Acetazolamide and CO₂ in hyperbaric oxygen toxicity

C. D. WOOD

Department of Pharmacology and Therapeutics, Louisiana State University School of Medicine, Shreveport, LA 71130

Wood CD. Acetazolamide and CO₂ in hyperbaric oxygen toxicity. Undersea Biomed Res; 1982; 9(1):15–20.—The role of CO₂ in hyperbaric oxygen toxicity was investigated by administering acetazolamide (Diamox®), Tris buffer [trishydroxymethyl]aminomethane], and sodium bicarbonate by i.p. injection, and by exposure of other groups of animals to an atmosphere of 5% CO₂ and 95% O₂. All animals were placed in a pressure chamber and maintained at 50 psig in 100% O₂ until death. The Tris buffer and the sodium bicarbonate buffer significantly extended time to onset of convulsions and to time of death. Acetazolamide and also 5% CO₂ shortened time to onset of convulsions and significantly shortened survival time. These results suggest that increased tissue levels of CO₂ play an important role in hyperbaric oxygen toxicity. The cause of death in our animals exposed to hyperbaric oxygen was pulmonary edema secondary to a systemic hypertension.

The toxicity of high levels of oxygen at both ambient and hyperbaric pressures is well established; however, the contribution of carbon dioxide in hyperbaric oxygen toxicity is at present still in question. Investigators such as Gesell (1), Bean (2), and Hill (3) proposed a major role for CO₂ in the toxicity of hyperbaric oxygen (HBO), while others such as Clark and Lambertsen (4) have relegated it to a minor position. The reactions collectively referred to as oxygen toxicity actually involve two separate mechanisms. Prolonged exposure to O₂, from 70% to 100% at pressures of 1 to 3 ATA, produces primarily pulmonary damage. Pressures of 100% oxygen at 3 ATA and higher produces central nervous reactions that include convulsions and a centrally activated hypertension in animals, resulting in pulmonary edema and death (5). Failure to clearly distinguish between the two separate reactions has produced some confusion in the literature concerning oxygen toxicity.

The central nervous system (CNS) is exquisitely protected from alterations in the systemic environment. Many substances are excluded from the CNS by this protection; however, other substances readily pass—such as O₂, CO₂, and many centrally active drugs including acetazolamide. In an attempt to determine the relative contribution of CO₂ and alterations of pH in hyperbaric oxygen toxicity, the drug acetazolamide, increased CO₂, and the buffers tris-(hydroxymethyl)aminomethane and sodium bicarbonate were used in this project.
METHODS

The experimental animals were male Sprague-Dawley rats ranging in weight from 200 to 300 g. The animals were placed singly in a 4-liter pressure chamber and the chamber was flushed with 100% oxygen before the pressure was increased to 50 psig (4.4 ATA, equivalent to 112 fsw). A flow of 100 cm³/min of oxygen was maintained through the chamber to prevent accumulation of CO₂. Levels of CO₂ and O₂ were monitored by Fyrite (Bacharach Instrument Co., Pittsburgh, PA) CO₂/O₂ indicators. Chamber temperature ranged from 70° to 75°F. A Plexiglas observation port permitted continuous observation of the animals and recording of the time of onset of seizures. The time of death was recorded at cessation of all respiratory movements. All medications were given by intraperitoneal (i.p.) injection. Acetazolamide was given in a 30-mg/kg dose 3 h prior to exposure to achieve peak effect. Tris-(hydroxymethyl)aminomethane was given as a 540-mg/kg dose with a 30-min absorption period. A 30-min absorption period was also allowed for animals receiving sodium bicarbonate, 6 meq/kg. The final experimental group was exposed to 5% CO₂ and 95% O₂ in the pressure chamber. A control group of 30 animals was exposed to 50 psig of 100% oxygen under the same conditions as the experimental groups.

RESULTS

The control groups had a mean time of onset of convulsions of 108 min when exposed to 100% O₂ at 50 psig. The groups injected with acetazolamide had a mean time to start of convulsions of 70 min. The group exposed to 5% CO₂ in 95% O₂ had a mean time to convulsions of 88 min. A statistically significant extension of time to convulsions was produced by Tris buffer with a mean of 140 min and by sodium bicarbonate with a mean time of 154 min. All animals died with frothy fluid filling the lungs and respiratory passages and draining from the nose and mouth. The mean time of death for the control groups was 263 min. The time of death was statistically significantly shortened with acetazolamide with a mean of 200 min, and in the animals exposed to 5% CO₂ in 95% O₂ with a mean death time of 193 min. The animals treated with Tris buffer had a mean death time of 343 min, and those treated with sodium bicarbonate a mean death time of 321 min. The extensions of survival time in 100% O₂ at 50 psig are both statistically significant. These results are summarized in Table 1 for convulsion times and in Table 2 for the survival times.

### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>No. of Animals</th>
<th>Minutes</th>
<th>SD</th>
<th>Range</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>108</td>
<td>28.61</td>
<td>(72–165)</td>
<td>—</td>
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<tr>
<td>Acetazolamide</td>
<td>30</td>
<td>70</td>
<td>24.83</td>
<td>(40–157)</td>
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<td>5% Carbon dioxide</td>
<td>10</td>
<td>88</td>
<td>14.25</td>
<td>(70–113)</td>
<td>0.02</td>
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<tr>
<td>Tris buffer</td>
<td>10</td>
<td>140</td>
<td>28.81</td>
<td>(105–175)</td>
<td>0.002</td>
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<tr>
<td>Sodium bicarbonate</td>
<td>10</td>
<td>154</td>
<td>54.35</td>
<td>(100–195)</td>
<td>0.008</td>
</tr>
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</table>

*Significance is probability in unpaired t test of 0.05 or less.
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TABLE 2

<table>
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<tr>
<th>Condition</th>
<th>No. of Animals</th>
<th>Minutes</th>
<th>SD</th>
<th>Range</th>
<th>(P^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>263</td>
<td>80.68</td>
<td>(135–432)</td>
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<tr>
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<td>200</td>
<td>52.56</td>
<td>(85–275)</td>
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<td>5% Carbon dioxide</td>
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<td>75.17</td>
<td>(135–390)</td>
<td>0.02</td>
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<tr>
<td>Tris buffer</td>
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<td>343</td>
<td>74.22</td>
<td>(240–440)</td>
<td>0.004</td>
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<tr>
<td>Sodium bicarbonate</td>
<td>10</td>
<td>321</td>
<td>87.84</td>
<td>(190–435)</td>
<td>0.029</td>
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</tbody>
</table>

\*Significance is probability in unpaired \(t\) test of 0.05 or less.

DISCUSSION

Two distinct mechanisms produce what is collectively known as oxygen toxicity. At partial pressures of O\(_2\) below 3 ATA the effect is usually pulmonary with replacement of type I pneumocytes with type II, disruption of the capillary endothelium, and leakage of blood proteins and fluids along with leukocytes into the interstitial spaces (4). Death is due to pulmonary insufficiency (5). In contrast, exposure to 100\% O\(_2\) at 3 ATA and higher (HBO) produces a central nervous system response that includes convulsions, coma, and a centrally activated systemic hypertension that causes increased pulmonary venous pressure and has resulted in pulmonary edema in animals (6). The mechanism of the pulmonary damage with 1 ATA pressure of O\(_2\) appears to be a direct chemical destruction of the integrity of the cells of capillary and alveolar membrane, allowing loss of proteins, fluids, and leukocytes. The pulmonary damage from exposure to HBO appears to be secondary to the hypertension that forces fluids and later intact erythrocytes through the alveolar and capillary membranes, causing the animals to die with frothy edema fluid running from their respiratory passages. In a previous study hypertension was routinely observed with HBO but never seen with exposure to 1 ATA pressures; rather, a decline in blood pressures to shock levels was observed (6). In a later study survival time in HBO appeared too brief for direct toxic effects of oxygen on lung tissues to be a contributing factor (5).

The role of excess O\(_2\) on the biochemical processes of the neurons of the central nervous system leading to the production of seizure activity has been the object of extensive research. Various mechanisms resulting in convulsions have been proposed, including inhibition of adenosine triphosphate (ATP) formation due to oxidation of pyridine nucleotides; production of H\(_2\)O\(_2\), superoxide anions, and hydroxyl radicals; lipid peroxide formation; oxidation of enzyme sulfhydryls; and alterations of glutathione/oxidized glutathione (GSH/GSSG) redox ratios (7). Reduction of glucose and O\(_2\) utilization in tissue has also been observed during hyperbaric O\(_2\) exposure (8). It has recently been proposed that increased ammonia production from more rapid degradation of catecholamines in the brain during exposure to HBO may produce seizures (9) and that the glutamate system appears sufficient to buffer this increased NH\(_3\) production for as much as one hour (10). Faiman et al. (7) reported that none of the current biochemical theories proposed to explain oxygen convulsions are supported by their in vivo experiments and that a new hypothesis is needed. This is in agreement with Davies and Davies (11), who state, "It has not been possible to discover any effect on any isolated enzyme system which occurs with sufficient rapidity to explain the time of onset of the convulsive seizures associated with oxygen poisoning in intact animals."

The possible role of CO\(_2\) in the production of convulsions resulting from exposure to 100\% O\(_2\) at 3 ATA and higher has been extensively investigated and discussed. Under conditions of
HBO the transport of CO₂ from the tissues is decreased. At oxygen pressures of 3 ATA, 7 volume percent of oxygen is physically dissolved in the plasma. This is a sufficient amount to supply the tissues, and the oxyhemoglobin is not reduced by releasing its load of oxygen. Reduced hemoglobin is then not available to buffer the hydrogen ions of carbonic acid, and a decreased amount of CO₂ is transported as the carbamino compound. Under normal conditions CO₂ is transported from the tissues as 7% physically dissolved, 23% in combination with hemoglobin, and 70% in the bicarbonate buffer system. Gesell (1) and later Bean (2) proposed that this impaired CO₂ transportation produced an increase of tissue CO₂ and H⁺ ions that was sufficient to produce convulsions. Clark and Lambertsen (3) felt that blood CO₂ levels in their investigations were too low to support such a position. However, Eisele (12) reported that increases in CO₂ resulting in a cerebral spinal fluid pH of 7.1 or less produced narcosis from the H⁺ ion effect secondary to the increased CO₂ of the cerebrospinal fluids (CSF), and that the pH of the cerebrospinal fluids (CSF) is independent of arterial pH levels due to the protective action of the blood-brain barrier. The intracellular environment of the cerebral neurons has a dual protection—the cell membrane and the blood-brain barrier. Ionized substances such as H⁺ and HCO₃⁻ are blocked, but CO₂ diffuses readily across both structures. The CSF is said to act as a weak solution of bicarbonate (its only buffer system) with a low CO₂ buffering power. The intracellular pH is quickly altered by respiratory changes due to the diffusibility of CO₂; systemic metabolic changes, however, produce little effect because of the protection of the blood-brain barrier against ionized substances (13). The CSF maintains a pH of 0.1 below arterial levels and a CO₂ content 6 to 7 mmHg higher (14). With a CSF pH of 7.4 the intracellular pH is reported to be below 7.0 in cerebral neurons (13). A 25% increase in cerebral metabolic rate is reported to result when CO₂ increases from 40 to 80 mmHg. At a level of 200–245 mmHg and an intracellular pH of 6.7, narcosis results and the cerebral metabolic rate decreases (15). Woodbury (16) reported a progression of effects with increased cerebral CO₂. At a level of 150 mmHg excitement is produced, which results in convulsions, and narcosis results at levels of 200 mmHg. The effects of lower pH due to hypercapnia include disturbed membrane ion transport, interference with glucose utilization, decreases in intracellular amino acids, and increased NH₃ formation in the cerebral neurons (13).

Breathing of increased levels (150–200 mmHg) of CO₂ in air at normal pressures will produce most of the symptoms of HBO toxicity, including excitement, convulsions, sympathetic activation producing centrally activated hypertension, and finally narcosis and death (13, 16). Increased production of CO₂ in the body shortens the time of onset of symptoms of oxygen toxicity produced by HBO. This has been demonstrated by several experimental techniques including hyperthermia, exercise in HBO (17), increasing metabolism by thyroid injections (2), and breathing of higher levels of CO₂ in oxygen (2). Procedures that lower cerebral metabolism (and thereby CO₂ production), such as hypothermia, anesthesia, and various sedative drugs, delay the onset of HBO symptoms (18). Therefore, alterations of intrinsic and extrinsic CO₂ appear to have a marked effect on onset of convulsions and survival time in HBO.

Carbon dioxide is about 20 times as soluble in plasma as oxygen and is 50% more soluble in lipid than in plasma (19). As an inert gas it has a large potential reservoir in the body; however, it is also a biologically active gas and combines with the buffers and enters into the cell systems. In breath-hold dives to 99 ft (3.7 ATA) the tensions of oxygen, nitrogen, and carbon dioxide increase fourfold, producing a gradient from the lungs into the tissues (20). During ascent CO₂ is slowly released from these storage sites. A similar tissue CO₂ buildup would be expected to occur in HBO because of blockage of CO₂ transport in hemoglobin and the increased pressure, and this may contribute to the central nervous system response to exposure to hyperbaric oxygen.
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In our research we attempted to alter the bicarbonate buffer system in our animals, as 70% of the CO₂ is usually transported from the tissue by this route. Bean (18) demonstrated that Tris buffer has a protective effect in HBO and extends the time of onset of seizures and also survival time. Tris buffer is 30% un-ionized in the body, and it is suggested that this portion can cross the cell membranes and act as an intracellular H⁺ ion acceptor. We also observed a delay in convulsive activity and an extension of survival time with Tris buffer and also with sodium bicarbonate. The buffering of the increased tissue CO₂ produced by exposure to HBO appears to be an effective method of achieving a significant extension of time to convulsions and death.

The most interesting and significant results in this project were produced by acetazolamide. It is thought that CO₂ is hydrated by the action of carbonic anhydrase to form HCO₃⁻ and H⁺ throughout the brain. Acetazolamide blocks this reaction in the brain and thereby contributes to an increase of tissue CO₂. Acetazolamide is used as an anticonvulsant and acts by lowering cerebral pH; however, in HBO where CO₂ is retained it accelerates the onset of seizures. The diuretic activity of this drug might be expected to offset the hypertension produced in HBO. It decreased survival time in this situation, however, where high cerebral CO₂ caused a central sympathetic activation, producing increased blood pressure and resulting in pulmonary edema. Our results indicate that acetazolamide is one of the most effective methods for shortening time of onset of seizures in HBO and that accumulation of CO₂ and not just increased H⁺ contributes to the seizure activity.

The results of our research are essentially in agreement with Gesell (1), Bean (2), and Hill (3), in that they indicate that increased tissue levels of CO₂ play a prominent if not a predominant role in the toxicity seen in HBO (5–6). Any marked decrease in intracellular pH would produce chaos in cellular enzyme systems, which are highly pH specific (21). However, all of the current biochemical theories advanced to explain HBO seizures suggest mechanisms that occur at too slow a rate to explain the convulsions (7–11). The CNS neurons metabolize glucose as an obligatory energy source and have a very limited anaerobic metabolic capacity. Seizures can rapidly be produced by hypoxia such as that associated with sublethal cyanide injection in cancer research (22) or with insulin injections that deprive the brain of glucose. A high level of CO₂ retained in the tissues could block the metabolic activity either by producing an intracellular pH lower than 6.7 or by excess CO₂ in the cell. The law of mass action states that as the end products of a chemical reaction build up in the reacting medium, the rate of the reaction approaches zero. It has also been demonstrated that glucose and O₂ utilization decreased during exposure to HBO (8, 11). Therefore there is ample supporting evidence for the role of increased tissue levels of CO₂ in the production of seizures and death in HBO. Alteration of the bicarbonate buffer system altered the time of onset of seizures in HBO. Acetazolamide, which blocks entry of CO₂ into this buffer system at tissue level, markedly reduced time of seizure onset and survival time, indicating that CO₂ levels, as well as the associated increase in hydrogen ion levels, contribute to this reaction. The influence of increased levels of CO₂ in the pressure chamber demonstrates that this mechanism can occur rapidly enough to account for the seizures in HBO. The results of our research indicate that increases of tissue CO₂, possibly in conjunction with direct effects of O₂, is an important part of the seizure mechanism in hyperbaric oxygen.

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C. D. WOOD

Istrant par voie i. p., de l'acétazolamide (Diamox®), du tampon Tris [tris(hydroxymethyl)amino-méthane], du bicarbonate de sodium, et en exposant d'autres groupes d'animaux à un mélange de 5% CO₂-95% O₂. Tous les animaux étaient placés dans un caisson hyperbare et maintenus à 50 psi relatifs (3.4 bar) dans 100% O₂ jusqu'à la mort. Les tampons Tris et le tampon bicarbonate de sodium allongeaient de façon significative la latence des convulsions et la durée de survie. L'acétazolamide et aussi le CO₂ à 5% diminuaient la latence des convulsions et diminuaient de façon significative la durée de survie. Ces résultats suggèrent qu'une concentration tissulaire de CO₂ élevée joue un rôle important dans la toxicité de l'oxygène hyperbare. La cause de la mort de nos animaux exposés à l'oxygène hyperbare était un œdème pulmonaire secondaire à une hypertension systémique.

toxicité de l'oxygène hyperbare

dioxyde de carbone

acétazolamide

convulsions hyperoxiques

REFERENCES