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(71) Applicants (for all designated States except US): **YALE UNIVERSITY** [US/US]; Two Whitney Avenue, New Haven, CT 06511 (US). **JOHNS HOPKINS UNIVERSITY** [US/US]; 111 Marketplace, Suite 906, Baltimore, MD 21202 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **CHOI, Augustine, M., K.** [US/US]; 2 Pole Bridge Lane, Guilford, CT 06437 (US). **OTTERBEIN, Leo, E.** [US/US]; 93 Mather Street, Hamden, CT 06517 (US).

(74) Agents: **FRASER, Janis, K.** et al.; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110 (US).

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(54) Title: CARBON MONOXIDE AS A BIOMARKER AND THERAPEUTIC AGENT

(57) Abstract: The present invention relates to the use of carbon monoxide (CO) as a biomarker and therapeutic agent of heart, lung, liver, spleen, brain, skin and kidney diseases and other conditions and disease states including, for example, asthma, emphysema, bronchitis, adult respiratory distress syndrome, sepsis, cystic fibrosis, pneumonia, interstitial lung diseases, idiopathic pulmonary diseases, other lung diseases including primary pulmonary hypertension, secondary pulmonary hypertension, cancers, including lung, larynx and throat cancer, arthritis, wound healing, Parkinson's disease, Alzheimer's disease, peripheral vascular disease and pulmonary vascular thrombotic diseases such as pulmonary embolism. CO may be used to provide anti-inflammatory relief in patients suffering from oxidative stress and other conditions especially including sepsis and septic shock. In addition, carbon monoxide may be used as a biomarker or therapeutic agent for reducing respiratory distress in lung transplant patients and to reduce or inhibit oxidative stress and inflammation in transplant patients.



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CARBON MONOXIDE AS A BIOMARKER AND THERAPEUTIC AGENT

TECHNICAL FIELD

The present invention relates to the use of carbon monoxide (CO) as a
5 biomarker and therapeutic agent of heart, lung, liver, spleen, brain, skin and kidney
diseases and other conditions and disease states including, for example, asthma,
emphysema, bronchitis, adult respiratory distress syndrome, sepsis, cystic fibrosis,
pneumonia, interstitial lung diseases, idiopathic pulmonary diseases, other lung
diseases including primary pulmonary hypertension, secondary pulmonary
10 hypertension, cancers, including lung, larynx and throat cancer, arthritis, wound
healing, Parkinson's disease, Alzheimer's disease, peripheral vascular disease and
pulmonary vascular thrombotic diseases such as pulmonary embolism. CO may be
used to provide anti-inflammatory relief in patients suffering from oxidative stress and
other conditions especially including sepsis and septic shock. In addition, CO may be
15 used to store organs prior to transplantation. In addition, carbon monoxide may be used
as a biomarker or therapeutic agent for reducing respiratory distress in lung transplant
patients, to reduce or inhibit oxidative stress, inflammation or rejection of transplants in
transplant patients.

BACKGROUND

20 Heme oxygenase (HO) catalyzes the first and rate limiting step in the
degradation of heme to yield equimolar quantities of biliverdin IXa, carbon monoxide
(CO), and iron (Choi et al., Am. J. Respir. Cell Mol. Biol. 15: 9-19; and Maines, Annu.
Rev. Pharmacol. Toxicol. 37:517-554). Three isoforms of HO exist; HO-1 is highly
inducible while HO-2 and HO-3 are constitutively expressed (Choi et al., supra,
25 Maines, supra and McCoubrey et al., E. J. Bioch. 247: 725-732). Although heme is the
major substrate of HO-1, a variety of non-heme agents including heavy metals,
cytokines, hormones, endotoxin and heat shock are also strong inducers of HO-1
expression (Choi et al., supra, Maines, supra and Tenhunen et al., J. Lab. Clin. Med.
75: 410-421). This diversity of HO-1 inducers has provided further support for the
30 speculation that HO-1, besides its role in heme degradation, may also play a vital
function in maintaining cellular homeostasis. Furthermore, HO-1 is highly induced by

a variety of agents causing oxidative stress including hydrogen peroxide, glutathione depletors, UV irradiation, endotoxin and hyperoxia (Choi, et al. supra, Maines, supra and Keyse, et al. Proc. Natl. Acad. Sci. USA . 86: 99-103). One interpretation of this finding is that HO-1 can serve as a key biological molecule in the adaptation and/or
5 defense against oxidative stress (Choi, et al., supra, Lee, et al., Proc Natl Acad Sci USA 93: 10393-10398; Otterbein et al., Am. J. J. Respir. Cell Mol .Biol. 13: 595-601; Poss et al., Proc. Natl. Acad. Sci. USA. 94: 10925-10930; Vile, et al., Proc. Natl. Acad. Sci. 91: 2607-2610; Abraham et al., Proc. Natl. Acad. Sci. USA. 92: 6798-6802; and Vile and Tyrrell, J. Biol. Chem. 268: 14678-14681. Our laboratory and others have shown
10 that induction of endogenous HO-1 provides protection both *in vivo* and *in vitro* against oxidative stress associated with hyperoxia and lipopolysaccharide-induced tissue injury (Lee et al., supra, Otterbein, et al., supra and Taylor et al., Am. J. Physiol. 18: L582-L591). We have also shown that exogenous administration of HO-1 via gene transfer can provide protection against oxidant tissue injury and elicit tolerance to hyperoxic
15 stress (Otterbein, et al., Am. J. Resp. Crit. Care Med. 157: A565 (Abstr)).

Carbon monoxide (CO) is a gaseous molecule with known toxicity and lethality to living organisms (Haldane, Biochem. J. 21: 1068- 1075; and Chance, et al., 1970, Ann. NY Acad Sci. 174: 193-204.). However, against this known paradigm of CO toxicity, there has been renewed interest in recent years in CO behaving as a regulatory
20 molecule in cellular and biological processes based on several key observations. Mammalian cells have the ability to generate endogenous CO primarily through the catalysis of heme by the enzyme heme oxygenase (HO) (Choi et al., supra and Maines, supra). The total cellular production of CO is generated primarily via heme degradation by HO (Marilena, Biochem. Mol. Med. 61: 136-142 and Verma, et al.,
25 1993, Science 259: 381-384). Moreover, CO, akin to the gaseous molecule nitric oxide, plays important roles in mediating neuronal transmission (Verma et al., supra and Xhuo, et al., Science 260: 1946-1950) and in the regulation of vasomotor tone (Morita, and Kourembanas, 1995, J. Clin. Invest. 96: 2676-2682.; Morita et al., 1995 Proc. Natl. Acad. Sci. USA 92: -1479; and Goda et al., 1998, J. Clin. Inv. 101: 604-12).
30 Other publication relating to the biological actions of CO include Pinsky et al., U.S. Patent No. 6,316,403, Sato et al., J. Immunol. 166: 4185-4194 (2001); Fujita et al., Nat. Med. 7(5): 598-604 (2001); Nachar et al., High Altitude Medicine & Biology 2:377-385 (2001); Vassalli et al., Crit. Care. Med. 29: 359-366 (2001); Otterbein et al., Am. J.

Physiol. Lung Cell Mol. Physiol. 276: L688-L694 (1999); Cardell et al., Brit. J. Pharmacol. 124: 1065-1068 (1998); Otterbein et al., Nat. Med. 6(4): 422-428 (2000); and Otterbein et al., Am. J. Physiol Lung Cell Mol. Physiol, 279: L1029-L1037 (2000).

5 Septic shock and sepsis syndrome, resulting from excessive stimulation of immune cells, particularly monocytes and macrophages, remains one of the leading causes of death in hospitalized patients. Parillo, et al., Ann. Intern. Med. 113, 991-992 (1992). The pathophysiological alterations observed in sepsis are often not due to the infectious organism itself, but rather to the uncontrolled production of pro-
10 inflammatory cytokines and chemokines including TNF- α , IL-1, and MIP-1 that leads to leukocyte recruitment, capillary leak and ultimately participates in the lethality of sepsis. Beutler, et al., 232, 977-980 (1986); Netea, et al., Immunology 94, 340-344 (1998); and Wolpe, et al., J. Exp. Med. 167, 570-581 (1988). Lipopolysaccharide (LPS), a constituent of the gram negative bacterial cell wall, is the leading cause of
15 sepsis, and when administered experimentally to macrophages or mice, mimics the same inflammatory response. Following LPS administration, there is a rapid but transient increase in these pro-inflammatory mediators which are subsequently down-modulated by a battery of anti-inflammatory cytokines including interleukin-10 (IL-10) and interleukin-4 (IL-4), which inhibit the synthesis of the pro-inflammatory cytokines
20 and chemokines. J. Exp. Med. 177, 1205-1208 (1993). LPS initially binds to the CD14 and toll-like receptor (TLR) 2 (or 4) at the cell surface, (Yang, et al., Nature. 395 : 284-288 (1998) and Chow, et al., J. Biol. Chem. 274 : 10689-10692 (1999) and has then been shown to activate the mitogen activated protein (MAP) kinase pathways including p38, p42/p44 ERK and JNK (MAP) kinases. Liu, et al., J. Immunol. 153, 2642-2652
25 (1994); Hambleton, et al., Proc. Natl. Acad. Sci. USA. 93, 2274-2778 (1996); Han, et al., J. Biol. Chem. 268, 25009-25014 (1993); Han, et al., Science 265, 808-811 (1994); Sanghera, et al., J. Immunol. 156, 4457-4465 (1996), and Raingeaud, et al., J. Biol. Chem. 270, 7420-7426 (1995). The relationship between the activation of these signaling molecules, downstream cytokine expression, and physiologic function
30 represents an active line of investigation.

The United States Government has provided support for research which led to the present invention under one or more of NIH grant numbers HL60234, AI42365 and HL55330. Consequently, the government retains certain rights in the invention.

SUMMARY

The present invention relates to novel pharmaceutical compositions for delivering to patients suffering from the effects of oxidative stress, the compositions comprising effective concentrations of carbon monoxide in a gaseous mixture comprising oxygen and optionally, nitrogen gas (as well as other minor optional gaseous components). An additional aspect of the present invention is directed to a method for delaying the onset of, inhibiting or alleviating the effects of oxidative stress, the method comprising delivering a therapeutic gas comprising carbon monoxide in an amount and for a time effective to delay the onset of, inhibit or alleviate the effects of oxidative stress in the patient. It has unexpectedly been discovered that the delivery of a therapeutic gas comprising low concentrations (i.e., concentrations ranging from about 1 ppb (part per billion) to about 3,000 ppm (preferably above about 0.1 ppm within this range) of the gas, preferably about 1 ppm to about 2,800 ppm, more preferably about 25 ppm to about 750 ppm, even more preferably about 50 ppm to about 500 ppm, e.g., about 250 ppm) of carbon monoxide is an extremely effective method for delaying the onset of, inhibiting or reversing the effects of oxidative stress in a patient. This is an unexpected result. It is noted here that in the method of the present invention, an amount of carbon monoxide in the therapeutic gaseous composition which is in excess of 0.3% may sometimes be used, depending upon the condition or disease state to be treated.

In another aspect, the present invention is directed to the use of carbon monoxide gas in the preparation of a medicament for use in treating a patient suffering from or at risk for emphysema, bronchitis, cystic fibrosis, pneumonia, interstitial lung disease, wound healing, arthritis, Parkinson's disease, and/or Alzheimer's disease.

In yet another aspect, the present invention is directed to the use of carbon monoxide gas in the preparation of a medicament for use in treating a patient suffering from or at risk for localized inflammation of the kidney, spleen, and/or skin.

The present invention also provides methods for using carbon monoxide as a biomarker for determining that a patient is suffering from oxidative stress or is at risk for or is suffering from a number of conditions or disease states that are secondary to or result in oxidative stress, for example, asthma, emphysema, bronchitis, adult respiratory distress syndrome, sepsis, cystic fibrosis, pneumonia, interstitial lung diseases, idiopathic pulmonary diseases, other lung diseases including primary pulmonary

hypertension, secondary pulmonary hypertension, cancers, including lung, larynx and throat cancer, arthritis, wound healing, Parkinson's disease, Alzheimer's disease, peripheral vascular disease and pulmonary vascular thrombotic diseases such as pulmonary embolism, among others. The method comprises detecting carbon

5 monoxide in a patient's breath to determine whether detectable levels of carbon monoxide occur in the breath. If detectable levels of carbon monoxide appear in the patient's breath, the patient may be diagnosed with oxidative stress or for being at risk for oxidative stress. The manifestations of oxidative stress may take the form of one or more of the above-referenced conditions or disease states. Appropriate therapeutic

10 steps or other steps may be taken after such diagnosis to alleviate or treat the condition that is responsible for the oxidative stress in the patient. In one embodiment, the method includes measuring carbon monoxide in breath exhaled by a patient, wherein an amount of carbon monoxide of at least about 1 ppm in the breath is indicative that the patient is at risk for sepsis or septic shock.

15 Another aspect of the present invention relates to the finding that in certain patients, the administration of carbon monoxide in effective amounts to the patient may be used to induce HO-1 enzyme in the patient and prevent or limit oxidative stress in the patient, especially oxidative stress caused by hyperoxia or sepsis. HO-1 enzyme is implicated in maintaining homeostasis in the cells of the patient.

20 Still another aspect of the present invention relates to the use of carbon monoxide to delay the onset of, inhibit or alleviate the effects of oxidative stress which occur in transplant patients, in particular, organ transplant patients, especially, but not exclusively lung transplant patients.

Another aspect of the present invention relates to a method for inhibiting the

25 production of pro-inflammatory cytokines such as TNF- α , IL-1 β , IL-6, and MIP-1 β and augmenting the production (expression) of the anti-inflammatory cytokine IL-10 and IL-4 in a patient, the method comprising administering to the patient an effective amount of CO.

Still another aspect of the present invention relates to a method to preserve

30 organs or tissue for transplants comprising adding to media in which the organs or tissue are stored a preservative effective amount or concentration of carbon monoxide. In this aspect of the present invention, the inclusion of carbon monoxide in effective amounts reduces, inhibits or alleviates the formation of reactive oxygen in the stored

organ or tissue, thus extending the period in which organ transplants can be effectively stored without suffering oxidative damage. In one embodiment, the method includes providing a medium comprising carbon monoxide and storing the organ in the medium, wherein the carbon monoxide is present in the
5 medium in an amount sufficient to enhance storage stability of the organ.

Another aspect of the present invention relates to a method to prevent or reduce the likelihood of damage caused by oxidative stress associated with hyperoxia in a patient comprising administering an effective amount of carbon monoxide to a hyperoxic patient.

10 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although suitable methods and materials for the practice or testing of the present invention are described below, other methods and materials similar or equivalent to those described herein, which are well known in the art, can also be used.
15 All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

DETAILED DESCRIPTION

20 The following definitions are used to describe the present invention.

The term “carbon monoxide” (or “CO”) as used herein describes molecular carbon monoxide in its gaseous state, compressed into liquid form, or dissolved in aqueous solution. The term “carbon monoxide composition” or “pharmaceutical composition comprising carbon monoxide” is used throughout the specification to
25 describe a gaseous or liquid composition containing carbon monoxide that can be administered to a patient and/or an organ. The skilled practitioner will recognize which form of the pharmaceutical composition, e.g., gaseous, liquid, or both gaseous and liquid forms, is preferred for a given application.

The terms “effective amount” and “effective to treat,” as used herein, refer to
30 the administration of carbon monoxide in an amount or concentration and for period of time including acute or chronic administration and periodic or continuous administration that is effective within the context of its administration for causing an

intended effect or physiological outcome. For gases, effective amounts of carbon monoxide generally fall within the range of about 0.0000001% to about 0.3% by weight, e.g., 0.0001% to about 0.25% by weight, preferably at least about 0.001%, e.g., at least about 0.005%, 0.010%, 0.02%, 0.025%, 0.03%, 0.04%, 0.05%, 0.06%, 0.08%,
5 0.10%, 0.15%, 0.20%, 0.22%, or 0.24% by weight carbon monoxide. Preferred ranges include, e.g., 0.001% to about 0.24%, about 0.005% to about 0.22%, about 0.005% to about 0.05%, about 0.010% to about 0.20%, about 0.02% to about 0.15%, about 0.025% to about 0.10%, or about 0.03% to about 0.08%, or about 0.04% to about 0.06%. For liquid solutions of CO, effective amounts generally fall within the range of
10 about 0.0001 to about 0.0044 g CO/100 g liquid, e.g., at least about 0.0001, 0.0002, 0.0004, 0.0006, 0.0008, 0.0010, 0.0013, 0.0014, 0.0015, 0.0016, 0.0018, 0.0020, 0.0021, 0.0022, 0.0024, 0.0026, 0.0028, 0.0030, 0.0032, 0.0035, 0.0037, 0.0040, or 0.0042 g CO/100 g aqueous solution. Preferred ranges include, e.g., about 0.0010 to about 0.0030 g CO/100 g liquid, about 0.0015 to about 0.0026 g CO/100 g liquid, or
15 about 0.0018 to about 0.0024 g CO/100 g liquid. An effective amount of carbon monoxide within the context of reducing the production or effect of inflammatory cytokines can be, for example, an amount sufficient to inhibit production and/or effect of TNF- α , IL-1, IL-6 and MIP-1, among others. Alternatively, it can be an amount sufficient to induce or increase production of anti-inflammatory cytokines such as IL-
20 10, among others. Within the context of transplant patients, an effective amount of carbon monoxide is that amount administered to the transplant patient to reduce the likelihood of rejection through an unfavorable immunological reaction. Within the context of preserving stored organs to be used for transplantation, an effective amount of carbon monoxide can be an amount that is added, e.g., bubbled, into the medium in
25 which the transplant organs are stored in order to enhance preservation of the organ and reduce the likelihood that the organ will be subject to some measure of oxidative damage. A skilled practitioner will appreciate that amounts outside of the ranges described above may be used depending upon the application.

The term "patient" is used throughout the specification to describe an animal,
30 human or non-human, to whom treatment according to the methods of the present invention is provided. Veterinary applications are clearly anticipated by the present invention. The term includes but is not limited to mammals, e.g., humans, other

primates, pigs, rodents such as mice and rats, rabbits, guinea pigs, hamsters, cows, horses, cats, dogs, sheep and goats.

The term “treat(ment),” is used herein to describe delaying the onset of, inhibiting, or alleviating the effects of a condition, e.g., emphysema, bronchitis, arthritis, cystic fibrosis, pneumonia, interstitial lung disease, Parkinson’s disease, Alzheimer’s disease, or inflammation of the kidneys, spleen, or skin. In the case of wound healing, the term describes the promotion of wound healing, e.g., the promotion of skin wound healing. Individuals considered at risk for developing a condition described herein may benefit particularly from the invention, primarily because prophylactic treatment can begin before there is any evidence of the condition. The skilled practitioner will appreciate that a patient can be determined to be at risk for any of the conditions described herein by any method known in the art, e.g., by a physician’s diagnosis.

The term “biomarker” is used to describe carbon monoxide produced in the breath of a patient in minor, detectable amounts, and which provides evidence that the patient is at risk for, is in the early stages of, or is suffering from oxidative stress is at risk for or is suffering from one or more of the conditions or disease states that are secondary to or that may result in oxidative stress. The amount of carbon monoxide in the breath of a patient that may function as a biomarker may be as low as 0.001 ppm, but is generally at least about 0.1 ppm.

Preparation of Gaseous Carbon Monoxide Compositions

A carbon monoxide composition may be a gaseous carbon monoxide composition. Compressed or pressurized gas useful in the methods of the invention can be obtained from any commercial source, and in any type of vessel appropriate for storing compressed gas. For example, compressed or pressurized gases can be obtained from any source that supplies compressed gases, such as oxygen, for medical use. The pressurized gas including carbon monoxide used in the methods of the present invention can be provided such that all gases of the desired final composition (e.g., CO, CO₂, O₂, N₂) are mixed in the same vessel. Optionally, the methods of the present invention can be performed using multiple vessels containing individual gases. For example, a single vessel can be provided that contains carbon monoxide, with or without other gases, the contents of which can be optionally mixed with room air or

with the contents of other vessels, e.g., vessels containing oxygen, nitrogen, carbon dioxide, helium, compressed air, or any other suitable gas or mixtures thereof.

Gaseous compositions administered to a patient according to the present invention typically contain 0% to about 79% by weight nitrogen, about 21% to about 5 100% by weight oxygen and about 0.0000001% to about 0.3% by weight (corresponding to about 1 ppb or 0.001 ppm to about 3,000 ppm) carbon monoxide. Preferably, the amount of nitrogen in the gaseous composition is about 79% by weight, the amount of oxygen is about 21% by weight, and the amount of carbon monoxide is about 0.0001% to about 0.25% by weight, preferably at least about 0.001%, e.g., at 10 least about 0.005%, 0.010%, 0.02%, 0.025%, 0.03%, 0.04%, 0.05%, 0.06%, 0.08%, 0.10%, 0.15%, 0.20%, 0.22%, or 0.24% by weight of carbon monoxide. Preferred ranges include 0.005% to about 0.24%, about 0.01% to about 0.22%, and about 0.08% to about 0.20%. It is noted that gaseous carbon monoxide compositions having concentrations of carbon monoxide greater than 0.3% (such as 1% or greater) may be 15 used for short periods (e.g., one or a few breaths), depending upon the application.

A gaseous carbon monoxide composition may be used to create an atmosphere that comprises carbon monoxide gas. An atmosphere that includes appropriate levels of carbon monoxide gas can be created, for example, by providing a vessel containing a pressurized gas comprising carbon monoxide gas, and releasing the pressurized gas 20 from the vessel into a chamber or space to form an atmosphere that includes the carbon monoxide gas inside the chamber or space. Alternatively, the gases can be released into an apparatus that culminates in a breathing mask or breathing tube, thereby creating an atmosphere comprising carbon monoxide gas in the breathing mask or breathing tube, ensuring the patient is the only person in the room exposed to 25 significant levels of carbon monoxide.

Carbon monoxide levels in an atmosphere can be measured or monitored using any method known in the art. Such methods include electrochemical detection, gas chromatography, radioisotope counting, infrared absorption, colorimetry, and electrochemical methods based on selective membranes (see, e.g., Sunderman *et al.*, 30 Clin. Chem. 28:2026-2032, 1982; Ingi *et al.*, Neuron 16:835-842, 1996). Sub-parts per million carbon monoxide levels can be detected by, e.g., gas chromatography and radioisotope counting. Further, it is known in the art that carbon monoxide levels in the sub-ppm range can be measured in biological tissue by a midinfrared gas sensor (see,

e.g., Morimoto *et al.*, Am. J. Physiol. Heart. Circ. Physiol 280:H482-H488, 2001).

Carbon monoxide sensors and gas detection devices are widely available from many commercial sources.

5 Preparation of Liquid Carbon Monoxide Compositions

A carbon monoxide composition may also be a liquid carbon monoxide composition. A liquid can be made into a carbon monoxide composition by any method known in the art for causing gases to become dissolved in liquids. For example, the liquid can be placed in a so-called "CO₂ incubator" and exposed to a
10 continuous flow of carbon monoxide, preferably balanced with carbon dioxide, until a desired concentration of carbon monoxide is reached in the liquid. As another example, carbon monoxide gas can be "bubbled" directly into the liquid until the desired concentration of carbon monoxide in the liquid is reached. The amount of carbon monoxide that can be dissolved in a given aqueous solution increases with
15 decreasing temperature. As still another example, an appropriate liquid may be passed through tubing that allows gas diffusion, where the tubing runs through an atmosphere comprising carbon monoxide (e.g., utilizing a device such as an extracorporeal membrane oxygenator). The carbon monoxide diffuses into the liquid to create a liquid carbon monoxide composition.

20 In one embodiment, the liquid can be any liquid known to those of skill in the art to be suitable administration to a patient (see, for example, Oxford Textbook of Surgery, Morris and Malt, Eds., Oxford University Press (1994)). In general, the liquid will be an aqueous solution. Examples of appropriate solutions include Phosphate Buffered Saline (PBS), Celsior™, Perfadex™, Collins solution, citrate solution, and
25 University of Wisconsin (UW) solution (Oxford Textbook of Surgery, Morris and Malt, Eds., Oxford University Press (1994)).

Any suitable liquid can be saturated to a set concentration of carbon monoxide via gas diffusers. Alternatively, pre-made solutions that have been quality controlled to contain set levels of carbon monoxide can be used. Accurate control of dose can be
30 achieved via measurements with a gas permeable, liquid impermeable membrane connected to a carbon monoxide analyzer. Solutions can be saturated to desired effective concentrations and maintained at these levels.

Treatment of Patients with Carbon Monoxide Compositions

A patient can be treated with a carbon monoxide composition by any method known in the art of administering gases and/or liquids to patient. Carbon monoxide compositions can be administered to a patient diagnosed with, or determined to be at risk, for example, for emphysema, bronchitis, arthritis, cystic fibrosis, pneumonia, interstitial lung disease, Parkinson's disease, Alzheimer's disease, or inflammation of the kidneys, spleen, or skin; or to promote wound healing, e.g., healing of skin wounds not associated with surgery. The invention contemplates the systemic administration of liquid or gaseous carbon monoxide compositions to patients (e.g., by inhalation and/or ingestion), and the topical administration of the compositions to the patient's lungs (e.g., by inhalation or intratracheal administration), joints (e.g., by infusion or transdermal administration) skin (e.g., by injection or by applying the composition to the surface of the skin), and other organs (e.g., by ingestion, insufflation, and/or introduction into the abdominal cavity).

15

Systemic Delivery of Carbon Monoxide

Gaseous carbon monoxide compositions can be delivered systemically to a patient. Gaseous carbon monoxide compositions are typically administered by inhalation through the mouth or nasal passages to the lungs, where the carbon monoxide may exert its effect directly or be readily absorbed into the patient's bloodstream. The concentration of active compound (CO) utilized in the therapeutic gaseous composition will depend on absorption, distribution, inactivation, and excretion (generally, through respiration) rates of the carbon monoxide as well as other factors known to those of skill in the art. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. Acute, sub-acute and chronic administration of carbon monoxide are contemplated by the present invention, depending upon, e.g., the severity or persistence of the condition in the patient. Carbon monoxide can be delivered to the patient for a time (including indefinitely) sufficient to treat the condition and exert the intended pharmacological or biological effect.

The following are examples of some methods and devices that can be utilized to administer gaseous carbon monoxide compositions to patients.

Ventilators

5 Medical grade carbon monoxide (concentrations can vary) can be purchased mixed with air or another oxygen-containing gas in a standard tank of compressed gas (e.g., 21% O₂, 79% N₂). It is non-reactive, and the concentrations that are required for the methods of the present invention are well below the combustible range (10% in air). In a hospital setting, the gas presumably will be delivered to the bedside where it will
10 be mixed with oxygen or house air in a blender to a desired concentration in ppm (parts per million). The patient will inhale the gas mixture through a ventilator, which will be set to a flow rate based on patient comfort and needs. This is determined by pulmonary graphics (i.e., respiratory rate, tidal volumes etc.). Fail-safe mechanism(s) to prevent the patient from unnecessarily receiving greater than desired amounts of carbon
15 monoxide can be designed into the delivery system. The patient's carbon monoxide level can be monitored by studying (1) carboxyhemoglobin (COHb), which can be measured in venous blood, and (2) exhaled carbon monoxide collected from a side port of the ventilator. Carbon monoxide exposure can be adjusted based upon the patient's health status and on the basis of the markers. If necessary, carbon monoxide can be
20 washed out of the patient by switching to 100% O₂ inhalation. Carbon monoxide is not metabolized; thus, whatever is inhaled will ultimately be exhaled except for a very small percentage that is converted to CO₂. Carbon monoxide can also be mixed with any level of O₂ to provide therapeutic delivery of carbon monoxide without consequential hypoxic conditions.

25

Face Mask and Tent

A carbon monoxide-containing gas mixture is prepared as above to allow passive inhalation by the patient using a facemask or tent. The concentration inhaled can be changed and can be washed out by simply switching over to 100% O₂.
30 Monitoring of carbon monoxide levels would occur at or near the mask or tent with a fail-safe mechanism that would prevent too high of a concentration of carbon monoxide from being inhaled.

Portable inhaler

Compressed carbon monoxide can be packaged into a portable inhaler device and inhaled in a metered dose, for example, to permit intermittent treatment of a recipient who is not in a hospital setting. Different concentrations of carbon monoxide
5 could be packaged in the containers. The device could be as simple as a small tank (e.g., under 5 kg) of appropriately diluted CO with an on-off valve and a tube from which the patient takes a whiff of CO according to a standard regimen or as needed.

Intravenous Artificial Lung

An artificial lung (a catheter device for gas exchange in the blood) designed for O₂ delivery and CO₂ removal can be used for carbon monoxide delivery. The catheter, when implanted, resides in one of the large veins and would be able to deliver carbon
5 monoxide at given concentrations either for systemic delivery or at a local site. The delivery can be a local delivery of a high concentration of carbon monoxide for a short period of time at the site of the procedure, e.g., in proximity to the spleen or kidney (this high concentration would rapidly be diluted out in the bloodstream), or a relatively longer exposure to a lower concentration of carbon monoxide (see, e.g., Hattler *et al.*,
10 Artif. Organs 18(11):806-812 (1994); and Golob *et al.*, ASAIO J., 47(5):432-437 (2001)).

Normobaric chamber

In certain instances, it would be desirable to expose the whole patient to carbon
15 monoxide. The patient would be inside an airtight chamber that would be flooded with carbon monoxide (at a level that does not endanger the patient, or at a level that poses an acceptable risk without the risk of bystanders' being exposed. Upon completion of the exposure, the chamber could be flushed with air (e.g., 21% O₂, 79% N₂), and samples could be analyzed by carbon monoxide analyzers to ensure no carbon
20 monoxide remains before allowing the patient to exit the exposure system.

Liquid Compositions

The present invention further contemplates that a liquid composition comprising carbon monoxide can be created for systemic delivery to a patient, e.g., for oral
25 delivery and/or by injection into the body, e.g., intravenously, intra-arterially, intraperitoneally, and/or subcutaneously.

Topical Treatment of Organs with Carbon Monoxide

Alternatively or in addition, carbon monoxide compositions can be applied
30 directly to organs, e.g., the skin, spleen, lung, and/or kidney(s). Gaseous compositions can be applied directly to the interior and/or exterior of the patient's body to treat the patient's organs. A gaseous composition can be directly applied to the internal organs of a patient by any method known in the art for insufflating gases into a patient. For

example, gases, e.g., carbon dioxide, are often insufflated into the abdominal cavity of patients to facilitate examination during laproscopic procedures (see, e.g., Oxford Textbook of Surgery, Morris and Malt, Eds., Oxford University Press (1994)). The skilled practitioner will appreciate that similar procedures could be used to administer carbon monoxide compositions directly to an internal organ of a patient. The skin and underlying joints can be treated topically with a gaseous composition by, for example, exposing the affected skin to the gaseous composition in a normobarometric chamber (described above), and/or by blowing the carbon monoxide composition directly onto the skin.

Liquid carbon monoxide compositions can also be administered topically to a patient's organs. Liquid forms of the compositions can be administered by any method known in the art for administering liquids to patients. As with gaseous compositions, liquid compositions can be applied directly to the interior and/or exterior of the body to treat a patient's organs. For example, the liquid compositions can be administered orally, e.g., by causing the patient to ingest an encapsulated or unencapsulated dose of the aqueous carbon monoxide composition. As another example, liquids, e.g., saline solutions containing dissolved CO, can be injected into the abdominal cavity of patients during laproscopic procedures. Alternatively or in addition, *in situ* exposures or organs, e.g., kidney(s) and spleen, can be performed by any method known in the art, e.g., by *in situ* flushing of the organ with a liquid carbon monoxide composition (see Oxford Textbook of Surgery, Morris and Malt, Eds., Oxford University Press (1994)). The skin can be treated topically with a liquid composition by, for example, injecting the liquid compositions into the skin. As a further example, the skin and underlying joints can be treated topically by applying the liquid composition directly to the surface of the skin, e.g., by pouring or spraying the liquid onto the skin and/or by submerging the skin in the liquid composition.

Disorders and Conditions

Carbon monoxide gas can be used in the preparation of a medicament for use in treating conditions or disease states such as asthma, emphysema, bronchitis, adult respiratory distress syndrome, sepsis, cystic fibrosis, pneumonia, interstitial lung diseases, idiopathic pulmonary diseases, other lung diseases including primary pulmonary hypertension, secondary pulmonary hypertension, cancers, including lung,

larynx and throat cancer, arthritis, Parkinson's disease, Alzheimer's disease, peripheral vascular disease and pulmonary vascular thrombotic diseases such as pulmonary embolism; and in treating a patient suffering from or at risk for localized inflammation of organs, e.g., the kidney, spleen, and/or skin. The present invention can also be used
5 to aid in wound healing, e.g., skin wound healing. Of particular interest is treatment of wounds not caused by surgery.

The present invention may also be used to delay the onset of, or alleviate the effects of oxidative stress in transplant patients, in particular organ transplant patients, especially lung transplant patients. The carbon monoxide compositions may also be
10 used to treat inflammatory conditions of the lungs or inflammation that occurs secondary to sepsis or rejection in transplant patients. While not being limited by way of theory, low dosage CO is believed to act as an anti-inflammatory agent by inhibiting the production and/or effect of pro-inflammatory cytokines such as TNF- α , IL-1, IL-6, MIP-1, and/or by inducing or promoting the action of anti-inflammatory cytokines IL-4
15 and IL-10.

The term "oxidative stress" is used to describe a condition resulting from the overwhelming production of reactive oxygen which cannot be quenched by endogenous antioxidants. Oxidative stress may result in permanent tissue damage caused by the action of the reactive oxygen species on the tissue. The physiological manifestation of
20 oxidative stress take the form of or occurs during various conditions or disease states that include asthma, emphysema, bronchitis, adult respiratory distress syndrome, sepsis or septic shock, cystic fibrosis, pneumonia, interstitial lung diseases, idiopathic pulmonary diseases, other lung diseases including primary pulmonary hypertension, secondary pulmonary hypertension, lung cancer and pulmonary vascular thrombotic
25 diseases such as pulmonary embolism or any inflammatory disease of the lungs.

The term "sepsis" is used to describe the presence of various pus-forming and other pathogenic organisms or their toxins (generally, lipopolysaccharides or LPS bacterial cell walls) in the blood tissues. Sepsis will often result in oxidative stress in those tissues exposed to the pathogens or their toxins. Sepsis often manifests itself in
30 the production of pro-inflammatory cytokines such as TNF- α , IL-1, IL-6 and MIP-1, the production of which is reduced or reversed by the administration of effective amounts of carbon monoxide.

The present invention may be used to treat inflammation. The term “inflammation” is used to describe the fundamental pathological process consisting of a dynamic complex of cytologic and histologic reactions that occur in the affected blood vessels and adjacent tissues in response to an injury or abnormal stimulation caused by a physical, chemical or biologic agent, including the local reactions and resulting morphologic changes, the destruction or removal of the injurious material, and the responses that lead to repair and healing. The term includes various types of inflammation such as acute, allergic, alterative (degenerative), atrophic, catarrhal (most frequently in the respiratory tract), croupous, fibrinopurulent, fibrinous, immune, hyperplastic or proliferative, subacute, serous and serofibrinous. Inflammation localized in the liver, heart, skin (e.g., dermatitis, inflammation due to bacterial, fungal, or viral infections and/or allergic or autoimmune reactions), spleen, brain, kidney (e.g., bacterial pyelonephritis, interstitial nephritis, and/or glomerulonephritis) and pulmonary tract, especially the lungs, and that associated with sepsis or septic shock is favorably treated by the methods according to the present invention.

The term “cancer” is used as a general term to describe any of various types of malignant neoplasms, most of which invade surrounding tissues, may metastasize to several sites and are likely to recur after attempted removal and to cause death of the patient unless adequately treated. Cancers which may be treated using the present compositions and methods include, for example, stomach, colon, rectal, liver, pancreatic, lung, breast, cervix uteri, corpus uteri, ovary, prostate, testis, bladder, renal, brain/CNS, head and neck, throat, Hodgkins disease, non-Hodgkins leukemia, skin melanoma, various sarcomas, small cell lung cancer, choriocarcinoma, mouth/pharynx, oesophagus, larynx, melanoma, kidney and lymphoma, among others.

The present invention can be used to treat conditions or disorders that involve the respiratory system, e.g., emphysema, bronchitis, cystic fibrosis, pneumonia, and interstitial lung disease. The term “emphysema” as used herein refers to a lung disease characterized by an enlargement of lung alveoli. In this condition, alveolar walls are destroyed, causing bronchioles to lose structural support and collapse during exhaling (see, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 6, Chapter 68). The term “bronchitis” refers to a lung disease characterized by inflammation of the tracheobronchial tree. Bronchitis may develop after infections, e.g., viral infections, e.g., the common cold; or bacterial infections, or after exposure to

an irritant (see, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 6, Chapter 69). The term “cystic fibrosis” refers to a genetic disease of the exocrine glands. The disease primarily affects the gastrointestinal tract and respiratory systems, and usually is characterized by chronic obstructive pulmonary disease (COPD) (see, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 19, Chapter 267). The term “pneumonia” refers to a lung disease affecting the parenchyma, and includes, e.g., bacterial, viral, and aspiration pneumonia (see, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 6, Chapter 73). The terms “interstitial lung disease” or “idiopathic interstitial lung disease” refer to a group of lung diseases of unknown etiology, which produce diffuse pathologic changes usually involving the interalveolar interstitial tissue (see, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 6, Chapter 78).

As used herein, the term “arthritis” refers to a condition characterized by inflammation of the joints, and includes, for example, rheumatoid arthritis (RA) (a chronic inflammatory polyarthritis that often leads to destruction of the joints), psoriatic arthritis (inflammatory arthritis associated with psoriasis), ankylosing spondylitis (inflammation of the axial skeleton and large peripheral joints), and ankylosis (immobility or fusion of the joint) (see, e.g., *The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 5, Chapter 50; Section 5, Chapter 51; Section 5, Chapter 51; and Section 9, Chapter 108, respectively).

The term “Parkinson’s disease” refers to “an idiopathic, slowly progressive, degenerative CNS disorder characterized by slow and decreased movement, muscular rigidity, resting tremor, and postural instability.” (*The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 14, Chapter 179). The term “Alzheimer’s disease” refers to a disease characterized by “a progressive, inexorable loss of cognitive function associated with an excessive number of senile plaques in the cerebral cortex and subcortical gray matter, which also contains b-amyloid and neurofibrillary tangles consisting of tau protein,” (*The Merck Manual of Diagnosis and Therapy*, 17th Edition, Section 14, Chapter 171).

Low dosage CO may also be used in the present invention to induce HO-1 enzyme in patients and prevent or limit oxidative stress, especially oxidative stress caused by hyperoxia or sepsis. Induced HO-1 is implicated in maintaining homeostasis in the cells of the patient.

Treatment of Organs and Tissues to Enhance Storage Stability.

The present invention also relates to the use of CO as a preservative for storing organs or tissues to be used in transplants. It is an unexpected result that the inclusion
5 of low dosage CO in the medium in which organs to be transplanted are stored will substantially reduce the likelihood of oxidative damage to the organs during storage and substantially enhance the storage time that organs to be transplanted may be safely stored without suffering irreversible oxidative damage. Thus, in one embodiment of the present invention, an effective amount of CO is bubbled into storage medium either
10 before or preferably when an organ is first placed in the media or shortly thereafter. CO may also be used to enhance the storage stability of organs which have been stored for some time in media, but in those instances, oxidative damage may have become irreversible, thus limiting the intended effect.

Accordingly, the present invention provides a method for enhancing the storage
15 stability of an organ or tissue. The storage stability is enhanced by exposing the organ or tissue to liquid and/or gaseous carbon monoxide compositions. Exposure of an organ or tissue to gaseous carbon monoxide compositions can be performed in any chamber or area suitable for creating an atmosphere that includes appropriate levels of carbon monoxide gas. Such chambers include, for example, incubators, and chambers
20 built for the purpose of accommodating an organ in a preservation solution. As another example, an appropriate chamber may be a chamber wherein only the gases fed into the chamber are present in the internal atmosphere, such that the concentration of carbon monoxide can be established and maintained at a given concentration and purity, e.g., where the chamber is airtight. For example, a CO₂ incubator may be used to expose an
25 organ to a carbon monoxide composition, wherein carbon monoxide gas is supplied in a continuous flow from a vessel that contains the gas.

With respect to liquid carbon monoxide compositions, the exposure may be performed in any chamber or space having sufficient volume for submerging the organ or tissue, completely or partially, in the carbon monoxide composition. In one
30 embodiment of the present invention, the organ may be exposed to a carbon monoxide composition by placing the organ in any suitable container, and causing the carbon monoxide composition to "wash over" the organ, such that the organ is exposed to a continuous flow of the carbon monoxide composition. In another embodiment, the

organ is perfused with a carbon monoxide composition. The term “perfusion” is an art recognized term, and relates to the passage of a liquid, *e.g.*, a carbon monoxide composition, through the blood vessels of an organ or tissue. Methods for perfusing organs *ex vivo* and *in situ* are well known in the art. An organ can be perfused with a carbon monoxide composition *ex vivo*, for example, by continuous hypothermic machine perfusion (see *Oxford Textbook of Surgery*, Morris and Malt, Eds., Oxford University Press (1994)). Optionally, the organ can be perfused with a wash solution, *e.g.*, UW solution without carbon monoxide, prior to perfusion with the carbon monoxide composition to remove the donor’s blood from the organ. Such a process could be performed to avoid competition for carbon monoxide by the donor’s hemoglobin. As another option, the wash solution can be a carbon monoxide composition. As still another example, an appropriate liquid may be passed through tubing that allows gas diffusion, which runs through an atmosphere comprising carbon monoxide (*e.g.*, through a chamber, such as with extracorporeal membrane oxygenation), to create a liquid carbon monoxide composition, which may then be passed into an organ (*e.g.*, perfused into the organ by connecting the tubing to the organ).

As another example, the organ may be placed, *e.g.*, submerged, in a medium or solution that does not include carbon monoxide, and placed in a chamber such that the medium or solution can be made into a carbon monoxide composition via exposure to a carbon monoxide-containing atmosphere as described herein. As still another example, the organ may be submerged in a liquid that does not include carbon monoxide, and carbon monoxide may be “bubbled” into the liquid.

The present invention contemplates that any or all of the above methods for exposing an organ to a liquid carbon monoxide composition, *e.g.*, washing, submerging, or perfusing, can be used in a given procedure, *e.g.*, used in a single procedure for enhancing the storage stability of an organ or tissue.

Carbon Monoxide as a Diagnostic Tool

In addition to using CO as a therapeutic agent, the measurement of CO may be a useful diagnostic tool, *e.g.*, a biomarker, to determine whether a patient is in oxidative stress or has a condition or a disease state where CO may be implicated, *e.g.*, sepsis or septic shock. In general, a patient suspected of being in oxidative stress or at risk for

oxidative stress is monitored to determine whether detectable levels of carbon monoxide may be measured in the exhaled breath of the patient. If detectable levels of carbon monoxide are seen (i.e., an amount of carbon monoxide of at least about 0.01 ppm in the patient's breath), then the attending physician or caregiver may then begin
5 to administer therapeutic doses of carbon monoxide to treat oxidative stress or any one or more of the conditions or disease states which are secondary to or result in oxidative stress.

In one embodiment, a patient will have his or her exhaled breath analyzed for the presence of CO. CO content in a patient's breath is measured by a CO monitor (for
10 example, using a Logan LR2000) which is sensitive to the detection of CO from 0 to about 1000 ppm (with a sensitivity as low as 1 ppb). In this method, the subjects exhale slowly from functional FVC into the breath analyzer with a constant flow (5-6 l/m) over a 20-30 second interval. Two successful recordings are made and mean values will be used for all calculations. Ambient CO levels are recorded before each
15 breath in order to provide control or background values. While any elevation in CO levels from background numbers may implicate an actual or incipient state of oxidative stress, an amount of CO of at least about 1 ppm provides a clear indication that the patient is in or is about to suffer oxidative stress.

A number of embodiments of the invention have been described. Nevertheless,
20 it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. Use of carbon monoxide gas in the preparation of a medicament for use in treating a patient suffering from or at risk for at least one disorder selected from the group consisting of: emphysema, bronchitis, cystic fibrosis, pneumonia, interstitial
5 lung disease, wound healing, arthritis, Parkinson's disease, and Alzheimer's disease; or to promote wound healing in the patient.
2. The method of claim 1, wherein the disorder is emphysema.
- 10 3. The method of claim 1, wherein the disorder is bronchitis.
4. The method of claim 1, wherein the disorder is cystic fibrosis.
5. The method of claim 1, wherein the disorder is pneumonia.
- 15 6. The method of claim 1, wherein the disorder is interstitial lung disease.
7. The method of claim 1, wherein the disorder is arthritis.
- 20 8. The method of claim 1, wherein the disorder is Parkinson's disease.
9. The method of claim 1, wherein the disorder is Alzheimer's disease.
10. The method of claim 1, wherein the carbon monoxide gas is used in the
25 preparation of a medicament for use in promoting wound healing in the patient.
11. Use of carbon monoxide gas in the preparation of a medicament for use in treating a patient suffering from or at risk for localized inflammation of at least one organ selected from the group consisting of: kidney, spleen, and skin.
- 30 12. The method of claim 11, wherein the organ is a kidney.

13. The method of claim 11, wherein the organ is a spleen.

14. The method of claim 11, wherein the organ is skin.

5 15. A method of enhancing the storage stability of an organ in a medium,
comprising:
 providing a medium comprising carbon monoxide; and
 storing the organ in the medium, wherein the carbon monoxide is
present in the medium in an amount sufficient to enhance storage stability of the organ.

10

16. A method of determining whether a patient is at risk for sepsis or septic
shock, comprising:

 measuring carbon monoxide in breath exhaled by a patient, wherein an amount
of carbon monoxide of at least about 1 ppm in the breath is indicative that the patient is
15 at risk for sepsis or septic shock.

AMENDED CLAIMS

**[Received by the International Bureau on 02 September 2003 (02.09.2003);
original claims 1-14 and 16 unchanged, original claim 15 cancelled
(1 page)]**

13. The method of claim 11, wherein the organ is a spleen.

14. The method of claim 11, wherein the organ is skin.

15. A method of determining whether a patient is at risk for sepsis or septic shock, comprising:

measuring carbon monoxide in breath exhaled by a patient, wherein an amount of carbon monoxide of at least about 1 ppm in the breath is indicative that the patient is at risk for sepsis or septic shock.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/14836

A. CLASSIFICATION OF SUBJECT MATTER														
IPC(7) : A61K 33/00 US CL : 424/613,699,718														
According to International Patent Classification (IPC) or to both national classification and IPC														
B. FIELDS SEARCHED														
Minimum documentation searched (classification system followed by classification symbols) U.S. : 424/613,699,718														
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched														
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet														
C. DOCUMENTS CONSIDERED TO BE RELEVANT														
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
X	Database NIOSHTIC on STN/CAS, Acc. No. 1997:109846, Doc. No. NIOSH-00153659, ABIDIN et al, The combined effect of carbon monoxide and normobaric hyperoxia on animals. Kosmicheskaya Biologiya i Aviakosmicheskaya Meditsina, 1978, No. 6, pp. 63-67), abstract.	1-14												
X	OTTERBEIN et al, Carbon monoxide provides protection against hyperoxic lung injury. Am. J. Physiol., 1999, 276, L688-L694, see entire document.	1-14												
X	Database BIOSIS on STN/CAS, Acc. No. 1998:178713, Doc. No. PREV199800178713, YUAN et al., Evidence of increased endogenous carbon monoxide production in newborn rat endotoxocosis. Chinese Medical Sciences Journal, 1997, Vol. 12, No. 4, pp. 212-215), abstract.	16												
Y	WO 98/08523 A1 (MESSER GRIESHEIM GMBH) 05 March 1998 (05.03.98), See entire document, especially the abstract, claims 4, 5, 7-12, see entire document.	1-14												
Y	Database BIOSIS on STN/CAS, Acc. No. 1996:420959, Doc. No. PREV199699143315, CHOI et al, Heme oxygenase-1: Function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. American Journal of Respiratory Cell and Molecular Biology, 1996, Vol. 15, No. 1, pp. 9-19), abstract.	1-14												
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.														
<table border="0"> <tr> <td colspan="2">* Special categories of cited documents:</td> </tr> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier application or patent published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			* Special categories of cited documents:		"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed	
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"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone													
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"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family													
"P" document published prior to the international filing date but later than the priority date claimed														
Date of the actual completion of the international search 08 August 2002 (08.08.2002)		Date of mailing of the international search report 30 SEP 2002												
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230		Authorized officer Valerie Bell-Harris for Frank Chor Telephone No. (703) 308-1235												

INTERNATIONAL SEARCH REPORT

PCT/US02/14836

Continuation of B. FIELDS SEARCHED Item 3:

WEST,STN/CAS

Search terms: carbon monoxide, disease, wound, emphysema, bronchitis, cystic fibrosis, pneumonia, interstitial lung disease, arthritis, Parkinson's, Alzheimer's, inflammation, organ, storage, sepsis, septic

INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database SCISEARCH on STN/CAS, Acc. No. 94:51937, LEFER et al, A comparison of vascular biological actions of carbon-monoxide and nitric-oxide. Methods and Findings in Experimental and Clinical Pharmacology, 1993, Vol. 15, No. 9, pp. 617-622), abstract.	1-14
Y	Database DRUGU on STN/CAS, Doc. No. 1999-19188, VASSALLI et al, Inhibition of hypoxic pulmonary vasoconstriction by carbon monoxide in dogs. Eur. Resp. J., 1998, 12, Suppl. 28, 237s), abstract.	1-14