

Carbon Monoxide Poisoning.

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INTRODUCTION

Carbon monoxide (CO) is an odorless, colorless gas and stable product of incomplete hydrocarbon combustion. CO is toxic, and the topic of CO poisoning is timely because it is still a common accidental poisoning in the United States. There are many ways to be poisoned, particularly by inhaling exhaust fumes from internal combustion engines. CO's high chemical stability at physiological temperatures is a major point of emphasis because this property determines its biochemical activity and toxicity in the body (1). And recently, the apparent capacity of CO to serve as a signaling molecule in basic cellular processes has renewed scientific interest in the gas. This presentation, however, focuses primarily on the toxic effects of CO in the brain, because the brain is the major organ in which lasting effects of CO do occur. If one examines the brains of people who die from CO poisoning, a diverse neuropathology is found. Different brain regions are also affected differently, but all types of structures in the brain, including the basal ganglia, hippocampus, white matter, and cortex are susceptible to injury by CO. This complicated neuropathology suggests that CO poisoning produces a multifaceted mechanism of brain injury.

History of CO in Biology

It is useful to digress to the history of CO in the study of heme proteins because heme protein binding is a key to understanding the mechanisms underlying the nature of CO's pathology (2). The history of CO in biology reaches back to Claude Barnard at the Sorbonne in the 1860's who discovered that the gas causes asphyxia by chemically combining with hemoglobin. At the turn of the last century J.S. Haldane proposed the use of canaries in mines to detect CO in settings where coal gas poisoning was a problem. Small birds are very sensitive to CO because they have a rapid circulation time and a small hemoglobin volume. Otto Warburg, the German biochemist of the 1920's discovered that CO reversibly inhibits cell respiration. Warburg also found that he could reverse the effects of CO on cells by illuminating them with specific wavelengths of light that turned out to correspond to the absorption peaks of cytochrome *c* oxidase (see 1).

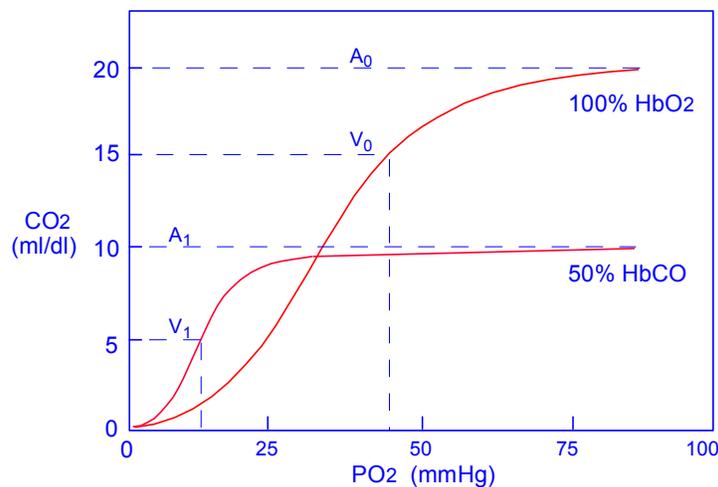
During and after World War II, CO-hemoglobin binding interactions were worked out, including its chemical ability to shift the oxygen dissociation curve of hemoglobin to the left. In 1950 cytochrome P₄₅₀ was discovered, a family of proteins named after their CO absorption peak, which appears in the UV region at 450 nanometers. Not long

thereafter, it was discovered that CO was made endogenously in the body (2)—during heme catabolism—subsequently shown to be an effect of heme oxygenases (3). Hyperbaric oxygen (HBO₂) was first proposed by Pace *et al.* in 1950 (4) as a therapy for poisoning some 85 years after Claude Bernard's original description of the classical asphyxia mechanism. Pace reported that the rate of CO elimination from hemoglobin could be greatly accelerated by HBO₂ administration. This idea was then put to use ten years later by Smith and Sharp (5).

Two mechanistic observations of notable mention involve the work of Ronald Coburn at the University of Pennsylvania (6). Coburn found that CO bound myoglobin in skeletal and cardiac muscle *in vivo* and that this binding occurred in proportion to the CO to O₂ ratio in the cell. This demonstrated Otto Warburg's important principle governing the uptake of CO by living tissues and was even used by Coburn to predict the cellular PO₂. Then in the late 1970s, Caughey and Young (7) discovered that mitochondria actually oxygenate CO to CO₂ and that this involves cytochrome *c* oxidase (8). Caughey and Young thus explain the 1930s observations of Fenn and Cobb that living muscles actually slowly burn CO (9). Thus, when this speaker came to study CO in 1980 there was already a great deal known about the cellular and biochemical activities of this important gas (see 10).

Mechanism of Action of CO

In the 1980's the prevailing opinion about the mechanism of CO poisoning was that it was entirely due to cellular hypoxia. Today we recognize that there is at least a dual poisoning mechanism, and perhaps more subtle effects of the gas related to interference with cell signaling processes. However, Bernard's chemical asphyxia mechanism, known as CO hypoxia, is a key initiator of the process. Carboxyhemoglobin (COHb) does not carry oxygen and the O₂-binding sites on the hemoglobin molecule that are not occupied by CO show an increased oxygen affinity. This is the allosteric mechanism responsible for the shift of the oxyhemoglobin dissociation curve to the left



(Figure 1). Thus, CO binding to hemoglobin causes both an anemia-like effect and an increase in the O₂ affinity of hemoglobin.

The relationship of the equilibrium CO binding to hemoglobin dates to Haldane in the late 19th century (11). This so-called Haldane relationship states that the steady-state

Fig. 1. Effect of carboxyhemoglobin formation on PO₂. Curves show the COHb-related decrease in the oxygen content of blood and left shift of the position of the oxyhemoglobin dissociation curve, which lower tissue PO₂ (see text for details).

carboxyhemoglobin to oxyhemoglobin ratio is M times the ratio of the partial pressures of CO and O₂. M is a binding constant which for human hemoglobin has a value of about 220. Thus, hemoglobin has a much greater affinity for CO than O₂:

$$\frac{\text{HbCO}}{\text{HbO}_2} = M \times \frac{\text{PCO}}{\text{PO}_2}$$

This biochemical mechanism has physiological importance because it causes asphyxia or tissue hypoxia. In the presence of COHb tissue, PO₂ must fall unless O₂ delivery (cardiac output) increases or metabolism (O₂ consumption) declines. This CO-related fall in tissue PO₂ was first measured experimentally in animals more than 30 years ago.

The familiar oxyhemoglobin dissociation curve of Figure 1 plots PO₂ on the abscissa and the oxygen content of blood (CO₂) on the ordinate. The top curve shows the normal oxyhemoglobin curve for 100 percent HBO₂ and the difference between the arterial and venous points shows that about a quarter of the oxygen is extracted from the blood at a normal cardiac output and oxygen uptake rate. The effect of CO is illustrated at 50 percent COHb, where the anemia-like effect reduces the arterial oxygen content by one half. This means that a normal O₂ extraction lowers venous O₂ content, and hence tissue PO₂ is considerably reduced relative to normal conditions (same blood flow and oxygen uptake rate).

The presence of tissue hypoxia clearly produces many direct cellular effects, but hypoxia also increases cellular CO uptake. This was first appreciated by Warburg when he was studying CO effects in yeast. Warburg discovered that he could relate the uptake of CO to a constant (Warburg constant) which is simply the fraction (n) bound to CO, divided by [1-n] times the ratio of gas partial pressures. Thus both uptake mechanisms, the hemoglobin binding mechanism and the cellular gas uptake mechanism, depend on the ratio of the partial pressures of CO to O₂.

When tissue hypoxia occurs during CO poisoning deviations from the effect of

Table 1. CO Interferes with Cell Function by Binding to Fe(II)

Hypoxia enhances CO uptake by heme proteins
 Guanylate cyclase
 myoglobin
 cytochrome *a, a₃*
 cytochrome P450
 Intracellular uptake of CO alters heme protein function and causes oxidative and nitrosative stress.
 Impaired heme protein function causes cell death; the mechanisms are complex and necrosis and apoptosis have been observed simultaneously.

simple hypoxia appear in part because the CO moving slowly into cells has inhibitory effects on cellular heme proteins such as myoglobin. CO's chemical stability means its main important biochemical effect is to bind to reduced transition metals. The body's most abundant transition metal, iron, is the main target and it binds CO only while in the ferrous (Fe²⁺)

state. Tissue hypoxia enhances CO uptake both by decreasing PO₂ relative to PCO and increasing the Fe²⁺ content of the cell. Thus, hypoxic conditions favor the binding of CO to heme proteins (6, 10). The heme proteins shown in Table 1 have been found to take up CO in living systems. In work done by Steven Brown in my laboratory about 12 years ago, cytochrome *a, a₃*-CO binding was shown to occur *in vivo* in the brain in the presence of a normal hemoglobin circulation (12). In principle, and as shown by experimental

measurement, intracellular CO alters heme protein function. In doing this, CO re-routes reducing equivalents (electrons) and creates oxidative and nitrosative stress (13-15), which will be discussed below.

The combined stress of hypoxia and too much intracellular CO leads to cell death, which derives from multiple factors, some of which are still unclear. In the brain, cell death of different types occurs at different times, and we have detected neuronal cell death of necrotic and apoptotic types (16). The precise cell death mechanisms are therefore complicated and not yet worked out at a molecular level, and they are a topic of an entirely separate discussion. It is sufficient to say that in brain and muscle, lower PO₂ will promote greater tissue uptake of CO *in vivo*, CO binding to heme proteins is observable, and on re-oxygenation, the presence of bound CO prolongs the period of energy deficit, increases the oxidative stress in the cell, and increases the probability of cell death (17).

One mechanism of oxidant production in CO poisoning is by the CO binding to cytochrome *a,a₃* in mitochondria, which not only interferes with respiration but increases the rate of reactive oxygen species generation during the re-oxygenation period because it reverses only slowly after the cell PO₂ is restored. Measurements in my laboratory in the 1990s showed that this effect was associated with an increase in mitochondrial hydrogen peroxide leakage and significant mitochondrial glutathione depletion (14).

An important implication of PO₂ dependence on tissue CO uptake has to do with how CO is distributed throughout the body tissues. Inhaled CO rapidly crosses the alveolar capillary membrane and enters the intravascular space where it binds primarily to hemoglobin. According to the Haldane relationship, most of the CO is bound to the red blood cell at steady state, but there is equilibration with tissues, mainly involving CO binding to myoglobin and heme protein enzymes. At equilibrium for the body, this settles out normally with about 80 percent of the body store of CO in the intravascular space and about 20 percent in the extravascular space. In addition, endogenous CO production a normal adult is about 12 milliliters per day. Some of this CO is bound to heme protein enzymes, some is oxygenated by mitochondria to CO₂ and the rest enters the blood and escapes from the lungs.

When the concentration of inspired CO increases, the apparent volume of distribution of CO compartments increases; there is more CO in the vascular space and more CO in the extravascular space (6). The body burden of CO thus increases, and the amount of CO in the tissue expands further as the PO₂ falls. The more that tissue PO₂ falls, the greater the CO burden will be in the extravascular compartment, particularly in heart and skeletal muscle. After CO poisoning, if O₂ is breathed, the PO₂ in the intravascular compartment increases first because it is easier to raise the blood PO₂ than it is to raise the tissue PO₂. Thus, the amount of CO in the vascular compartment (COHb) declines before the CO in the tissues dissipates.

It is under these circumstances that we often encounter our CO poisoned patients. They frequently present with little or no elevation of COHb level. If they've been breathing oxygen the COHb is low, yet the clinical examination is abnormal, and there is likely an appreciable body store of CO, although this still needs experimental confirmation. Thus, further oxygen breathing or hyperbaric oxygen therapy should in theory clear the tissues of CO, and the clinical status can be restored to normal.

At this point, a few more words about oxidant mechanisms are useful because it provides special insight into one of the mechanisms of injury. In particular, new injury mechanisms have been identified related to the relationships between CO, reactive nitrogen species (RNS), and reactive oxygen species (ROS). Again, a key to understanding these principles is by their relationship to molecular iron (Figure 2).

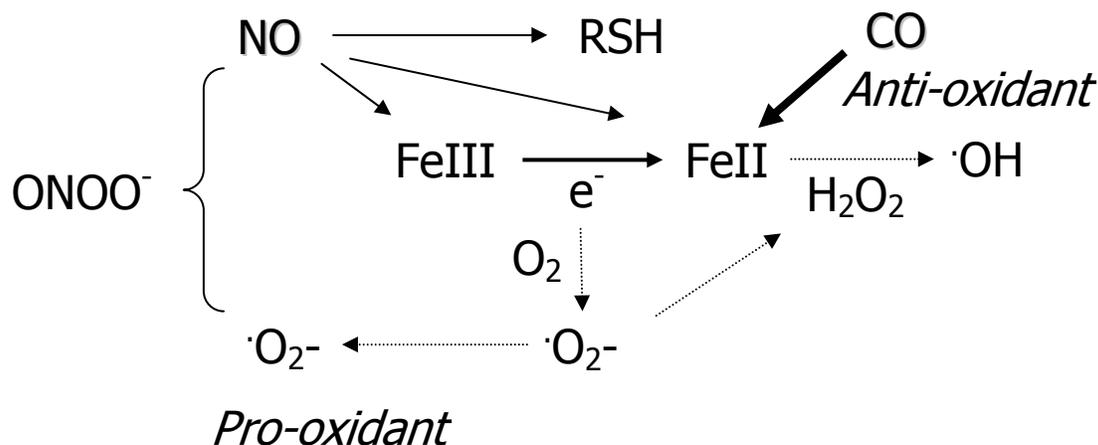


Fig. 2. Interactions between carbon monoxide (CO) and nitric oxide (NO) lead to changes in oxidative and nitrosative stress, which are based primarily on the redox state of iron in the cell.

In vivo, Fe^{2+} is generally well-sequestered; non-sequestered Fe^{2+} is harmful because it increases the generation of hydroxyl radical ($\cdot\text{OH}$) in the presence of hydrogen peroxide (Fenton reaction). Because CO binds only Fe^{2+} it been proposed by some to have an antioxidant effect by preventing free Fe^{2+} from participating in Fenton reactions. However, the body's mechanisms for sequestering Fe^{2+} are highly effective; this then is a hypothetical mechanism for which the evidence is not yet great. However, in the presence of nitric oxide (NO) the chemistry of CO changes because NO has possible alternative fates. First, NO is capable of binding either Fe^{2+} or Fe^{3+} . And though NO binds Fe^{2+} it is slowly displaced from the iron by CO. Thus, CO binding to Fe^{2+} supervenes over NO binding to Fe^{2+} . Second, reduced protein or peptide thiols are excellent NO-ligands (nitrosothiols, SNO), and these SNO compounds have both positive and negative effects on cell function. In situations where both CO and NO are present in the cell, what happens chemically depends to a great degree on the O_2 concentration. For example, re-oxygenation of a tissue after CO poisoning may allow electron transport systems that have been blocked to re-route electrons directly to O_2 forming superoxide anion, which then via enzymatic or spontaneous dismutation, leads to H_2O_2 production. Superoxide may also interact with NO to produce the very strong oxidant peroxynitrite (15). These are clearly pro-oxidant effects that damage constitutive cellular macromolecules. Furthermore, they may disrupt cell signaling processes in the brain that rely on endogenous CO production (18).

Clinical aspects of CO poisoning

The combined effects of cellular hypoxia, CO itself, and oxidative and nitrosative stress produce the pathology of CO poisoning and its clinical manifestations as well as the poor correlation between tissue damage and blood COHb. Normal individuals breathing clean air have COHb levels of 1 to 2% from endogenous CO production. If one lives in a large city like San Diego or Los Angeles, the COHb level is about 2%. If one rides on the freeway to work every day COHb may reach 3 to 5%. In smokers, for each pack of cigarettes consumed each day, the COHb level rises roughly 5%. However, in the absence of heart or lung disease, symptoms of CO poisoning do not generally appear until the COHb level reaches about 15%.

In severe CO poisoning, most patients have reached levels of more than 20% COHb; they have had significant tissue hypoxia and a significant increase in tissue CO burden. Therefore, the signs and symptoms of CO poisoning do not correlate with COHb level. They are nonspecific and variable. The most common symptoms are headache, nausea, vomiting, confusion, and flu-like illness. There are no reliable physical signs. It is notable that cherry red skin is rare. Also, in older adults, cardiac damage is common and may be overlooked easily. During the wintertime it has been estimated that 5 to 20% of emergency department patients with flu-like illnesses have occult CO poisoning. Thus, a high index of suspicion for the poisoning always should be maintained. The diagnosis is confirmed by Co-oximetry, performed equally well on an arterial sample or a venous sample of blood.

A major concern of treating victims of CO poisoning is the delayed neuropsychiatric syndrome (DNS). This interesting syndrome is characterized by a variable lucid interval followed by new neurological signs or symptoms that develop some days to weeks after acute poisoning. DNS is seen in 3 to 20% of acute CO poisoning victims, most often in older or more severely poisoned patients. Loss of consciousness has appeared as an independent risk factor for DNS in many of the reports in the literature. The long-term cognitive manifestations of the delayed syndrome can be very troubling and even disabling. Depression and memory loss are most common but dementia, Parkinson-like syndromes, seizures, and blindness have all been reported.

The prognosis of DNS patients has been hard to determine as will be discussed in the section on clinical trials. However, a few prognostic factors are clear. A poor outcome is predicted by advanced age, loss of consciousness, lengthy CO exposures, and metabolic acidosis. Independently, hypotension and cardiac arrest are poor prognostic factors and predict permanent disability and death. The long-term neurological effects of untreated CO poisoning are appreciable. The first to point this out were Smith and Brandon in 1973 (19). These authors, however, did not discriminate between the DNS and patients that had permanent sequelae from a severe initial poisoning. What is interesting about this series is that a significant number of patients, 13%, had gross neuropsychiatric abnormalities, about 30% had deterioration of personality and more than 40% had memory problems. This also was the first work to point out the relationship between loss of consciousness and persistent neurological sequelae.

Therapy of CO Poisoning

The mainstay of therapy for CO poisoning has traditionally been the administration of normbaric oxygen (NBO₂). The original rationale for HBO₂ was to

hasten elimination of COHb, and this rationale still holds today. But today the goals of HBO₂ therapy are more comprehensive because HBO₂ is intended to reverse the ongoing cellular energy deficit and prevent late cell death by a range of mechanisms; this has been the modern promise of HBO₂.

The potential benefits of HBO₂ are as follows: 1- eliminate COHb rapidly, 2- maintain adequate cerebral oxygen delivery, 3- eliminate CO from tissue heme proteins and restore their functions, e.g. improve energy metabolism, 4 - decrease cerebral edema, 5 - decrease leukocyte adherence, and 6 - decrease oxidative stress, e.g. interrupt lipid peroxidation and glutathione depletion. Most of these potential salutary effects of HBO₂ cannot be achieved with NBO₂.

The current UHMS treatment recommendations are simple and based on clinical empiricism. The UHMS has recommended HBO₂ for loss of consciousness or any other neuropsychiatric signs or symptoms (not headache alone) or evidence of cardiovascular compromise (20). Recommended treatment pressures have been between 2.4 and 3.0 ATA for 90 to 120 minutes. Residual neurological effects are treated for up to a maximum of 5 sessions, after which by peer review is recommended. The UHMS treatment guidelines are also practical because HBO₂ is relatively expensive, access to it is limited, and potential side effects such as O₂ toxicity have sometimes made it controversial. Since 1989 the question of efficacy of HBO₂ in acute CO poisoning has been addressed in six randomized control trials (RCT), which vary greatly in quality, cogency of study design, endpoint selection and outcomes.

The first RCT was that of Raphael *et al.* from Paris in 1989, who randomized 343 patients without loss of consciousness to receive either six hours in NBO₂ or two hours of HBO₂ at 2 ATA plus four hours of NBO₂ (21). In a second arm, 286 patients with loss of consciousness were randomized to one or two HBO₂ sessions at 2 atmospheres. Raphael *et al.* found no difference in outcome in either arm of the study. But they found very high residual neurological effects in all groups, 32 to 34% without loss of consciousness, which is similar to what Smith and Brandon reported in 1973, and 46 to 48% in patients with loss of consciousness (19). These high residual effect rates raised a number of criticisms including overly broad entry criteria, adequacy of the 2 ATA schedule for HBO₂, effect of treatment delays of up to 12 hours, and weak outcome measurements. But the study was a catalyst for better designed RCTs trials, which began appearing over the next few years.

The second RCT was a trial of 26 patients by Ducasse, also from France (22). Two-thirds of patients had loss of consciousness and surrogate outcome measurements were used. In other words no measurements of cognitive function were done; the investigators simply assessed symptoms, EEG and cerebral blood flow responses to acetazolamide. Ducasse reported significant positive effects of the HBO₂ treatment at three weeks but this result was not widely accepted. The work was published only as an abstract, it was a small study and the follow-up period was quite short. There was no blinding and the validity of the surrogate outcome measurements was questioned.

The third RCT of Thom *et al.* at Pennsylvania included 60 patients with moderate CO poisoning, excluding loss of consciousness and cardiac dysfunction (23). Thom *et al.* used oxygen at 2.8 atmospheres versus NBO₂ and performed follow-up evaluations with serial, neuropsychological tests. The study was stopped early due to detection of benefit

in the patients who received HBO₂ therapy. These results fit with the clinical wisdom of the time, and with results of case series of HBO₂ treatment of CO poisoning.

The next study by Scheinkestel *et al.* from Australia was highly controversial, and in a sense, opened Pandora's Box (24). Scheinkestel randomized 191 patients of different poisoning severities to receive either daily HBO₂ at 3 atmospheres for 60 minutes and then three to six days of high-flow NBO₂ or high-flow NBO₂ for three to six days. Outcome was assessed by neuropsychological testing after the treatment course and at one month after poisoning. No significant HBO₂ treatment effects were detected.

The problems of design and implementation of the Scheinkestel study are so serious as to call into question any "findings". These design flaws, however fatal, were initially overlooked by many clinicians, and the study was quoted as "evidence" that HBO₂ was not effective. In addition to patient enrollment problems, the O₂ doses did not meet clinical standards; the difference in O₂ dose between study arms was negligible and only 46% of the patients were followed-up. Although the study fails to provide clinically useful information it points out one of the problems of negative clinical trials; the trial can be negative for a host of the wrong reasons including poor design.

The fifth RCT, Daniel Matthieu's study in France, is still ongoing (25). At an interim analysis 575 patients had been randomized to a single HBO₂ treatment (2.5 ATA for 90 minutes) versus 12 hours of NBO₂. The patients are being followed serially for a year. At the three month follow-up there was a significant statistical effect of HBO₂ of about twofold with a strong p-value. The difference diminished at six months, a trend was present but without statistical significance. The trend was lost by one year, when outcome in both groups was the same. Matthieu has continued this trial to try to identify subgroups of patients that are most likely to benefit from HBO₂. The results of Matthieu raise some interesting points that will be covered below after discussing Weaver's study.

Weaver *et al.* from Salt Lake City have published a large RCT, where patients were stratified by age, exposure time, treatment delay, and history of loss of consciousness in which they detected a significant benefit of HBO₂ therapy (26). The Weaver study was double-blind, randomized and placebo controlled; patients were treated in a monoplace chamber three times at 6- to 12-hour intervals with HBO₂ or sea level O₂ (NBO₂). Weaver operated on an intention to enroll 200 patients; 152 were actually enrolled, with one-to-one randomization. The trial was interrupted at the third interim analysis because of a difference in favor of HBO₂.

The poisonings in Weaver's study patients were fairly severe, mean COHb of 25% and half of the patients had suffered loss of consciousness. The HBO₂ therapeutic advantage held up after adjusting for pretreatment differences, i.e. cerebellar dysfunction, and for stratification. In patients with complete follow-up data (94%), 24% of the HBO₂ group had cognitive sequelae compared to 43% of the NBO₂ group. It is worth noting again that 43% typifies the literature reports of residual effects in people who don't receive HBO₂; thus 24% is a significant decrease in cognitive sequelae.

The Weaver trial has a number of great strengths. The investigators preserved the double blind design, defined their endpoints *a priori*, and corrected the neuropsychiatric tests for age, gender, and education. The patients were treated as soon as possible after CO poisoning, the follow-up rate, 94% is exceptional, and the analysis was done by intention to treat.

Despite the excellence of the Weaver study and the positive results, there are still some unresolved treatment issues. These are put forward now (Table 2) for later consideration. In short, clinical research on CO poisoning still suffers from the lack of objective criteria or tests to identify high-risk patients or to predict risk of both delayed and permanent neurological sequelae.

Table 2. Unresolved Issues in Treatment of CO Poisoning

- 1) Are 3 HBO₂ treatments in 24 h necessary?
- 2) If 1 treatment is used, should the O₂ dose be greater than 2.5 ATA or longer than 90 min?
- 3) Should patients with milder CO poisoning receive HBO₂? If so, what criteria are appropriate?
- 4) Should patients be given HBO₂ more than 12 or 24 hours after poisoning?
- 5) Are O₂ toxicity and other side effects of HBO₂ significantly greater with multiple treatments?
- 6) How should cost/benefit of multiple treatments be assessed?

This problem has three parts. First, at the basic level, no one yet understands the exact mechanisms of cell death or the etiology of the delayed neurological syndrome. Second, no one yet knows the optimal dose of HBO₂, for example, number of treatments or best treatment pressure. Third, no one knows the time after which HBO₂ is no longer effective. Most of the trials have treated as soon as possible based on the six-hour window of opportunity proposed in Goulon's 1969 retrospective study (27).

What follows is a synopsis of clinical issues that have arisen primarily since Weaver's study data became available (28): 1) Are three HBO treatments in 24 hours necessary? Most of the benefit in the Weaver study was found after the first treatment. 2) If one or more treatments are used, must the oxygen dose be greater than 2.5 ATA or longer than 90 minutes? This point is specifically in reference to our practice at Duke in which our treatment outcomes were good before the results of Weaver were published. 3) Should patients with mild CO poisoning receive HBO₂ and if so, what treatment selection criteria should be used? 4) What treatment should be given to patients who are not selected for HBO₂? 5) Should patients be given HBO₂ more than 12 to 24 hours after the discovery of the poisoning and is the cost-benefit of HBO₂ reasonable after such delays? This issue becomes a notable problem when a patient has to be transported a long distance. 6) Is the cost-benefit of multiple HBO₂ treatments worthwhile? In other words are side effects of multiple HBO₂ sessions like O₂ toxicity important problems? These are questions that need to be addressed and may require one or more future randomized control trials.

A validated definition of "severity of poisoning" has been lacking, which if defined, certainly could be incorporated usefully into a future study design. Also, treatment protocols that are implemented should be clinically reasonable and commonly available; one could logically argue three treatments in 24 hours as clinically unnecessary for the majority of CO poisoned patients.

The nature and timing of exit evaluations are important considerations that need to be defined *a priori* because lack of appropriate long-term follow-up has been a limiting problem in a number of studies. A strategy being discussed for a multi-center RCT of HBO₂ among investigators at several large centers is one that would consider stratification by a valid definition of severity of poisoning, (e.g. high versus low risk), randomization of the patients to either one, two, or three treatments; stratification by

treatment delay, (e.g. less than or equal to 6, 6 to 12 or 12 to 24 hours), and rigorous follow up at multiple time points, including a one-year analysis.

A summary of this discussion can be made in three fairly straightforward points: First, the basic science studies of CO poisoning demonstrate multiple toxicity mechanisms involving the brain. This is by no means a simple problem but fortunately many of the toxicity mechanisms appear to be amenable to timely HBO₂; this conclusion is based both on a sound biochemical rationale and on rigorous experimental data. Second, well designed clinical trials now strongly support the use of HBO₂ therapy in selected patients. Third, there are significant unresolved treatment issues, including how to identify patients at high risk for DNS, determining the optimal number of treatments, and defining the effect of treatment delay on the patient's clinical outcome.

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