect on mortality on the mice tested in Example 1) and B (AQGV (SEQ ID NO:2), also having superior effect on BUN of the mice tested in Example 1) were also tested in a dose-response manner in the mice test as described above. Peptides were tested at 0.3, 1, 3, 10 and 30 mg/kg dosages given as described in Example 1. The results can be seen in FIG. 2. P values (calculated by Mann Whitney U-test (SPSS for Windows)) of serum urea levels of PBS compared to peptide D groups at 72 hours post-clamping were at 0.3 mg/kg 0.001, at 1 mg/kg 0.009, at 3 mg/kg 0.02, at 10 mg/kg 0.000, and at 30 mg/kg 0.23, for peptide B groups these P-values were 0.88, 0.054, 0.000, 0.001 and 0.003. As can be seen, peptide D (AQG) did reduce BUN levels surprisingly well at the lower dosages tested, as compared with peptide B (AQGV (SEQ ID NO:2)), while the beneficial effect on mortality was also still notable at the lower dosages tested.

TABLE 3

Mortality in dose-response experiment		
	24 h	72 h
PBS	0-9	4-8
AQG 0.3 mg/kg	0-10	2-8
AQG 1.0 mg/kg	0-10	1-8
AQG 3.0 mg/kg	0-10	0-10
AQG 10.0 mg/kg	0-8	1-10
AQG 30.0 mg/kg	0-8	1-8

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TABLE 3-continued

Mortality in dose-response experiment		
	24 h	72 h
AQGV (SEQ ID NO: 2) 0.3 mg/kg	0-9	2-10
AQGV (SEQ ID NO: 2) 1.0 mg/kg	0-10	1-8
AQGV (SEQ ID NO: 2) 3.0 mg/kg	1-10	0-10
AQGV (SEQ ID NO: 2) 10.0 mg/kg	0-10	0-8
AQGV (SEQ ID NO: 2) 30.0 mg/kg	0-8	3-10

TABLE 4

Urea Levels in dose-response experiment		
	24 h	72 h
PBS	57.8	85.4
Peptide D (AQG) 0.3 mg/kg	38.4	30.4
Peptide D (AQG) 1.0 mg/kg	48.4	38.4
Peptide D (AQG) 3.0 mg/kg	39.3	40.3
Peptide D (AQG) 10.0 mg/kg	46.8	25.8
Peptide D (AQG) 30.0 mg/kg	52.8	58.9
Peptide B (AQGV (SEQ ID NO: 2)) 0.3 mg/kg	62.4	86.7
Peptide B (AQGV (SEQ ID NO: 2)) 1.0 mg/kg	50.0	52.6
Peptide B (AQGV (SEQ ID NO: 2)) 3.0 mg/kg	37.4	19.6
Peptide B (AQGV (SEQ ID NO: 2)) 10.0 mg/kg	41.2	37.1
Peptide B (AQGV (SEQ ID NO: 2)) 30.0 mg/kg	47.8	38.0
standard error		
PBS	7.1	14.7
Peptide D (AQG) 0.3 mg/kg	8.6	3.5
Peptide D (AQG) 1.0 mg/kg	7.2	10.2
Peptide D (AQG) 3.0 mg/kg	3.5	10.7
Peptide D (AQG) 10.0 mg/kg	8.0	3.4
Peptide D (AQG) 30.0 mg/kg	9.5	12.9
Peptide B (AQGV (SEQ ID NO: 2)) 0.3 mg/kg	10.8	14.1
Peptide B (AQGV (SEQ ID NO: 2)) 1.0 mg/kg	11.7	14.3
Peptide B (AQGV (SEQ ID NO: 2)) 3.0 mg/kg	7.6	2.6
Peptide B (AQGV (SEQ ID NO: 2)) 10.0 mg/kg	8.5	6.9
Peptide B (AQGV (SEQ ID NO: 2)) 30.0 mg/kg	5.8	7.8

TABLE 5

statistical significance/p values (Mann Whitney U-Test) of serum urea levels in dose-response experiment 72 hours post-clamping. PBS control compared to peptide administered groups. (See, FIG. 3).	
	72 h
PBS	NA
AQG 0.3 mg/kg	0.001
AQG 1.0 mg/kg	0.009
AQG 3.0 mg/kg	0.02
AQG 10.0 mg/kg	0.000
AQG 30.0 mg/kg	0.23
AQGV (SEQ ID NO: 2) 0.3 mg/kg	0.88
AQGV (SEQ ID NO: 2) 1.0 mg/kg	0.054
AQGV (SEQ ID NO: 2) 3.0 mg/kg	0.000
AQGV (SEQ ID NO: 2) 10.0 mg/kg	0.001
AQGV (SEQ ID NO: 2) 30.0 mg/kg	0.003

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 6

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<211> LENGTH: 4
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthesized Peptide

<400> SEQUENCE: 1

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<210> SEQ ID NO 2
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<223> OTHER INFORMATION: Synthesized Peptide

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Val Leu Pro Ala Leu Pro Gln
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<210> SEQ ID NO 6
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<223> OTHER INFORMATION: Synthesized Peptide

-continued

<400> SEQUENCE: 6

Val Leu Pro Ala Leu Pro
1 5

What is claimed is:

1. An isolated oligopeptide consisting of the amino acid sequence of SEQ ID NO: 2 or a pharmaceutically acceptable salt thereof.

2. A pharmaceutical composition comprising a peptide consisting of the amino acid sequence of SEQ ID NO: 2 or a pharmaceutically acceptable salt thereof.

3. The pharmaceutical composition of claim 2 further comprising a pharmaceutically acceptable carrier, adjuvant, diluent, excipient or any mixtures thereof.

4. An isolated oligopeptide consisting of the amino acid sequence of SEQ ID NO: 2.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : RE43,279 E
APPLICATION NO. : 13/065316
DATED : March 27, 2012
INVENTOR(S) : Robbert Benner et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page:

In ITEM (73) Assignee: change “**Biotemp**” to --**Biotempt**--

In ITEM (56) References Cited:

OTHER PUBLICATIONS

Page 1, 2nd column,

1st line of the 2nd entry (line 45), change “Fai□ure, Amer□can” to --Failure, American--

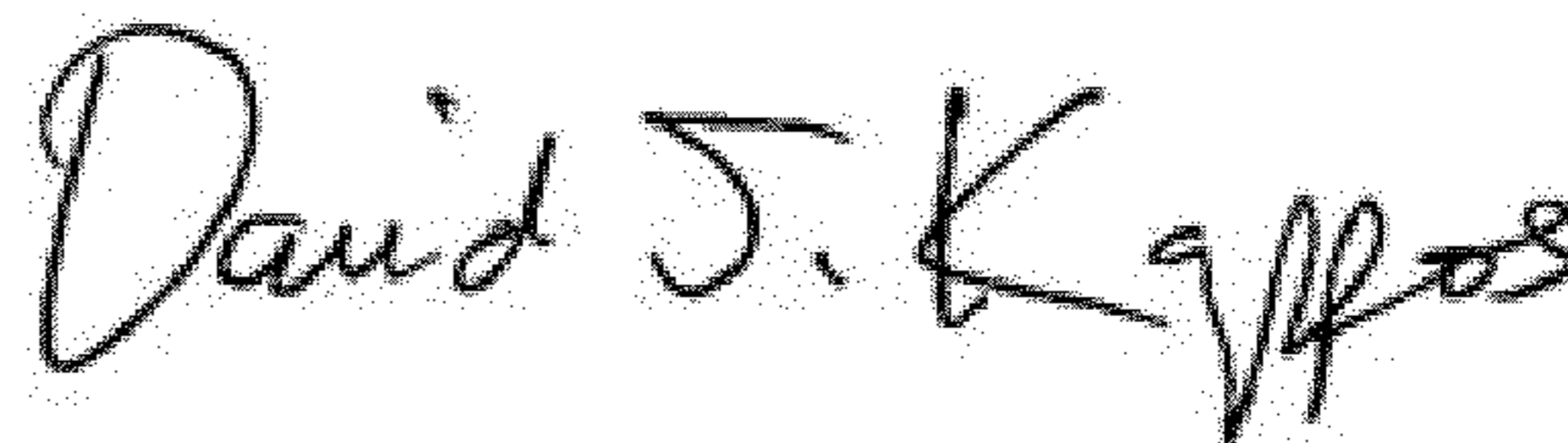
Page 2, 1st column,

3rd line of the 5th entry (line 72), change “Interferon□gamma.” ” to --Interferon-gamma.”--

Page 3, 2nd column,

2nd line of the 3rd entry (line 9), change “VersUS -Host Disease by Human Chorionic gonadotropin,”23(1)” to --versus Host-Disease by Human Chorionic Gonadotropin,” 23(1)--

Signed and Sealed this
Seventh Day of August, 2012



David J. Kappos
Director of the United States Patent and Trademark Office