US006916471B2

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

(10) **Patent No.: US 6,916,471 B2**
(45) **Date of Patent: *Jul. 12, 2005**

(54) **RED BLOOD CELLS LOADED WITH S-NITROSOTHIOL AND USES THEREFOR**

(75) Inventors: **Jonathan S. Stamler**, Chapel Hill, NC (US); **Joseph Bonaventura**, Beaufort, NC (US); **John R. Pawloski**, Raleigh, NC (US); **Timothy J. McMahon**, Durham, NC (US)

(73) Assignee: **Duke University**, Durham, NC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 138 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **10/045,603**

(22) Filed: **Oct. 23, 2001**

(65) **Prior Publication Data**

US 2003/0008300 A1 Jan. 9, 2003

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/724,305, filed on Nov. 28, 2000, now abandoned, which is a continuation of application No. 08/873,679, filed on Jun. 12, 1997, now Pat. No. 6,203,789, which is a continuation-in-part of application No. PCT/US96/14664, filed on Sep. 13, 1996, which is a continuation of application No. 08/616,255, filed on Mar. 15, 1996, now Pat. No. 6,153,186.

(60) Provisional application No. 60/003,801, filed on Sep. 15, 1995.

(51) **Int. Cl.**[7] **C12N 5/06**

(52) **U.S. Cl.** **424/93.73**; 424/93.7

(58) **Field of Search** 424/93.73, 93.7

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,900,719	A	2/1990	Means et al.	
5,380,758	A	1/1995	Stamler et al.	
5,405,919	A	4/1995	Keefer et al.	
5,427,797	A	6/1995	Frostell et al.	
5,480,866	A	1/1996	Bonaventura et al.	
5,574,068	A	11/1996	Stamler et al.	
5,593,876	A	1/1997	Stamler et al.	
6,153,186	A	* 11/2000	Stamler et al.	424/93.73
6,197,745	B1	3/2001	Stamler et al.	
6,291,424	B1	9/2001	Stamler et al.	

FOREIGN PATENT DOCUMENTS

EP	0604143	6/1994
WO	WO 93/09806	5/1993
WO	WO 93/12068	6/1993
WO	WO 94/22306	10/1994
WO	WO 94/22482	10/1994
WO	WO 94/22499	10/1994
WO	WO 96/15797	5/1996
WO	WO 96/16645	6/1996
WO	WO 96/17604	6/1996
WO	WO 96/30006	10/1996
WO	WO 97/10265	10/1997
WO	WO 98/34955	8/1998

OTHER PUBLICATIONS

Stamler, J. et al., "S–Nitrosylation of Proteins with Nitric Oxide: Synthesis and Characterization of Biologically Active Compounds," *Proc. Natl. Acad. Sci. USA*, 89:444–448 (1992).

Langford, E.J. et al., "Inhibition of Platelet Activity by S–Nitrosoglutathione During Coronary Angioplasty," *The Lancet*, 344:1458–1460 (1994).

Ribeiro, J.M.C. et al., "Reversible Binding of Nitric Oxide by a Salivary Heme Protein from a Bloodsucking Insect," *Science*, 260:539–541 (1993).

Olsen, S.B. et al. "Enhancement of Platelet Deposition by Cross–Linked Hemoglobin in a Rat Carotid Endarterectomy Model," *Circulation*, 93 (2) :327–332 (1996).

Scharfstein, J. et al., "In Vivo Transfer of Nitric Oxide Between a Plasma Protein–Bound Reservoir and Low Molecular Weight Thiols," *J. Clin. Invest.*, 94:1432–1439 (1994).

Charache, S. et al., "Evaluation of Extracorporeal Alkylation of Red Cells as a Potential Treatment for Sickle Cell Anemia," *Blood*, 47(3):481–488 (1976).

Kosaka, H. et al., "ESR Spectral Transition by Arterio-venous Cycle in Nitric Oxide Hemoglobin of Cytokin-e–Treated Rats," *Am. J. Physiol.*, 266(5) :1400–1405 (1994).

Doyle, M.P. et al., "Structural Effects in Alkyl Nitrite Oxidation of Human Hemoglobin," *J. Biol. Chem.*, 259(1) : 80–87 (1984).

Greenburg, A.G. and Kim, H.W., "Nitrosyl Hemoglobin Formation In–Vivo After Intravenous Administration of a Hemoglobin–Based Oxygen Carrier in Endotoxemic Rats," *Artif. Cells, Blood Substitutes, Immobilization Biotechnol.*, 23(3) :271–276 (1995).

Stamler, J.S., "Redox Signaling: Nitrosylation and Related Target Interactions of Nitric Oxide," *Cell*, 78:931–936 (1994).

Arnelle, D.R. and Stamler J.S., "NO^+, NO^-, and NO^- Donation by S–Nitrosothiols: Implications for Regulation of Physiological Functions by S–Nitrosylation and Acceleration of Disulfide Formation," *Arch. of Biochem. and Biophys.*, 318(2): 279–285 (1995).

(Continued)

Primary Examiner—Jean C. Witz
(74) *Attorney, Agent, or Firm*—McDonnell Boehnen Hulbert & Berghoff LLP

(57) **ABSTRACT**

Red blood cells can be loaded with low molecular weight nitrosylating agents, such as S-nitrosothiols, to act as a delivery system for NO^+ groups to tissues. Loaded red blood cells can be used in methods of therapy for conditions which are characterized by abnormal O_2 metabolism of tissues, oxygen-related toxicity, abnormal vascular tone, abnormal red blood cell adhesion, or abnormal O_2 delivery by red blood cells. Such treatment of red blood cells can be extended to in vivo therapies, with the object to achieve an increase in the ratio of red blood cell S-nitrosothiol to hemoglobin.

30 Claims, 24 Drawing Sheets