

# Metabolic modulation of carbon monoxide toxicity

Stanley T. Omaye \*

*Department of Nutrition and Environmental Toxicology and Health Track, Environmental Sciences and Health Graduate Program,  
University of Nevada, Reno, NV 89557, USA*

## Abstract

Carbon monoxide (CO) gas is a product of the incomplete combustion of carbon-based fuels and substances. From a public health perspective, CO poisoning may be the cause of more than 50% of fatal poisonings in many industrial countries. The adverse effects of CO poisoning may be more widespread because of unreported situations and delayed neurologic effects, which may be linked to CO exposure. Chronic CO effects that are subtle, such as the adverse effects on vascular diseases, may increase the number of people at risk. The apparent role of CO as an important mediator of cell signaling is a paradox and may represent an example of hormesis, i.e. beneficial effects at low concentration but adverse effects at higher concentrations. Nevertheless, because CO can form ligands with iron (heme) and copper sites, the potential for metabolic intervention is likely. Furthermore, CO-induced oxidative stress opens the opportunity for modulating the adverse effects of CO with antioxidants (both water- and lipid-soluble compounds) and various factors involved with reducing oxidative stress. However, consideration must be given to the micro-environment in some situations that could potentially create more oxidation and subsequent metabolic damage if the combinations and concentrations of antioxidants are not correct, i.e. pro oxidant effects. Likewise, it is important that we take precautions in the development of antioxidant adjuvants to use with oxygen therapies in CO poisoning. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

*Keywords:* CO poisoning; Hormesis; CO-induced oxidative stress

## 1. Introduction

If the increase in recent years of the use of home carbon monoxide (CO) detectors is any indication, the consumer has become more aware of the dangers of CO poisoning. Acute episodes of CO exposure are the leading cause of poisoning in the United States and may account for more than 50% of fatal poisonings reported in many industrial countries (Cobb and Etzel, 1991; Meredith et al.,

1988; Yang et al., 1998; Varon et al., 1999). CO, a product of incomplete combustion (oxidation) of carbon compounds, is a toxic gas that is colorless, odorless, and tasteless. Motor vehicles, various appliances that use carbon-based fuels, and fireplaces used for heating homes are the main sources of CO.

There has also been an increase in clinical awareness that a link exists between chronic exposure to CO and the etiology of vascular or cardiovascular diseases (Stern et al., 1988; Kleinman et al., 1989; Sheps et al., 1990; Allred et al., 1989). An underpinning supposition is CO derived

\* Tel.: +1-775-784-6447; fax: +1-775-784-6449

E-mail address: [omaye@unr.edu](mailto:omaye@unr.edu) (S.T. Omaye).

from smoke, either from active tobacco smoking or from environmental tobacco smoke exposure (Menear, 1993; Freund et al., 1993; Milei et al., 1998), may be a critical risk factor for chronic diseases. Thus, there is certainly a greater sense of public and medical awareness regarding the dangers of CO exposure.

Coincidentally, there has been recent scientific interest in purported roles of CO in signal transduction, somewhat analogous to the role of nitric oxide (NO). Overall, we have a good understanding about the physiological response and underlying metabolic effects for CO toxicity, and these roles will only be briefly reviewed here (Cobb and Etzel, 1991; Meredith et al., 1988; Yang et al., 1998; Varon et al., 1999; Penney, 2000). The intent of this review is to discuss recent findings focused on the physiological and metabolic responses to CO, particularly, with respect to how we might better use such knowledge to design ways to modulate the toxic response. Such knowledge will enable us to develop efficacious adjuvants in treating CO poisoning and better understand the role of CO in chronic diseases.

## 2. Environmental sources of CO and magnitude of exposure

Car exhaust fumes, smoke from fires, gas-powered engines, wood-burning fire places, and methylene chloride containing paints are the most common sources of CO. Some common environmental CO sources are listed in Table 1. An improperly vented natural gas heater in a small room can make the air unsafe to breathe within a matter of minutes. Cases of CO poisoning have occurred outdoors when faulty equipment was

Table 1  
Common environmental sources of CO

Propane engines	Radiant heaters	Portable generators
Kerosene heaters	Gas fireplaces	Natural gas appliances
Motor vehicle exhaust	Fires	Paint
Hibachi cookers	Tobacco smoke	

involved. Solvents, such as found in paint strippers, or those used for degreasing machinery, can form methylene chloride. Methylene chloride vapors are readily absorbed by the lung into the circulation and, as shown in Fig. 1, upon reaching the liver can be converted to CO. Methylene chloride can be deposited in fatty tissue during chronic exposures. Individuals who inhale sufficient quantities, may be overcome by CO toxicity some time after exposure due to the slow release of methylene chloride from adipose tissue and subsequent metabolism in the liver to CO.

The exhaust from gasoline internal combustion engines contains between 3 and 7% CO. Emission standards for motor vehicles in the United States set CO limitation to 0.5%. More than 50% of deaths due to CO can be attributed to motor vehicle exhaust mostly associated with running the engine in stationary vehicles, in closed garages. Smoke inhalation from all types of fire ranks second as the leading cause of CO poisoning, particularly for firefighters. Epidemics of CO poisoning are more common during the winter months, particularly when power outages occur forcing people to use alternative wood-burning sources for heat. Transient concentrations of CO can be high in tunnels and parking garages due to the accumulation of motor exhaust fumes (Stern et al., 1988).

An estimated 10 000–40 000 people each year will seek medical attention or miss work due to CO poisoning in the United States (Schaplowsky et al., 1974; Hampson, 1998). Mortality rates between 1 and 31% have been reported; however, the true incidence of CO poisoning is not known, since many non-lethal incidences are not reported. Complete, timely data are often difficult to obtain, particularly for developing countries. A significant number of missed diagnoses may occur because of patients' association of the symptoms with viral illness or clinical depression. Fortunately, deaths from CO have declined consistently in the United States during the past two decades. The decline in



Fig. 1. Hepatic conversion of methylene chloride to CO. Methylene chloride ( $\text{CH}_2\text{Cl}_2$ ).

CO-related deaths is attributed, in part, to transportation-related emission controls, improved safety of cooking and heating appliances, and intensive education to increase consumers' awareness of the dangers of CO poisoning (Varon et al., 1999; Raub et al., 2000).

One can reach lethal concentration of CO within 10 min from a motor vehicle by running an automobile in a closed garage. Acute CO toxicity can occur when ambient CO air levels reach 0.1% or 1000 ppm, i.e. unconsciousness, respiratory failure, and death after 1 h of exposure. Although CO accumulation with chronic exposure does not occur, the anoxia can cause central nervous system damage. CO exposure is particularly dangerous to pregnant women and to their fetuses. Women with increased exposure to CO have reduced blood hemoglobin concentrations and an increased ventilation rate and are more vulnerable to CO. Fetal blood has a greater affinity for CO and the CO to hemoglobin complex, carboxyhemoglobin, can be 10–15% higher compared with the mother (Farrow et al., 1990). The most common source of CO to the mother is from smoking and may be related to the reason smoking is a major factor in increased incidence of stillbirths, premature deliveries, and reduced birth weights of babies. The avidly bound CO to fetal hemoglobin results in slow transplacental transport, resulting in fetal CO level decreases that are much slower than those occurring in the mother. Thus, causing fetal deaths in situations where CO was nonfatal to the mothers. Individuals may be exposed to 400 ppm of CO from a typical cigarette or about 4% carboxyhemoglobin levels for 5 min smoking bouts. A heavy smoker could have carboxyhemoglobin levels as high as 15%. One study reported that cigarette smoke contains 42 000 ppm or 4.2% of CO and can significantly elevate the level of carboxyhemoglobin (Abelson, 1967). It has also been reported that nonsmokers exposed to environmental tobacco smoke can have an incremental doubling of their carboxyhemoglobin (normal is about 1%). Incidentally, smokers do show some adaptive increased red-cell volumes and reduced plasma volumes to elevated carboxyhemoglobin levels, a response to chronic smoking.

At-risk-populations for CO related health effects include: individuals with anemia, hematological disorders, compromised blood flow, and those with chronic cardiopulmonary diseases, particularly the elderly (Varon et al., 1999; Raub et al., 2000).

### 3. Endogenous CO formation

As red cells age, they are removed from circulation and their heme is degraded. Heme degradation begins with oxidative cleavage of the porphyrin between rings A and B, forming the green tetrapyrrole, biliverdin, shown in Fig. 2. As shown in Fig. 3, the methenyl bridge carbon between porphyrin rings A and B is released as CO. Heme oxygenase is the rate-limiting enzyme in the heme catabolic pathway. Subsequently, biliverdin can be reduced to the red–orange bilirubin. CO will form a resilient heme ligand and approximately 1% of hemoglobin's oxygen binding sites are blocked by CO from endogenous sources, even in the absence of any air pollution. In contrast to higher concentration of CO, at 1%, steric conditions at the molecular level favors an overall reduced affinity of heme for CO compared with oxygen, permitting the CO to be exhaled slowly. In the situation where mutant hemoglobin Zurich, a substitution of Arg for His results in 10% of the heme carrying CO.

The turnover of other hemoproteins contribute to CO, such as from myoglobin, catalases, cytochromes, and peroxidases. It has been estimated that approximately 79% of the CO produced is from the heme of RBCs, including some degraded cells during erythropoiesis in the bone marrow. Up to 21% of the endogenous CO is derived from the

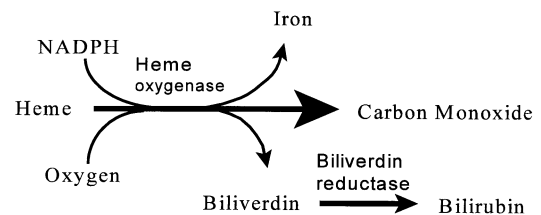


Fig. 2. Heme degradation, sources of endogenous CO.

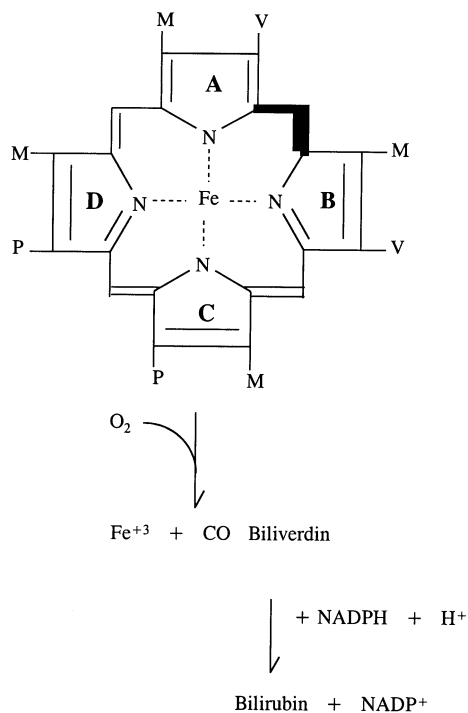


Fig. 3. Origin of endogenous CO (bolded bond). Methyl (M), vinyl (V), propionyl (P) and ethyl (E) groups.

degradation of other hemoproteins and other sources.

Minor amounts of CO may be derived from the process of lipid peroxidation, such as from NADPH-dependent oxidation of microsomal lipids (Nishibayashi et al., 1968),  $\text{Fe}^{3+}$ -ascorbate catalyzed oxidation of microsomes (Wolff et al., 1976), or carbon tetrachloride induced degradation of membrane lipids (Lindstrom et al., 1978).

CO, along with nitric oxide (NO), appear to modulate intracellular cGMP levels, platelet aggregation, and smooth muscle relaxation. Overall, CO has a lower affinity for soluble guanylyl cyclase (a heme protein) than NO (Fig. 4). It has been suggested that decreased production or

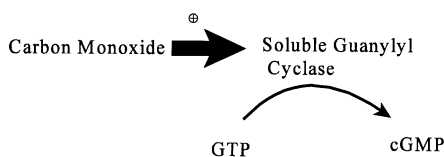


Fig. 4. Interaction of CO with guanylyl cyclase.

sensitivity to NO in atherosclerosis may be compensated for by an induction of heme oxidase, producing bilirubin, an antioxidant and CO as a vasodilator (Siow et al., 1999). Such adaptive responses may contribute to the maintenance of vascular tone in atherosclerotic vessels and compensate for diminished NO generation and activity. Such findings imply that CO may promote vascular health at low concentration. Apparent paradoxical affects of CO seem perplexing in terms of defining a mode of action. However, the biphasic effects of CO might be another example of hormesis in action. Hormesis is the phenomenon whereby a substance has been found to exhibit beneficial effects at a low or very low concentration in contrast to detrimental responses found at a higher concentration (Furst, 1987; Heiby, 1988; Calabrese et al., 1999). The phenomenon of a response opposite to that which is expected can be observed, is well documented, and has been referred to as the 'Arndt-Schulz law', the 'reverse effect' or simply, 'paradoxical reaction' (Furst, 1987; Heiby, 1988). Many examples can be found with agents such as: alcoholic beverages, anesthetic gases, barbiturates, some tranquilizers, many vitamins, caffeine, nicotine, salicylates, and some metals. For ingestion alcohol, the first dose results in the stimulation of respiration and elevation of body temperature; but, at a higher dose, respiration is slowed and body temperature decreases. For ingestion of nicotine, there is a transient stimulation of autonomic ganglia at low doses, followed by a persistent depression of these autonomic ganglia. Heart rates are first elevated and then slowed.

## 4. Mechanisms of CO toxicity

### 4.1. Hemoglobin

CO diffuses freely in air and does not layer significantly. CO has a significant affinity for all iron- or copper-containing sites and competes with oxygen at these active sites. Red blood cell hemoglobin is a major target site for CO. CO combines with hemoglobin to form carboxyhemoglobin, a molecule that is incapable of carrying

oxygen to tissue sites, resulting in tissue hypoxia. The binding of CO to hemoglobin is reversible and, removing an individual from the source of CO will lead to eventual removal of CO from the body. The CO hemoglobin ligand has an affinity 240 times greater than that of oxygen. Also, the CO hemoglobin ligand on any one of the four oxygen binding sites of hemoglobin results in the complex having a greater affinity for oxygen at the remaining binding sites. Thus, oxygen bound to the CO hemoglobin produces a complex that does not give oxygen to peripheral body tissue sites. The increased affinity for oxygen by the carboxyhemoglobin complex is known as the Haldane effect. The Haldane effect causes a leftward shift of the oxygen hemoglobin dissociation curve. Not only does the oxygen–hemoglobin dissociation curve shift to the left but the curve is distorted from a sigmoidal shape to a hyperbola. In addition, the decreased oxygen delivery becomes sensed at the central nervous system, resulting in ventilatory stimulation and further increased uptake of CO, elevation in carboxyhemoglobin, and eventually respiratory alkalosis. Thus, CO is toxic because of its capacity to reduce both the oxygen-carrying capacity and the oxygen-unloading function of the hemoglobin molecule.

#### 4.2. Myoglobin

Other hemeprotein have an affinity for CO (Fig. 5). Muscle myoglobin has an affinity for CO, which is 40 times greater than that for oxygen. Like hemoglobin, CO association with myoglobin will display leftward oxygen dissociation. CO also binds to cardiac myoglobin. Cardiac myoglobin binds three times more CO than skeletal myoglobin. A delayed return of CO symptoms has been described and appears to result when a recurrence of increased carboxyhemoglobin levels, presumably due to late release of CO from myoglobin,

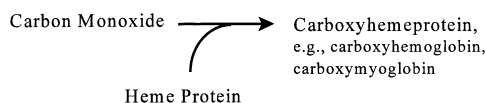


Fig. 5. Interaction of CO and hemeproteins with the formation of carboxyhemeproteins.

subsequently binds CO to hemoglobin. A reduction in oxygen delivery because of the elevated carboxyhemoglobin level, exacerbated by impaired perfusion, which results from hypoxic cardiac dysfunctions, will impair cellular oxidative metabolism, i.e. ischemia. The hypoxia and reduction in blood flow allow CO to bind to cytochrome *c* oxidase, interfering with cellular respiration at the mitochondrial level, i.e. aerobic adenosine triphosphate synthesis. This disturbance in electron transport also increases the production of reactive oxygen species (ROS) and induces oxidative stress. Because of the continued inhibition of respiration by carboxyhemoglobin, energy production and mitochondrial function are restored slowly (Hendrik et al., 2000; Penney, 2000).

Much of the late changes associated with CO poisoning is similar to post ischemic reperfusion injuries, including oxidative stress. In rats, allopurinol prevents the late effects of CO exposure. Likewise, rats made leukopenic do not demonstrate the late effects of CO poisoning (Miro et al., 1999; Hendrik et al., 2000; Penney, 2000).

#### 4.3. Cytochrome oxidase

Cyanide and CO bind to and inhibit cytochrome oxidase (Complex IV)(Fig. 6). The consequence is all components preceding cytochrome oxidase, the terminal electron complex, become reduced; and it is not possible to pump protons. Unless immediate intervention occurs, cells respond by switching to anaerobic metabolism, resulting in lacticacidosis and eventual death. Methylene blue can be used to alleviate the inhibition of cytochrome oxidase by accepting electrons from cytochrome *c* reductase (Complex III) permitting Complex I and cytochrome *c* reductase to pump protons, so that ATP can continue to be synthesized (Baynes et al., 1999).

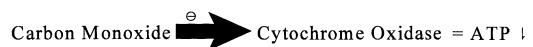


Fig. 6. Interaction of CO with cytochrome oxidase.



#### 4.4. Cytochrome P450

Although the physiologic significance is not known, CO can bind to cytochrome P450 hemoprotein. This relationship was used to study the spectroscopic properties of cytochrome P450 enzymes. The heme group of such proteins forms a strong chromophore with spectroscopic properties that are sensitive to the nature of the ligands bound, to the iron oxidation state, and the protein environment with which the heme is associated.

### 5. Pathophysiology of CO

Principal manifestation of CO poisoning is dyspnea. Earliest signs in mild exposure are nausea, vomiting, and dizziness. Moderate exposures result in tachycardia, tachypnea, weakness and ataxia. More severe CO poisoning results in syncope, seizures, hypotension, coma and death. The heart is readily affected by CO, demonstrating arrhythmia, premature ventricular contractions, atrial fibrillation, heart block and ischemic changes. The brain is the most sensitive target site, manifesting neurologic symptoms.

Effects of CO exposure vary with the concentration and duration, and range from rather subtle vascular and neurologic changes to unconsciousness and death. Patients who die of CO poisoning are characterized by diffuse petechia and hemorrhages with edematous brains. Ischemic anoxia is prominent in those patients who initially survive acute CO poisoning and die within a few weeks. Ambient CO exposure can cause pulmonary cell damage, as a direct result of CO uptake and not because of a CO to hemoglobin interaction. [Table 2](#) lists examples of ambient CO concentrations, estimated carboxyhemoglobin levels that might result as steady-state exposure, and related human health effects. Keep in mind that individuals may experience different symptoms of CO toxicity even under similar exposure conditions. Below carboxyhemoglobin levels of 10%, most subjects are asymptomatic. A carboxyhemoglobin concentration of 2.5% has been proposed as the no-effect level. The proposed level is lower than the 3.5%

carboxyhemoglobin level that the American Conference of Governmental Industrial Hygienists suggested as the best estimate of a no effect concentration among industrial workers ([ACGIH, 1991](#)). It also is a level below 6.3% carboxyhemoglobin, which noted above ([Stern et al., 1988](#)), is the concentration associated with increased ischemic heart disease risk in tunnel workers or 3.9% carboxyhemoglobin found to have an effect on exercise-induced arrhythmias in patients with preexisting coronary artery disease ([Sheps et al., 1990](#)). Increases of carboxyhemoglobin levels above 20%, resulting in the patient developing headache, dizziness, confusion and nausea. Patients with cardiopulmonary problems, such as chronic obstructive pulmonary diseases or angina, may have exacerbated effects at low levels of carboxyhemoglobin. CO binds with high affinity to cardiac myoglobin and exhausts cell oxygen reserves. Patients may complain of chest pain, which can be attributed to cardiac dysrhythmias due to myocardial ischemia. Carboxyhemoglobin levels above 20% can result in the impairment of task performance and neurophysiologic functions in healthy subjects. Seizures and coma can be common in carboxyhemoglobin levels greater than 40% and unconsciousness or death is likely above 60%. Carboxyhemoglobin related hypoxic stress appears to be responsible for cardiac injuries, fatalities, and acute neurological abnormalities found in approximately 15% of those who survive CO poisoning. Other systemic complications which occur as a result of CO poisoning include: skeletal muscle necrosis, renal failure, pancreatitis, and hepatocellular injury ([Mennear, 1993](#); [Horner, 2000](#)).

### 6. Delayed neurological damage

An insidious effect of CO poisoning is the development of late neuropsychiatric damage, or delayed neurological syndrome. Between 5 and 40% of CO exposed patients may manifest cognitive difficulties, such as poor concentration, memory loss and cognitive impairment within several days to a month after exposure. Up to 40% of patients develop memory impairment, with dete-

Table 2  
Ambient CO, carboxyhemoglobin and associated human health effects

Ambient CO		COHb in blood (%)	Human health, symptoms and effects
%	ppm		
0.001	10	2	Asymptomatic
0.007	70	10	Exhaustion in healthy people, more angina in patients, headaches
0.012	120	20	Dizziness, nausea, dyspnea
0.022	220	30	Visual disturbances
0.035–0.052	350–520	40–50	Confusion, syncope, seizures and coma
0.080–0.195	1950	60–80	Cardiopulmonary dysfunction and death

Modified from; Varon et al., 1999; Raub et al., 2000; Horner, 2000.

rioration of personality. It appears many cases may be consequences of missed diagnosis. This is unfortunate because complete recovery may be obtained when intervention by supplemental oxygen is initiated soon after diagnosis. Neuronal death in the cortex, hippocampus, substantia nigra and globus pallidus is all an effect with CO poisoning. Demyelination of the cerebral cortex and a broken blood–brain barrier are common abnormalities found in patients of CO poisoning.

### 6.1. Cardiovascular diseases

Workers exposed to automobile exhaust (50 ppm of CO) in tunnels were reported to have 35% excess in ischemic heart disease death (Stern et al., 1988). We have looked at the association between hospitalization for cardiovascular system illnesses and ambient levels of CO in the Reno–Sparks area of Northern Nevada. The Reno–Sparks area is unique because of its high altitude and low concentration of sulfur dioxide. According to our statistical models, a consistent positive relationship exists between the ambient CO level and different groups of cardiovascular disease hospital admissions, although the male group and the > 60 years old group tended to have the highest correlations of all groups studied (Yang et al., 1998; Chen et al., 2001). Our findings are consistent with those of other studies (Schwartz et al., 1995; Schwartz, 1997; Burnett et al., 1997a,b), supporting the role of ambient CO in hospital admissions for heart disease. Carboxyhemoglobin level changes from 1.5 to 3% (exposure to 100 ppm

CO for 1 h) caused more rapid onsets of exercised-induced anginal pain in male subjects with stable angina (Kleinman et al., 1989). Others have found that 5.8% carboxyhemoglobin saturation resulted in increased frequency and complexity of post-exercise ventricular arrhythmias, compared with 3.9% carboxyhemoglobin saturation (Sheps et al., 1990). Thus, several studies in humans strongly suggest that exposure to high concentrations of CO may elevate risk of ischemic heart disease. As noted above, such effects are consistent with the production of systemic anoxia and impaired myocardial tissue. In addition, tobacco smoke or smoke resulting from the environment of smokers, could contribute to the CO load of individuals; however, the significance of such contribution has been debated (Mennear, 1993).

## 7. Biochemical changes.

Other mechanisms not related to tissue hypoxia have been described as involved in the adverse effects of CO poisoning, such as reoxygenation injury (Zhang et al., 1992), products from the xanthine dehydrogenase/oxidase reaction, or nitric oxide derived oxidants (Thom, 1992; Thom et al., 1997).

### 7.1. Mitochondrial dysfunction

Cytochromes contained in complex III (succinate-coenzyme Q reductase) and complex IV (cytochrome *c* oxidase) of the mitochondrial

respiratory chain are targets for CO because they contain heme groups. It has been reported that cytochrome *c* oxidase activity returned to normal level much more slowly than did carboxyhemoglobin levels following treatment. Thus, cytochrome *c* oxidase inhibition could be crucial for some of the symptoms ascribed to CO toxicity, such as delayed neuronal injury. In rats, it has been demonstrated that cerebral oxidative injury is not the direct effect of hypoxia, but rather due to ROS generated in brain mitochondria (Zhang et al., 1992). Similar results have been found in circulating lymphocytes from patients acutely intoxicated by CO (Miro et al., 1999). Enhanced oxidative damage of lipid membranes related to inhibition of mitochondrial enzymes could play a key role in the pathophysiology of CO toxicity.

### 7.2. Interaction of CO and NO

As little as 22 nM of CO can elevate tissue nitric oxide ( $\bullet$ NO), which can occur when interstitial CO partial pressure is approximately 20 ppm with a 7% carboxyhemoglobin level. The  $\bullet$ NO level increase is not due to increased enzyme synthesis but because competitive binding with CO for intracellular heme protein binding sites (Fig. 7). CO has a stronger affinity than  $\bullet$ NO for heme protein binding sites. Subsequently, increased vascular  $\bullet$ NO will promote oxidative stress, leakage of various mediators, and trigger phagocyte adherence/activation.

## 8. Nutrient interactions

### 8.1. Oxygen

Patients with CO poisoning respond to treatment with 100% oxygen, both normal or hyperbarometric situations. The mean half-life of

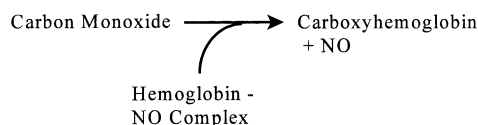


Fig. 7. Displacement of nitric oxide by CO.

carboxyhemoglobin is 5.3 h. Administration of 100% oxygen at one atmosphere reduces the half life to 1.3 h, and with 100% oxygen at three atmospheres will reduce the half life to 0.4 h. Adding carbon dioxide may also be beneficial because it reduces the half-life of carboxyhemoglobin to 12 min in a patient breathing hyperbaric oxygen. This is due to the stimulation, by carbon dioxide, of alveolar ventilation and acidemia, which increases the dissociation of carboxyhemoglobin.

### 8.2. Iron and copper

Both iron and copper-containing proteins are important targets for forming the CO ligand. Obviously, dietary deficiencies of either element can have accentuating consequences in CO poisoning. Iron intakes are frequently inadequate in some population groups, noted above overlap with the high risk groups for CO poisoning. These groups include: infants and young children, adolescents, females during childbearing years, and pregnant women. Other conditions associated with increased need for intake due to iron losses or impaired iron absorption: hemorrhage prone, protein calorie malnutrition, renal disease, decreased gastrointestinal transit time, steatorrhea, intestinal parasites, achlorhydria and prolonged use of alkaline-based drugs, e.g. antacids. The impact of iron deficiency on anemia is not really evident until iron depletion is severe. However, there are significant health effects that can be attributed to iron deficiency without anemia. Iron deficiency in children can result in pallor, behavioral disturbances, lack of ability in cognitive tasks, some irreversible impairment of learning ability, and shorter attention span. In older people, iron deficiency without anemia, can lead to decreased work performance and productivity. Likely modes of action may be by either impairing degradation of gamma-amino butyric acid (GABA), an inhibitory neurotransmitter in the brain, or inhibiting dopamine producing neurons. Thus, it is easy to conclude that iron deficiency could further compromise an individual exposed to CO, with respect to oxygen carrying ability and various health indices that involve iron metabo-



lism. Currently, there is little information available about the benefit of iron supplementation as an adjunct in CO therapy, with the possible exception of individuals with iron deficiency.

Various clinical symptoms are associated with human copper deficiency, which are somewhat similar to those described for iron. Recognized symptoms include hypochromic anemia, leukopenia, hypopigmentation or depigmentation of skin and hair, impaired immune function and bone abnormalities. In addition, the risk of atherosclerosis is higher with copper deficiency. In copper-deficient animals, hypercholesterolemia is found. Elevated blood pressure and plasma cholesterol have been seen in some humans with low copper status. Therefore, CO poisoning superimposed on copper deficiency would further compromise subcellular energy production and elevate the level of oxidative stress.

### 8.3. Antioxidants

Oxidative stress plays an intimate role in the progression of CO-induced tissue damage, but particularly during the ischemic and reperfusion phase of CO-induced injury. Also, oxidative stress induced by CO toxicity may play a significant role in various chronic diseases, such as cardiovascular diseases. Evidence of high intakes of vitamin E associated with lower risk of heart disease has been shown in studies involving large groups of men and women. An increased risk of ischemic heart disease has been shown with low plasma concentration of antioxidants, primarily vitamin E, but to a lesser extent with carotene, vitamin C and vitamin A. Antioxidant supplementation studies have shown some promising results, such as diminished risk of heart disease. Recovery of tissue following ischemic-reoxygenation injuries also appear to benefit from the use of antioxidants.

Oxygen therapy, both normal and hyperbaric levels, are the accepted means for CO treatment. It is well established that such treatments increase the risk of further complications due to increased oxidative stress. Patients who are subject to oxygen therapy might benefit by the use of antioxidant supplements, i.e. prevention of side effects due to increased oxidative stress. Thus, it is

likely that antioxidant nutrients, such as vitamins C and E have important roles as adjuvants in CO-induced damage.

In addition to CO effects, smoke is rich in ROS and can induce the respiratory tract production of endogenous oxidants and ROS via the inflammatory response. It is likely that smoke exposure can levy a heavy toll on the body's natural antioxidant defense systems, depleting tissue pools of critically needed antioxidants. This has been demonstrated in several nutrition surveys for smokers, showing that this population suffers from low blood levels of antioxidants. In part, low antioxidant reserves reflect poor dietary habits and a consumption lack of fruits and vegetables. Several clinical trials have explored the use of selected antioxidant supplements in preventing various adverse effects; however, the findings have been less than promising in support for the use of supplements. For example, results from the Physicians' Health Study (PHS) and the Beta-Carotene and Retinol Efficacy Trial (CARET) indicated that supplements of vitamin E had little affect and supplements of  $\beta$ -carotene increased the incidence of lung cancer (Hennekens et al., 1996; Omenn et al., 1996). Thus, use of antioxidants may be efficacious for treatment of the adverse effects of CO; but caution is warranted in the use of scientific approaches to designing such treatment, rather than using hit-or-miss or shot-gun approaches.

Ascorbic acid is the most important water-soluble, essential antioxidant present in human serum and blood. Relatively high concentrations of ascorbic acid are found extracellularly, and this vitamin functions well as an antioxidant and scavenger of superoxide anion radical, singlet oxygen, hydroxyl radical and in preventing lipid peroxidation (Elsayed et al., 2001). Ascorbic acid has been shown to protect DNA and other macromolecules from oxidative-induced damage. As the major water-soluble antioxidant, ascorbic acid serves as the first-line-of-defense against damaging byproducts of oxidative stress. Ample evidence exists supporting the role of ascorbic acid in regenerating the antioxidant properties of  $\alpha$ -tocopherol, the important lipid-soluble antioxidant. Other important water-soluble antioxidants

include: glutathione, lipoic acid and other thiol compounds.

It is likely that  $\alpha$ -tocopherol is the key lipid-soluble, oxidation chain breaker and quenching free radical compound. In addition to its antioxidant role,  $\alpha$ -tocopherol provides membrane stability. Other important lipid-soluble antioxidants include:  $\beta$ -carotene, coenzyme Q10, and uric acid.

#### 8.4. Potential interactions promoting prooxidant effects

Due to several unanticipated clinical findings testing the efficacy of antioxidants in modulating chronic disease, a number of recent studies have found that antioxidants can exhibit adverse effects. Vitamin E compounds can act as pro-oxidants, particularly the tocopheroxyl radical (Pokorny et al., 1987; Thomas et al., 2000). When the concentration of tocopheroxyl radical is high enough, it is possible for a number of undesirable side reactions to occur, leading eventually to enhancing lipid peroxidation. Under mild oxidation conditions, vitamin E can accelerate the lipid peroxidation of low-density lipoproteins (LDLs) isolated from human blood (Bowry et al., 1992, 1993; Ingold et al., 1993). Such situations where LDL oxidation is initiated by reactions with various attacking aqueous radicals, vitamin E residing at or near the surface of the lipid membranes of the lipoprotein particles, may form vitamin E radicals. Subsequently, the LDL particle forces it to propagate the radical chain by its reaction with polyunsaturated fatty acids (PUFA) within the particle, and such vitamin E radicals are not able to escape and propagate the radical chain with PUFA within the particle, particularly, if another reductant (co-antioxidant), such as ascorbic acid, is not available in the aqueous phase outside the LDL particle (Thomas et al., 2000).

The antioxidant/pro-oxidant activities of  $\beta$ -carotene and carotenoids are related to environmental conditions, such as the concentrations of oxygen,  $\beta$ -carotene, and the presence of other oxidants (Burton et al., 1984; Lieber et al., 1996; Zhang et al., 1998). At 150 Torr (composition of air),  $\alpha$ -tocopherol is about 40- to 50-fold better than  $\beta$ -carotene as an antioxidant. However, at 15 Torr

(approximately the level of oxygen found in living tissues), the difference in effectiveness decreases by 40%; that is,  $\beta$ -carotene has enhanced antioxidant activity at lower oxygen tension. At even higher oxygen tension (760 Torr or 100% oxygen), any antioxidant activity of  $\beta$ -carotene appears to be offset by  $\beta$ -carotene acting as a pro-oxidant. Recent studies verifying the pro-oxidant effect of  $\beta$ -carotene have also shown the oxidative modification of protein, fatty acids, and DNA (Zhang et al., 2000, 2001a,b). Extrapolating such findings to in vivo situations, the potential harm versus benefits of  $\beta$ -carotene is alarming.  $\beta$ -Carotene would be useful when oxygen was less available (hypoxia, such as might be induced by CO) compared with ambient situations. However,  $\beta$ -carotene would be contraindicated in situations of high oxygen tension, such as hyperoxia, oxygen therapy, and in reperfusion/reoxygenation injury, which might occur in the latter stages of CO poisoning or in attempts to use oxygen intervention.

It has become more evident that extensive interactions between antioxidant compounds, both synergistic and antagonistic, both antioxidant and pro-oxidant, occur in vivo. No longer can we assume that the outcome of their total action is equal to the sum of each antioxidant entity alone. For example, the intake of excessive amounts of one antioxidant compound in the presence of other oxidants may lead to more overall oxidation rather than some synergistic response. Thus, studies on a single antioxidant may be misleading if likely interactions are not considered. If we are to consider the use of antioxidant compounds as adjuvants to CO therapy, then we must insure that we consider ramifications of potential interactions between such compounds.

## 9. Conclusion

The effects of CO appear to be biphasic, a poison at high concentration with some apparent benefits at low doses. The role of CO toxicity and heme protein is well established and provides the basis for current therapies. The metabolically

beneficial effects of low dose CO involving interactions with NO and signal transduction are just beginning to be appreciated, as is our understanding about the significance of the role of oxidative stress. It seems plausible that metabolic intervention may be useful in modulating CO toxicity, particularly as we develop a better understanding about how different metabolic compounds interact, such as oxidants, antioxidants, cellular messengers, and various biomolecules. Improved understanding will be advantageous in the design of more efficacious methods in the treatment of CO poisoning.

## References

- Abelson, P.H., 1967. A damaging source of air pollution. *Science* 158, 1527.
- Allred, E.N., Bleecker, E.R., Chaitman, B.R., Dahms, T.E., Gottlieb, S.O., Hackney, J.D., Pagno, M., Selvester, R.H., Walden, S.M., Warren, J., 1989. Short-term effects of carbon monoxide exposure on the exercise performance of subjects with coronary artery disease. *New Engl. J. Med.* 321, 1426–1432.
- American Conference of Governmental Industrial Hygienists (ACGIH) 1991. Threshold Limit Values for Chemical Substance and Physical Agents and Biological Exposure Indices 1991–1992, ACGIH, Cincinnati, OH.
- Baynes, J., Dominiczak, M.H., 1999. *Medical Biochemistry*. Mosby, London, pp. 83–96.
- Bowry, V.W., Ingold, K.U., Stocker, R., 1992. Vitamin E in human low-density lipoprotein. *Biochemistry* 288, 341–344.
- Bowry, V.W., Stocker, R., 1993. Tocopherol-mediated peroxidation: the pro-oxidant effect of vitamin E on the radical-initiated oxidation of human low-density lipoprotein. *J. Am. Chem. Soc.* 115, 6029–6044.
- Burnett, R.T., Cakmak, S., Brook, J.R., Krewski, D., 1997a. The role of particulate size and chemistry in the association between summertime ambient air pollution and hospitalization for cardiorespiratory diseases. *Environ. Health Perspect.* 105, 614–620.
- Burnett, R.T., Dales, R.E., Brook, J.R., Raizenne, M.E., Krewski, D., 1997b. Association between ambient carbon monoxide levels and hospitalizations for congestive heart failure in the elderly in 10 Canadian cities. *Epidemiology* 8, 162–167.
- Burton, G.W., Ingold, K.U., 1984.  $\beta$ -Carotene: an unusual type of lipid antioxidant. *Science* 224, 569–573.
- Calabrese, E.J., Baldwin, L.A., 1999. Chemical hormesis: its historical foundations as a biological hypothesis. *Toxicol. Pathol.* 27, 195–216.
- Chen, L., Omaye, S.T., 2001. Air pollution and health effects in Northern Nevada. *Rev. Environ. Health* 16, 133–149.
- Cobb, N., Etzel, R., 1991. Unintentional carbon monoxide-related deaths in the United States, 1979–1988. *J. Am. Med. Assoc.* 266, 659–663.
- Elsayed, N.M., Bendich, A., 2001. Dietary antioxidants: potential effects on oxidative products in cigarette smoke. *Nutr. Res.* 21, 551–567.
- Farrow, J., Davis, G., 1990. Fetal death due to nonlethal maternal carbon monoxide poisoning. *J. Forensic* 35, 1448–1452.
- Freund, K.M., Belanger, A.J., D'Agostino, R.B., Kannel, W.B., 1993. The health risks of smoking. The Framingham study: 34 years of follow-up. *Ann. Epidemiol.* 3, 417–424.
- Furst, A., 1987. Hormetic effects in pharmacology: pharmacological inversions as prototypes for hormesis. *Health Phys.* 52, 527–530.
- Hampson, N.B., 1998. Emergency department visits for carbon monoxide poisoning in the Pacific Northwest. *J. Emerg. Med.* 16, 695–698.
- Heiby, W.A., 1988. *The Reverse Effect: How Vitamins and Minerals Promote Health and Cause Disease*. MediScience Publisher, Deerfield, IL.
- Hendrik, J.V., Wong, R.J., Stevenson, D.K., 2000. Carbon monoxide in breath, blood and other tissues. In: Penney, D.G. (Ed.), *Carbon Monoxide Toxicity*. CRC Press, Boca Raton, pp. 19–60.
- Hennekens, C.H., Buring, J.E., Manson, J.E., Stampfer, M., Rosner, B., Cook, N.R., Belanger, C., LaMotte, F., Gaziano, J.M., Ridker, P.M., Willett, W., Peto, R., 1996. Lack of effect of long-term supplementation with  $\beta$ -carotene on the incidence of malignant neoplasms and cardiovascular disease. *New Engl. J. Med.* 334, 1145–1149.
- Horner, J.M., 2000. Anthropogenic emissions of carbon monoxide. *Rev. Environ. Health* 13, 289–298.
- Ingold, K.U., Bowry, V.W., Stocker, R., Walling, C., 1993. Autoxidation of lipids and antioxidant of  $\alpha$ -tocopherol and ubiquinol in homogeneous solution and in aqueous dispersions of lipids: unrecognized consequences of lipid particle size as examined by oxidation of human low density lipoprotein. *Proc. Natl. Acad. Sci.* 90, 45–49.
- Kleinman, M.T., Davidson, D.M., Vandagriff, R.B., Caiozzo, V.J., Whittenberger, J.L., 1989. Effects of short-term exposure to carbon monoxide in subjects with coronary artery disease. *Arch. Environ. Health* 44, 361–369.
- Lieber, D.C., McClure, T.D., 1996. Antioxidant reactions of  $\beta$ -carotene: identification of carotenoid-radical adducts. *Chem. Res. Toxicol.* 9, 8–11.
- Lindstrom, A.D., Anders, M.W., 1978. The effect of phenobarbital and 3-methylcholanthrene treatment on carbon tetrachloride-stimulated heme degradation and carbon monoxide expiration. *Toxicol. Lett.* 1, 307–314.
- Menear, J.H., 1993. Carbon monoxide and cardiovascular disease: an analysis of the weight of evidence. *Regul. Toxicol. Pharmacol.* 17, 77–84.
- Meredith, T., Vale, A., 1988. Carbon monoxide poisoning. *Br. Med. J.* 296, 77–78.
- Milei, J., Grana, D.R., 1998. Mortality and morbidity from smoking-induced cardiovascular diseases: the necessity of

- the cardiologist's involvement and commitment. *Int. J. Cardiol.* 67, 95–109.
- Miro, O., Alonso, J.P., Casademont, J., Jarreta, D., Urbano-Marquez, A., Cardellach, F., 1999. Oxidative damage on lymphocyte membranes is increased in patients suffering from acute carbon monoxide poisoning. *Toxicol. Lett.* 110, 219–223.
- Nishibayashi, H., Tomura, T., Sota, R., Estabrook, R.W., 1968. Structure and Function of Cytochromes. University Park Press, Baltimore, MD.
- Omenn, G.S., Goodman, G.E., Thornquist, M.D., Balmes, J., Cullen, M.R., Glass, A., Keogh, J.P., Meyskens, F.L., Valanis, B., Williams, J.H., Barnhart, S., Hammar, S., 1996. Effects of a combination of  $\beta$ -carotene and vitamin A on lung cancer and cardiovascular disease. *New Engl. Med.* 334, 1150–1155.
- Penney, D.G., 2000. Carbon Monoxide Toxicity. CRC Press, Boca Raton.
- Pokorny, J., 1987. Major factors affecting the autoxidation of lipids. In: Chang, H.W.S. (Ed.), *Autoxidation of Unsaturated Lipids*. Academic Press, London, pp. 141–206.
- Raub, J.A., Mathieu-Nolf, M., Hampson, N.B., Thom, S.R., 2000. Carbon monoxide poisoning—a public health perspective. *Toxicology* 145, 1–14.
- Schaplowsky, A.F., Oglesbay, F.B., Morrison, J.H., Gallagher, R.E., Berman, W., Jr, 1974. Carbon monoxide contamination of the living environment: a national survey of home air and children's blood. *J. Environ. Health* 36, 569–573.
- Schwartz, J., 1997. Air pollution and hospital admissions for cardiovascular disease in Tucson. *Epidemiology* 8, 371–377.
- Schwartz, J., Morris, R., 1995. Air pollution and hospital admission for cardiovascular disease in Detroit, Michigan. *Am. J. Epidemiol.* 142, 23–35.
- Sheps, D.S., Herbst, M.C., Hinderliter, A.L., Adams, K.F., Ekelund, L.G., O'Neil, J.J., Goldstein, G.M., Bromberg, P.A., Dalton, J.L., Ballenger, M.N., Davis, S.M., Koch, G.G., 1990. Production of arrhythmias by elevated carboxyhemoglobin in patients with coronary artery disease. *Ann. Intern. Med.* 113, 343–351.
- Siow, R.C.M., Sato, H., Mann, G.E., 1999. Heme oxygenase-carbon monoxide signalling pathway in atherosclerosis: anti-atherogenic actions of bilirubin and carbon monoxide. *Cardiovasc. Res.* 41, 385–394.
- Stern, F., Halperin, W., Hornung, R., 1988. Heart disease mortality among bridge and tunnel officers exposed to carbon monoxide. *Am. J. Epidemiol.* 128, 1276–1288.
- Thom, S.R., 1992. Dehydrogenase conversion to xanthine oxidase and lipid peroxidation in brain after carbon monoxide poisoning. *J. Appl. Physiol.* 73, 1584–1589.
- Thom, S.R., Kang, M., Fisher, D., Ischiropoulos, H., 1997. Release of glutathione from erythrocytes and other markers of oxidative stress in carbon monoxide poisoning. *J. Appl. Physiol.* 82, 1424–1432.
- Thomas, S.R., Stocker, R., 2000. Molecular action of vitamin E in lipoprotein oxidation: Implication for atherosclerosis. *Free Radic. Biol. Med.* 28, 1795–1805.
- Varon, J., Marik, P.E., Fromm, R.E., Jr, Gueller, A., 1999. Carbon monoxide poisoning: a review for clinicians. *J. Emerg. Med.* 17, 87–93.
- Wolff, D.G., Bidlack, W.K., 1976. The formation of carbon monoxide during peroxidation of microsomal lipids. *Biochem. Biophys. Res. Commun.* 73, 850–859.
- Yang, W., Jennison, B.L., Omaye, S.T., 1998. Cardiovascular disease hospitalization and ambient levels of carbon monoxide. *J. Toxicol. Environ. Health* 55, 185–196.
- Zhang, J., Piantadosi, C.A., 1992. Mitochondrial oxidative stress after carbon monoxide hypoxia in the rat brain. *J. Clin. Invest.* 90, 1193–1199.
- Zhang, P., Omaye, S.T., 1998. Phytochemical interactions:  $\beta$ -carotene, tocopherol and ascorbic acid. In: Bidlack, W.R., Omaye, S.T., Meskin, M.S., Jahner, D. (Eds.), *Phytochemicals: A New Paradigm*. Technomic Press, Lancaster, Penn, pp. 53–75.
- Zhang, P., Omaye, S.T., 2000.  $\beta$ -Carotene and protein oxidation: effects of ascorbic acid and  $\alpha$ -tocopherol. *Toxicology* 146, 37–48.
- Zhang, P., Omaye, S.T., 2001a. DNA strand breakage and oxygen tension: effect of  $\beta$ -carotene,  $\alpha$ -tocopherol and ascorbic acid. *Food Chem. Toxicol.* 39, 239–246.
- Zhang, P., Omaye, S.T., 2001b.  $\beta$ -Carotene: interactions with  $\alpha$ -tocopherol and ascorbic acid in microsomal lipid peroxidation. *J. Nutr. Biochem.* 12, 38–45.