Hyperbaric Oxygen Increases Survival Following Carotid Ligation in Gerbils

John A. Reitan, MD, Nguyen D. Kien, PhD, Steven Thorup, MD, and Guy Corkill, MD

We studied the effects of graded exposure to hyperbaric (1,875 mm Hg) oxygen therapy in an acute stroke model prepared by unilateral carotid artery interruption in gerbils. Pentobarbital alone, superoxide dismutase alone, two periods of hyperbaric oxygen alone, and each agent combined with hyperbaric oxygen were administered to investigate possible mechanisms of protection from cerebral ischemia. Survival rates and neurologic deficit scores over 5 days in all treated groups were compared with those in a control group. Survival rates in the groups subjected to 2 (63.9±4.0%) and 4 hours (70.1±5.2%) of hyperbaric oxygen alone were significantly higher than in the control group (53.6±4.2%). The group treated with pentobarbital alone also demonstrated increased survival (69.8±7.0%), but the combination of therapeutic regimens offered no apparent additive protection. By 5 days there were no differences in the neurologic deficit scores of the survivors in the groups. The toxic pulmonary effects of hyperbaric oxygen were assessed in a pilot LD₅₀ study. The pressure used caused no mortality during 4 hours of exposure, and the calculated LD₅₀ was 7.26 hours. This investigation demonstrates that graded doses of hyperbaric oxygen given after the insult increase survival in a gerbil model of stroke. (Stroke 1990;21:119-123)

Hyperbaric oxygen (HBO) therapy has been advocated as an adjunct to surgical revascularization of the brain, for treatment of acute cerebral edema or stroke, and as a predictive tool for staging reversible stroke with and without surgical intervention. More recently, HBO has been advocated as therapy for multiple sclerosis, however, this use has been challenged and is of questionable value. In fact, there have been major criticisms of the use of HBO in most disease processes other than carbon monoxide poisoning and decompression sickness. The single major criticism of most HBO studies has been the lack of controlled prospective analysis. Consequently, we prospectively studied the efficacy of HBO for the treatment of evolving stroke in an animal model.

The gerbil is a unique rodent in that it has unilateral hemispheric blood flow from the carotid artery. Unilateral carotid artery ligation results in ipsilateral signs of stroke in approximately 50% of the animals; hence gerbils have become the subject of numerous studies. Gerbils have been used to evaluate barbiturate therapy in stroke, to delineate neurotransmitter amine levels following carotid artery ligation, and to study the effects of steroids, naloxone, and calcium antagonists on induced cerebral ischemia. We chose the gerbil as our animal model for unilateral cerebral ischemia because of the animal's accessibility, the reproducibility of the resulting brain lesion, and the ease of animal manipulation in the HBO environment.

HBO itself is not without peril due to pulmonary and cerebral toxicity. Free radical liberation with HBO may cause significant damage, primarily due to free radical peroxidation of reactive lipids, including neuronal membranes. To attenuate these toxic effects of HBO, we evaluated the protective effects of superoxide dismutase, a specific and efficient scavenger of superoxide, during stroke and HBO therapy. Lastly, we compared the effects of pentobarbital, a known protectant against cerebral ischemia in gerbils, to those of HBO and superoxide dismutase to help delineate possible mechanisms of protection by these therapies.

Materials and Methods

All 695 gerbils studied were male, 8–12 weeks old (mean±SEM 10.4±0.5), and weighed 50.4±0.9 g. Investigations were performed between 1 PM and 6 PM to avoid circadian variations. The gerbils were divided into several groups: 615 were subjected to various therapeutic regimens, 10 were subjected to...
sham operation, and 70 were used to delineate the LD$_{50}$ of HBO.

All gerbils were fasted for 12 hours, and all except the 70 used in the LD$_{50}$ study were anesthetized initially with 1.5% inspired halothane in air and placed on a dissecting table with continued halothane insufflation. The left carotid artery was exposed through an anterior neck incision, and in all but the 10 sham-operated gerbils, the artery was doubly ligated and divided by cautery. After closure of the wound, the gerbils were randomly assigned to a specific therapeutic regimen. Table 1 lists the treatment and number of gerbils in each group. In most groups, approximately 20 gerbils were studied at a time. Two investigators worked in tandem for the carotid interruption procedure, and the therapeutic regimen was begun 40 minutes after the median time for carotid interruption in each group.

The hyperbaric chamber was a small diving decompression device (approximately 0.36 m$^3$ in volume) that was modified to permit visual observation and constant temperature regulation by a heating blanket and a heat lamp. The rectal temperature of selected gerbils was monitored inside the chamber, and the rectal temperature of all gerbils was measured before and after therapy. All gerbils rested on a heating blanket, and a heat lamp was applied if their rectal temperature fell below 38.0°C. The oxygen pressure applied to the gerbils was 1,875 mm Hg, sufficient for cellular respiration from dissolved oxygen alone without the need for hemoglobin transport. The hyperbaric chamber was flushed with 75 l/min oxygen for approximately 10 minutes before therapy began. The HBO-treated gerbils were placed in the hyperbaric chamber and subjected to HBO conditions for either 2 or 4 hours, whereupon they were decompressed and placed in multiple animal cages. Full pressurization took approximately 7.5 minutes. Decompression was performed over 20 minutes.

Pentobarbital-treated gerbils were given 50 mg/kg of the drug intraperitoneally and were lethargic or unconscious for approximately 2 hours, whereupon they slowly revived to the predrug state. Their rectal temperatures were kept constant with heating blankets and heat lamps. Those pentobarbital-treated gerbils that also received HBO therapy were given the barbiturate immediately before entering the hyperbaric chamber. The superoxide dismutase-treated gerbils received 5 mg/kg of the enzyme (Cu,Zn-superoxide dismutase, 3800 units/mg, Diagnostic Data, Inc., Mountain View, California) intraperitoneally every 6 hours for 24 hours and were similarly observed and kept at a constant rectal temperature. The gerbils that received superoxide dismutase plus HBO therapy were given the first dose of enzyme before entering the hyperbaric chamber.

All gerbils were observed in their original cages for 5 days, and their survival rates were noted periodically over this interval. Gerbils in which a progressive, but significant, stroke occurred usually died within 3 days.

The degree of physical impairment in the surviving gerbils was monitored using a neurologic deficit score$^{15}$ illustrated in Table 2.

Because excessive oxygen is known to be a cerebral and pulmonary toxin$^{22}$ and because specific information concerning its effect on gerbils is not available in the literature, a pilot mortality study was performed. Groups of 10 gerbils each were subjected to 0, 1, 2, 4, 6, 8, or 10 hours of 1,875 mm Hg oxygen and followed for 5 days. The number of gerbils surviving for 5 days in each group versus their HBO exposure time was plotted to produce a simple LD$_{50}$ curve.

The animal experiments and all procedures were performed in accordance with the guidelines of the

<table>
<thead>
<tr>
<th>Sign</th>
<th>Score</th>
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<tbody>
<tr>
<td>Ptosis</td>
<td>1</td>
</tr>
<tr>
<td>Head tilt</td>
<td>1</td>
</tr>
<tr>
<td>Drowsy</td>
<td>2</td>
</tr>
<tr>
<td>Reduced motility</td>
<td>2</td>
</tr>
<tr>
<td>Splayed hind limb</td>
<td>2</td>
</tr>
<tr>
<td>Circling</td>
<td>3</td>
</tr>
<tr>
<td>Rolling</td>
<td>4</td>
</tr>
<tr>
<td>Coma</td>
<td>4</td>
</tr>
<tr>
<td>Maximum</td>
<td>19</td>
</tr>
</tbody>
</table>

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Animal Use and Care Administrative Advisory Committee at the University of California, Davis, California.

Survival among the groups was compared using analysis of variance and life table regression analysis. Differences in neurologic deficit scores among groups were investigated using χ² analysis.

Results

Figure 1 illustrates the LD₅₀ for exposure to HBO in 70 gerbils. The LD₅₀ was calculated as 7.26 hours. No gerbil died after 4 hours of HBO exposure (the longest exposure in our therapeutic groups). All gerbils died within 2 days after 10 hours of exposure. In no gerbil were convulsions observed, and death was commonly preceded by gasping respiration suggestive of pulmonary toxicity.

The survival for each group over 5 days is shown in Table 3. No sham-operated gerbil died, while only 53% of the control group survived. However, survival increased with HBO therapy, and survival in the 2- and 4-hour HBO groups differed significantly from that in the control group by 5 days (p<0.05). Figure 2 shows the apparent dose-dependency of HBO therapy in increasing the survival of gerbils following unilateral carotid artery interruption. Gerbils with evidence of seizure activity or a circling gait often returned to their normal ambulatory, inquisitive behavior during the HBO therapy. This remission from signs of neural damage was usually transient, although more permanent recovery did occur. There was no significant difference in the rectal temperatures of gerbils before and after HBO therapy and among treated gerbils and controls.

Treatment with superoxide dismutase alone afforded no apparent lasting protection from stroke (did not increase survival) in this gerbil model. While survival in the pentobarbital-treated group differed significantly from that in the control group at 5 days, survival of gerbils treated with superoxide dismutase or pentobarbital plus HBO therapy did not differ significantly from that of gerbils treated with HBO therapy alone after 5 days.

After 5 days, neurologic deficit score in no group differed from that in the control group (Table 4), although at 8 hours scores in the superoxide dismutase alone, pentobarbital alone, and pentobarbital plus 2 hours HBO groups differed significantly from that in the control group.

Discussion

Our investigation demonstrates that acute postschismic treatment with HBO improves the survival of gerbils following stroke induced by unilateral carotid artery interruption. Our data are consistent with

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>1</th>
<th>8</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
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<tbody>
<tr>
<td>Control</td>
<td>138</td>
<td>90.6±2.3</td>
<td>81.2±3.3</td>
<td>72.5±3.8</td>
<td>59.4±4.2</td>
<td>58.0±4.2</td>
<td>55.1±4.2</td>
<td>53.6±4.2</td>
</tr>
<tr>
<td>2-hr HBO</td>
<td>147</td>
<td>91.2±2.3</td>
<td>78.9±3.4</td>
<td>74.8±3.6</td>
<td>66.7±3.9</td>
<td>63.9±4.0</td>
<td>63.9±4.0</td>
<td>63.9±4.0*</td>
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<tr>
<td>4-hr HBO</td>
<td>77</td>
<td>90.9±3.3</td>
<td>81.8±4.4</td>
<td>77.9±4.7</td>
<td>72.7±5.1</td>
<td>70.1±5.2</td>
<td>70.1±5.2</td>
<td>70.1±5.2*</td>
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<tr>
<td>SOD</td>
<td>79</td>
<td>98.7±1.3</td>
<td>89.9±3.4</td>
<td>73.4±5.0</td>
<td>60.8±5.5</td>
<td>55.7±5.6</td>
<td>53.2±5.6</td>
<td>53.2±5.6</td>
</tr>
<tr>
<td>2-hr HBO+SOD</td>
<td>45</td>
<td>100±0.0</td>
<td>88.9±4.7</td>
<td>82.2±5.7</td>
<td>66.7±7.0</td>
<td>64.4±7.1</td>
<td>64.4±7.1</td>
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</tr>
<tr>
<td>4-hr HBO+SOD</td>
<td>45</td>
<td>95.6±3.1</td>
<td>73.3±6.6</td>
<td>71.1±6.8</td>
<td>71.1±6.8</td>
<td>71.1±6.8</td>
<td>71.1±6.8</td>
<td>71.1±6.8*</td>
</tr>
<tr>
<td>P</td>
<td>43</td>
<td>97.7±2.3</td>
<td>93.5±3.2</td>
<td>86.0±5.3</td>
<td>69.8±7.0</td>
<td>69.8±7.0</td>
<td>69.8±7.0</td>
<td>69.8±7.0*</td>
</tr>
<tr>
<td>2-hr HBO+P</td>
<td>20</td>
<td>100±0.0</td>
<td>95.0±4.9</td>
<td>80.0±8.9</td>
<td>60.0±11.0</td>
<td>60.0±11.0</td>
<td>60.0±11.0</td>
<td>60.0±11.0</td>
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<tr>
<td>4-hr HBO+P</td>
<td>21</td>
<td>95.2±4.7</td>
<td>95.2±4.7</td>
<td>85.7±7.6</td>
<td>81.0±8.6</td>
<td>71.4±9.9</td>
<td>71.4±9.9</td>
<td>71.4±9.9*</td>
</tr>
</tbody>
</table>

HBO, hyperbaric oxygen; SOD, superoxide dismutase; P, pentobarbital. Data are mean±SEM % survival.

*p<0.05 different from control by life table regression analysis.
shown superoxide dismutase to be ineffective in attenuating the effects of complete cerebral ischemia in dogs,35 and our study demonstrated a similar lack of long-term efficacy in the more focal lesion of this gerbil model. Interestingly, there was apparent increased survival in the superoxide dismutase-treated group during the first 8 hours following carotid artery interruption, but even with continued intraperitoneal injection of the enzyme, the survival rate returned to the control level after 24 hours.

While there was a dose-dependent relation between survival and increasing HBO exposure, no such additive effect occurred between pentobarbital or superoxide dismutase and HBO. Since a secondary effect of HBO is the formation of free oxygen radicals, which may increase peri-infarct tissue injury,21 and since both pentobarbital and superoxide dismutase are free radical scavengers, the two treatments combined with HBO might act in consort to reduce stroke damage. Our data for the combined therapies showed no such effect, and the combined treatment was not different from HBO alone. Apparently pentobarbital, superoxide dismutase, and HBO work by means of separate pathways that are not additive or synergistic in gerbils.

The effects of oxygen therapy on experimental subjects is paradoxical. On one hand oxygen causes marked tissue damage and toxicity, while on the other hand it provides apparent treatment for neuronal ischemia, depending on the total dose and pressure. For example, Mickel and colleagues36 found that therapy with 100% oxygen at atmospheric pressure was harmful to gerbils following transient global ischemia.

The primary mechanism of action for HBO therapy remains enigmatic, although it has been suggested that the oxygen-mediated constriction of the cerebral vasculature reduces intracranial volume and therefore intracranial pressure falls sufficiently to attenuate some ischemic changes.37 From our study it is obvious that the primary beneficial effects of HBO outweigh the secondary production of deleterious free radicals. Recent studies in both cats38 and gerbils39 that were treated during transient cerebral ischemia have shown that HBO increased survival. This same therapy instituted after the cerebral insult (similar to our investigation) has been reported to increase glucose utilization in ischemic brain, even after discontinuation of therapy.40 While this information does not present distinct mechanistic evidence, it does show that postinjury treatment with HBO causes lasting changes in cerebral metabolism, and these changes may be related to recovery from ischemia. Indeed, by Day 5 after the insult the average neurologic deficit scores of our HBO-treated groups were not different from that of the control group in spite of treated rats' increased survival. There was an apparent maintenance of "quality of life."

The pulmonary toxicity of hyperoxia has been well summarized recently,22 and our cursory LD50 study at 1,875 mm Hg oxygen was performed to demonstrate the upper limits of exposure to HBO for 100%

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Table 4. Neurologic Deficit Scores of Groups of Gerbils Surviving Unilateral Carotid Artery Interruption

<table>
<thead>
<tr>
<th>Group</th>
<th>Score 8 hr</th>
<th>Score 120 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.2±0.8</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>2-hr HBO</td>
<td>1.7±0.9</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>4-hr HBO</td>
<td>3.8±0.8</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>SOD</td>
<td>5.2±1.1*</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>2-hr HBO+SOD</td>
<td>4.7±1.0</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>4-hr HBO+SOD</td>
<td>1.7±0.5</td>
<td>0.6±0.2</td>
</tr>
<tr>
<td>P</td>
<td>5.6±1.0*</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>2-hr HBO+P</td>
<td>5.2±0.9*</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>4-hr HBO+P</td>
<td>4.9±0.9</td>
<td>0.5±0.1</td>
</tr>
</tbody>
</table>

HBO, hyperbaric oxygen; SOD, superoxide dismutase; P, pentobarbital. Score is mean±SEM.

*p<0.05 different from control by χ2 analysis.

those of numerous clinical trials1-8 in regard to the value of this transient therapy.

The use of gerbils as an animal model for the delineation of stroke and stroke treatment is well documented by histologic and chemical studies.24-26 Kahn14 has shown that 100% of gerbils demonstrating evidence of significant stroke died within 4½ days after carotid artery ligation. Consequently, our experiment was designed to use this ultimate end point, death, and required a large number of gerbils to study this sort of therapy. Our results confirm the reported mortalities over time of other investigators; in fact, all our gerbils with fatal cerebral ischemia died by the end of 4 days after carotid artery interruption.

After treatment with pentobarbital, our gerbils' survival increased 16.2% over control values. These numbers agree with earlier data from Lawner et al,16 who showed that pentobarbital increased the proportion of neurologically intact animals from 50% (control) to 72%. This agreement occurred even though our gerbils received the barbiturate after the insult and Lawner's gerbils were anesthetized with pentobarbital for the carotid artery ligation. On the other hand, halothane, which we used for anesthesia, is known to increase cerebral blood volume, raise intracranial pressure, and oppose a favorable outcome following stroke.27 Apparently, postinjury treatment with both pentobarbital and HBO partially overcame the deleterious effects of this anesthetic agent, and our results in gerbils confirm the worth of this delayed therapy in other species.27,28

For protecting myocardial tissue from ischemia and reperfusion injury, superoxide dismutase has been considered both a success29,30 and a failure.31,32 Superoxide dismutase is presumed to act by catalyzing the production of H2O2 from free oxygen radicals, and the H2O2 is degraded by naturally occurring catalases.33 Chan et al44 have verified that superoxide dismutase is effective in attenuating the brain levels of superoxide radicals and in preserving the integrity of the blood–brain barrier following cold-induced brain edema in rats. However, previous work has
survival. The first death occurred at 6 hours, 2 hours beyond our longest therapeutic exposure to HBO. One must assume that some pulmonary changes did result from the longer HBO exposure, but it was beyond the scope of our investigation to uncover it.

The attractiveness of HBO treatment for stroke and other neuroischemic conditions continues because of its noninvasiveness and relatively low risk compared with other intervention modalities such as barbiturate coma or surgery. Our study has demonstrated that HBO is an effective and reasonably safe therapy for acute focal cerebral ischemia in gerbils.

Acknowledgments

The authors would like to thank Mr. Richard Martucci for his expert technical assistance and advice in the investigation and Charles E. Franti, PhD, for his help with statistical analysis and study design.

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Hyperbaric oxygen increases survival following carotid ligation in gerbils.
J A Reitan, N D Kien, S Thorup and G Corkill

Stroke. 1990;21:119-123
doi: 10.1161/01.STR.21.1.119

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