



- [54] **RED BLOOD CELLS LOADED WITH S-NITROSTHIOL AND USES THEREFOR**
- [75] Inventors: **Jonathan S. Stamler**, Chapel Hill;
Joseph Bonaventura, Durham, both of
N.C.
- [73] Assignee: **Duke University Medical Center**,
Durham, N.C.
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[56] **References Cited**

U.S. PATENT DOCUMENTS

4,900,719	2/1990	Means et al.	514/18
5,380,758	1/1995	Stamler et al.	514/562
5,405,919	4/1995	Keefer et al.	525/377
5,427,797	6/1995	Frostell et al.	424/434
5,480,866	1/1996	Bonaventura et al.	514/6
5,574,068	11/1996	Stamler et al.	514/562
5,593,876	1/1997	Stamler et al.	435/188

FOREIGN PATENT DOCUMENTS

0604143	6/1994	European Pat. Off. .
WO 93/09806	5/1993	WIPO .
WO 93/12068	6/1993	WIPO .
WO 94/22306	10/1994	WIPO .
WO 94/22482	10/1994	WIPO .
WO 94/22499	10/1994	WIPO .
WO 96/15797	5/1996	WIPO .
WO 96/16645	6/1996	WIPO .
WO 96/17604	6/1996	WIPO .
WO 96/30006	10/1996	WIPO .

OTHER PUBLICATIONS

- Pietraforte et al., *Biochemistry* 34: 7177-7185 (1995).
- Kruszyna et al., *Toxicology and Applied Pharmacology* 91: 429-438 (1987).
- Kumura et al., *Neurosci. Lett.* 177(1-2): 165-167 (1994).
- Jia et al., *Nature* 380(6571): 221-226 (1996).
- Garel, M.C., et al., "Covalent Binding of Glutathione to Hemoglobin," *The Journal of Biological Chemistry*, 261:14704-14709 (1986).
- Wennmalm, Å., et al., "Dependence of the Metabolism of Nitric Oxide (NO) in Healthy Human Whole Blood on the Oxygenation of Its Red Cell Haemoglobin," *Br. J. Pharmacol.*, 106:507-508 (1992).
- Garel, M.C., et al., "Binding of 21 Thiol Reagents to Human Hemoglobin in Solution and in Intact Cells," *Eu. J. Biochem.*, 123:513-519 (1982).
- Stamler, Jonathan S. 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, José M.C. et al., "Reversible Binding of Nitric Oxide by a Salivary Heme Protein from a Bloodsucking Insect," *Science*, 260:539-541 (1993).

Simon, Daniel I. et al., "Effect of Nitric Oxide Synthase Inhibition of Bleeding Time in Humans," *Journal of Cardiovascular Pharmacology*, 26:339-342 (1995).

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

Arnelle, Derrick R. and Stamler, Jonathan S., "NO⁺, NO⁻, and NO⁻ Donation by S-Nitrosothiols: Implications for Regulation of Physiological Functions by S-Nitrosylation and Acceleration of Disulfide Formation," *Archives of Biochemistry and Biophysics*, 318(2):279-285 (1995).

Kondo, T. et al., "Thiol Transport from Human Red Blood Cells," *Methods in Enzymology*, 252:72-82 (1995).

Feelisch, M. and Stamler, J.S., "Donors of Nitrogen Oxides," *Methods In Nitric Oxide Research*, John Wiley & Sons Ltd. (1996).

Stamler, J.S. and Feelisch, M., "Preparation and Detection of S-Nitrosothiols," *Methods in Nitric Oxide Research*, John Wiley & Sons Ltd. (1996).

Clancy, R.M. et al., "Nitric Oxide Reacts with Intracellular Glutathione and Activates the Hexose Monophosphate Shunt in Human Neutrophils: Evidence for S-Nitrosoglutathione as a Bioactive Intermediary," *Proc. Natl. Acad. Sci. USA*, 91:3680-3684 (1994).

Ignarro, Louis J. et al., "Mechanism of Vascular Smooth Muscle Relaxation by Organic Nitrates, Nitrites, Nitroprusside and Nitric Oxide: Evidence for the Involvement of S-Nitrosothiols as Active Intermediates," *The Journal of Pharmacology and Experimental Therapeutics*, 218(3):739-749 (1981).

Primary Examiner—Jean C. Witz

Attorney, Agent, or Firm—Hamilton, Brook, Smith & Reynolds, P.C.

[57] **ABSTRACT**

Nitric oxide (NO) interacts with hemoglobin (Hb) at its metal centers, whereas S-nitrosothiols (RSNOs) can donate the NO group to β93 cysteine residues, thereby shielding the NO functionality from heme inactivation. S-nitrosylation of Hb is under the allosteric control of oxygen and the oxidation state of heme. NO group release from SNO-Hb is further facilitated by intracellular low molecular weight thiols, forming RSNOs which can be exported from the erythrocyte to regulate blood pressure. Red blood cells can be loaded with low molecular weight RSNOs to act as a delivery system for NO⁺ groups. Loaded red blood cells can be used in methods of therapy for conditions which are characterized by abnormal O₂ metabolism of tissues, oxygen-related toxicity, abnormal vascular tone, abnormal red blood cell adhesion, or abnormal O₂ delivery by red blood cells.