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Treatment of Hydrogen Cyanide and Hydrogen Chloride Inhalation in Mice with Hyperbaric Oxygen Therapy versus Supplemental Oxygen

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Running Title: HBO Treatment for HCl/HCN Inhalation
ABSTRACT

Hyperbaric oxygen therapy promotes faster tissue healing by increasing the amount of oxygen carried to the tissues. HBO is currently used to treat carbon monoxide poisoning as well as smoke inhalation. Based on this information, it was believed that HBO would also promote faster healing for hydrogen cyanide gas or hydrogen chloride gas inhalation, which are encountered in burning material. To test this theory, mice were exposed to HCN or HCl gas. The mice were then placed in hyperbaric oxygen or supplemental oxygen treatments for five days. Weight change and overall survival rate were used to establish what method of treatment was ideal. It was determined that mice treated with supplemental oxygen healed as quickly as those treated with hyperbaric oxygen. Therefore, in the case of hydrogen cyanide or hydrogen chloride inhalation, supplemental oxygen is the better treatment.

INTRODUCTION

Hyperbaric Oxygen Therapy (HBO) is a medical treatment using oxygen administered at greater than normal pressure to a patient in order to treat specific medical indications (10). Pressurized air has been used to treat medical conditions since 1662, but the first hyperbaric chamber was not built until 1834 (10). In the last decade the number of hyperbaric chambers in the U.S. has risen to 510 from 383 in 1993 (4). HBO treatment is used to treat medical problems including decompression sickness, arterial gas embolism, and severe carbon monoxide poisoning. It is used to improve skin graft and flap healing as well as clostridial myonecrosis (5). Once it was used in conjunction with nitrite to treat potassium cyanide poisoning (1).

HBO is beneficial in recovery because it increases the amount of oxygen reaching the tissues. Under normal conditions, atmospheric pressure is 760 mm Hg (1 atm, 14.7 psi). At this pressure, the average adult consumes six lbs of oxygen/day with about two lbs entering the blood
for transport to tissue cells. This oxygen in the blood is called oxygen tension or partial pressure (pO2) and normally measures 80-100 mm Hg (7). However, by the time oxygen reaches the tissues, pressure has dropped to 39 mm Hg. The increased pressure environment of the hyperbaric chamber doubles the number of oxygen molecules consumed. The red blood cells instantly fill with oxygen and the extra oxygen dissolves directly into the blood fluid. This extra oxygen in the plasma builds up tissue oxygen levels increasing the tissue pO2 to 50 – 80 mm Hg, which is the optimal level for healing.

It is well known that HBO therapy is used to treat carbon monoxide poisoning and smoke inhalation. Carbon monoxide binds to hemoglobin with an affinity 240 times that of oxygen, so the individual dies from anoxia, loss of oxygen (5). Hyperbaric oxygen provides an alternative source of tissue oxygenation through oxygen dissolved in the plasma. In this situation the theoretical benefits of hyperbaric oxygen include a faster reduction in carboxyhemoglobin levels, increased intracellular delivery of oxygen, and reduced neutrophil activation and adherence, thereby reducing lipid peroxidation (9).

The three primary processes that cause inhalation injury due to a fire include: thermal injury, inhalation of asphyxiants, and toxin-induced cellular damage (2). In a typical house fire, five main toxic gases are produced: carbon dioxide, carbon monoxide, hydrogen chloride, hydrogen cyanide, and phosgene (3). Since HBO therapy is known to be beneficial for carbon monoxide poisoning and smoke inhalation, I was curious about its effects on the other gases. Carbon dioxide is a product of free burning so it is found in all fires. Its IDLH level, immediately dangerous to life and health, is 40,000 ppm, which rates it as a relatively safe gas compared to the others and therefore inconsequential (3). Phosgene is extremely lethal with an IDLH level of 2 ppm (3). Due to its danger and almost immediate health risk this gas was not
included in the experiment. This left HCl gas and HCN gas. Both gases are colorless, have a bad odor, and have a low IDLH rating (6). HCl causes respiratory problems because it adheres to mucous membranes and forms corrosive acids and alkalis that results in the death of mucosal cells, ulceration and further edema (2,8). Hydrogen cyanide inhibits the final step of oxidative phosphorylation by binding the cytochrome aa3 complex and halting mitochondrial aerobic metabolism. This results in lactic acidosis and cellular asphyxia, despite adequate arterial blood oxygen content (2,8).

The purpose of this experiment was to determine if HBO therapy increased the recovery rate and improved the overall survival chances for the victim after exposure to HCl or HCN gases.

MATERIALS AND METHODS

Mice Population

Fifty-three young adult male mice were used for this experiment. They were kept in six cages (15” x 18”) at temperatures around 30°C. Bedding, food, and water were changed three times per week. Bedding consisted of wood shavings and food was Natural Harvest™ dry dog food.

Smoke Production

The smoke chamber consisted of an aluminum can with a 3” x 2” door in the side to insert the fuel load. This can was topped with a funnel. The can and funnel were placed atop a propane burning camp stove, which served as the ignition source. A 3 ft clear plastic hose was connected to the funnel. The hose passed through a cold water bath and attached to the side of a
7" x 5" x 5" plastic dish with a lid. A thermometer and an Ohmeda 5120 oxygen monitor were inserted through parts drilled in the chamber (See Fig 1).

To produce hydrogen chloride gas, samples of polyvinyl chloride (PVC) piping were burned. The PVC piping was 3/4" in diameter and was cut into 1" sections. Approximately ten pieces of piping were burned for each trial. To produce hydrogen cyanide gas, polyurethane foam was burned. The foam was ripped into 4" x 3" pieces with four pieces burned for each trial (See Table 1). Smoke production was standardized by using pre-measured materials and observing oxygen concentration.

Hyperbaric Chamber

The hyperbaric chamber was made out of a 12" x 22" x 8" pressure cooker. Two 4" holes were cut out of the lid. Lexan glass was placed in these holes and sealed tight. A hose connected the HBO chamber to an oxygen flow meter, which inserted into the lid of a 7" x 5" x 5" plastic dish. This second chamber served as the site for treatment with high oxygen levels but without the pressure. Oxygen levels were monitored by an Ohmeda 5120 oxygen monitor (See Fig 2).

Smoke Exposure

Four mice at a time were exposed to the smoke. One or two mice went to each treatment area (See Fig 3). This was repeated three times. Before exposure they were weighed. Exposure time for hydrogen chloride was ~ 4 min with oxygen saturation reaching 15%. Exposure time for hydrogen cyanide was ~ 1 min with oxygen saturation reaching 17%.

Recovery

For each exposure: four mice received treatment with hyperbaric oxygen, four mice received supplemental oxygen (varying from 80-95% oxygen saturation), and four mice received
no treatment at all. The mice were placed in their respective treatment areas after being weighed and no more than 15 min after exposure to the gas. Mice placed in the hyperbaric chamber were treated for 30 min at 22 psi. They were brought to this pressure at a rate of 2 psi per minute. Mice treated with supplemental oxygen received oxygen for a total of 20-30 min. Mice receiving no treatment were placed directly into their cages. Treatment was administered once a day for five days. The mice were weighed each day before and after their respective treatment. For two to four days after completion of treatment, the mice were also weighed.

Dissection

Upon completion of the experiment the mice were dissected and their lungs were removed. Six mice from each gas exposure were chosen. Two mice were from HBO treatment, two were from supplemental oxygen treatment, and two had received no treatment. The lung samples were sent to Dr. Daniel Wunderlich, MD at Mid Michigan Medical Center, Clare, MI where microscope slides were prepared.

RESULTS

Mice exposed to hydrogen chloride tended to drop weight after exposure to smoke. Four out of the twenty-four mice did gain approximately 0.1 – 0.2 g, but the majority lost between 0.1 – 0.8g of weight. The average weight loss was 0.16 g (See Fig 4). Mice exposed to hydrogen cyanide also dropped in weight after their exposure. The average amount of weight lost was 0.63 g (See Fig 4). Weight loss ranged from 0.2 – 1.02 g.

Mice exposed to hydrogen chloride showed a drastic (5 g -7 g) weight loss for the first several days. Mice treated with supplemental oxygen gained a little bit of weight on the fifth day. For all three groups, a consistent increase in weight did not begin until the 7th or 8th day.
For all five days of treatment, each mouse weighed slightly less after treatment than before being treated. (See Fig 5) Mice exposed to hydrogen cyanide had weight gain/loss of about 2.0 g for the first five days. At this time mice treated with hyperbaric oxygen showed a dramatic weight gain/ weight loss pattern. While being treated, weight after treatment was slightly less than before treatment (See Fig 6).

Mice that had not been involved in the experiment were weighed to determine their typical weight pattern. The average weight range for these mice varied between 27.03 g and 28.51 g. During their observation these control mice gained weight, rather than losing weight.

Survival rate for mice exposed to HCl was as follows: survival rate for mice treated with supplemental oxygen was 88% (See Fig 7). Those treated with hyperbaric oxygen only had a survival rate of 75%, while those with no treatment at all had only a 62% chance for survival. Survival rate for mice exposed to hydrogen cyanide was much better. Mice receiving treatment with either supplemental oxygen or hyperbaric oxygen had a survival rate of 100%. However mice that did not receive any treatment had only a 50% chance of surviving.

Slides of the mice’s lungs — taken about ten days after initial exposure to smoke— showed little effects of the original gas. Some had slight areas where hemorrhaging occurred. Mice treated with HBO had slightly more hemorrhaging than those treated with supplemental oxygen (See Fig 8).

DISCUSSION

The exposure to hydrogen chloride caused a dramatic weight loss in a short period of time (See Fig 4). A possible reason for this could be a reaction to stress from handling, difficulty breathing, or due to increased heart rate and amount of energy expended during exposure. Mice
exposed to hydrogen chloride had eyes that were either swollen shut or covered with a white film. This is because the hydrogen chloride reacts with the secretions in the eye. The mice also had difficulty breathing, because hydrogen chloride adheres to mucous membranes causing ulceration. The weight loss was a result of the mice’s reaction to the effects of the gas. After several days the gas was completely out of the system and the mice gradually regained their weight (See Fig 5).

Treatment with oxygen seemed to greatly help the recovery time of the mice. Physical appearance of eyes and breathing returned to normal within a couple of hours to a couple of days. Those mice that did not receive oxygen as treatment had a longer recovery time and some even permanently lost their eyesight. The increased hemorrhaging in the lungs of mice treated with HBO could be a result of too much pressure, but according to Leach if pressures do not exceed 300kPa and the length of treatment is less than 120 min, hyperbaric oxygen therapy is safe (5).

Initial weight loss for mice exposed to hydrogen cyanide had an even more dramatic weight loss than those exposed to hydrogen chloride (See Fig 4). The same belief, that weight loss is a result of stress, applies. The reason for the greater weight loss may be that mice exposed to hydrogen cyanide were under even greater stress than those exposed to hydrogen chloride, because while exposure time was less, toxicity of cyanide is greater and therefore more disturbing.

While the initial weight loss (0.6 g vs. 0.15 g) immediately after HCN exposure was more obvious, overall weight loss was steadier than for those exposed to HCl (See Fig 6). This may be because the initial effects of cyanide are more drastic but can be corrected faster by the body. Treatment with oxygen seems to have little effect on the weight change. However, after
treatment with hyperbaric oxygen was suspended, a large fluctuation in weight occurred. This could be a side effect of the HBO. The 100% survival rate for mice receiving treatment indicates that treatment with oxygen is a necessity. Lung samples of these mice are similar to those with hydrogen chloride poisoning. The mice treated with hyperbaric oxygen again seemed to have more hemorrhaging than those treated just with oxygen.

In both cases, treatment with supplemental oxygen of some form is ideal. It offers a better chance of survival and a faster recovery. Hyperbaric oxygen can be beneficial in some instances, such as faster healing of the eyes in HCl exposure, but it generally seems to cause more problems than benefits. Since hyperbaric oxygen is expensive, and faster recovery does not seem to be conclusive, it is not a recommended treatment for inhalation of HCl or HCN gas.

ACKNOWLEDGEMENTS

I would like to thank my father, Russell Rader, for helping design and build the hyperbaric chamber and the smoke chamber. I would also like to thank Dr. Daniel Wunderlich and his lab assistants for preparing the microscopic slides for me.
LITERATURE CITED


Table 1: Toxic Atmospheres Associated with Fire (3).

<table>
<thead>
<tr>
<th>Gas</th>
<th>IDLH (ppm)</th>
<th>Caused by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon Dioxide (CO₂)</td>
<td>40,000</td>
<td>Free-burning</td>
</tr>
<tr>
<td>Carbon Monoxide (CO)</td>
<td>1200</td>
<td>Incomplete combustion</td>
</tr>
<tr>
<td>Hydrogen Chloride (HCl)</td>
<td>50</td>
<td>Burning plastics (e.g. PVC)</td>
</tr>
<tr>
<td>Hydrogen Cyanide (HCN)</td>
<td>50</td>
<td>Burning of wool, nylon, polyurethane, foam, rubber, &amp; paper</td>
</tr>
<tr>
<td>Phosgene (COCl₂)</td>
<td>2</td>
<td>When refrigerants (Freon) contact flame</td>
</tr>
</tbody>
</table>

Fig 1: Smoke Chamber: (a) 12 oz aluminum can where fuel load was placed, (b) hose connecting to smoke chamber, (c) cold water bath, (d) smoke chamber.
Fig 2: Hyperbaric Chamber and Supplemental Oxygen Chamber: (a) oxygen tank, (b) hyperbaric oxygen chamber, (c) hose connecting HBO chamber to supplemental chamber, (d) oxygen flow monitor, (e) supplemental oxygen chamber.

Fig 3: Flowchart depicting the distribution of mice exposed to HCl to their relative treatment areas.
Fig 4: Weight of mice before and after exposure to gas.

Fig 5: Mean weight of mice exposed to HCl.
Fig 8: Light micrographs of lung tissue removed from mice: a) lung biopsy of normal mice not exposed to smoke showing well-defined alveolar spaces, b) lung biopsy of mice exposed to HCl receiving no treatment showing inflammation and hemorrhaging, c) lung biopsy of mice exposed to HCl receiving hyperbaric oxygen treatment showing inflammation and hemorrhaging, d) lung biopsy of mice exposed to HCl receiving supplemental oxygen showing little hemorrhaging.
Southern Scholars Senior Project

Name: Renée Rader  Date: 1-9-02  Major: Biology

Senior Project

A significant scholarly project, involving research, writing, or special performance, appropriate to the major in question, is ordinarily completed the senior year. The project is expected to be of sufficiently high quality to warrant a grade of A and to justify public presentation.

Under the guidance of a faculty advisor, the Senior Project should be an original work, should use primary sources when applicable, should have a table of contents and works cited page, should give convincing evidence to support a strong thesis, and should use the methods and writing style appropriate to the discipline.

The completed project, to be turned in in duplicate, must be approved by the Honors Committee in consultation with the student's supervising professor three weeks prior to graduation. Please include the advisor's name on the title page. The 2-3 hours of credit for this project is done as directed study or in a research class.

Keeping in mind the above senior project description, please describe in as much detail as you can the project you will undertake. You may attach a separate sheet if you wish:

I am writing a research paper on a project that I completed this summer. I studied the benefits of hyperbaric oxygen therapy vs. 100% oxygen vs. normal breathing air on the healing process of hydrogen cyanide + hydrogen chloride toxicity. For this project (with my dad's help) I constructed a hyperbaric oxygen chamber (from a pressure cooker) and a smoke chamber. I worked with mice and exposed them to toxic gases. The mice were then immediately put into treatment with either HBO, 100% O2, or nothing. I wanted to determine whether HBO helps to speed up the recovery time. HBO treatment worked well.

Signature of faculty advisor

Expected date of completion: April 10, 2002

Approval to be signed by faculty advisor when completed:

This project has been completed as planned: Yes  No

This is an "A" project: Yes  No

This project is worth 2-3 hours of credit: Yes  No

Advisor's Final Signature

Chair, Honors Committee  Date Approved:

Dear advisor, please write your final evaluation on the project on the reverse side of this page. Comment on the characteristics that make this "A" quality work.
Prisc designed and developed her own research project and implemented the project with great success. The same time and excellent job well done! Her project is presenting herd results at the Tennessee Academy of Science meeting, April 29, 2022.