Brain Research Bulletin, Vol. 3, pp. 221–225. Printed in the U.S.A.

Ameliorating Effects of Hyperbaric Oxygenation on Experimental Allergic Encephalomyelitis

LEON D. PROCKOP

Section of Neurology, Department of Internal Medicine

AND ROBERT J. GRASSO

Department of Medical Microbiology, College of Medicine, Medical Center
University of South Florida, Tampa, FL 33612

(Received 12 September 1977)

PROCKOP, L. D. AND R. J. GRASSO. *Ameliorating effects of hyperbaric oxygenation on experimental allergic encephalomyelitis.* BRAIN RES. BULL. 3(3) 221–225, 1978. – The effect of hyperbaric oxygenation (OHP) on survival and quality of survival of guinea pigs afflicted with experimental allergic encephalomyelitis (EAE) was investigated. EAE was induced in Hartley and Strain 13 animals by intradermal injections of whole guinea pig spinal cord in complete Freund's adjuvant. The inoculated animals were divided into control and treatment groups; the treated animals received OHP in a variety of treatment schedules. Clinical signs of EAE were quantitated and mean survival times were measured. When Hartley animals were exposed to 100% O_2 at 2.5 atmospheres absolute (ATA) for 2 hr/day from 5–19 days postinoculation, the mean survival time (± SE) was 19.1 ± 1.6 days relative to 15.7 ± 0.7 days in the control ($p<0.050$). When Strain 13 guinea pigs were treated with 100% O_2 at 2 ATA for 4 hr/day on 5–16 days, the mean survival time was 21.6 ± 0.6 days compared to 16.0 ± 0.4 days for the control ($p<0.001$). Clinical sign measurements demonstrated that the onset of EAE in the treated animals of both strains occurred between 4–6 days after these signs became detectable in control animals. These results suggest that OHP therapy can ameliorate EAE in afflicted guinea pigs.

Experimental allergic encephalomyelitis Hyperbaric oxygenation therapy Guinea pigs Clinical signs Survival times

DEMYELINATION in the central nervous system results in degenerative diseases such as experimental allergic encephalomyelitis (EAE) in laboratory animals and multiple sclerosis in humans. These two neurological disorders differ from each other in one important respect. In EAE, perivascular infiltration and other cellular inflammatory responses precede myelin destruction whereas the reverse occurs in multiple sclerosis [10]. Nevertheless, the progressive destruction of myelin occurs ultimately in both disease states.

Hyperbaric oxygenation (OHP) therapy has been shown to improve neurological deficit resulting from cerebral edema [5,13], cerebral ischemia [4,11], head injury [7,8], and cerebrospinal cord injury [3]. In EAE, demyelination is accompanied by inflammation and cerebral edema [6,9]. There are no previous reports describing the effects of OHP therapy on EAE. If beneficial effects can be demonstrated, the potential implications for the treatment of demyelinating diseases such as multiple sclerosis are obvious. Therefore, this study was initiated to determine whether OHP therapy would ameliorate EAE clinical signs and extend survival times of afflicted animals.

METHOD

Animals

Healthy Hartley male and female guinea pigs (Camm Research Institute, Wayne, NJ) and Strain 13 male guinea pigs (University of Missouri Medical Center, Columbia, MO) weighing>600 g were maintained in our vivarium for at least 5 days before the animals were inoculated.

Induction of EAE

Three frozen adult guinea pig spinal cords (Pel-Freez Bio-Animals, Inc., Rogers, AK) were ground to a paste in 3 ml normal saline and lyophilized to a dry powder. On the day of inoculation, the powder was suspended in normal saline plus complete Freund's adjuvant (Grand Island Biological Co., Grand Island, NY) containing additional heat-killed *Mycobacterium tuberculosis* strain H37–RA (Difco Laboratories, Detroit, MI) and phenol. Each 0.5 ml of this antigen-adjuvant mixture contained 16 mg lyophilized spinal cord antigen, 0.25 ml normal saline, 0.25 ml complete Freund's adjuvant, an additional 2.5 mg *M. tuberculosis* and 0.25% phenol. Each guinea pig was injected

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with 0.1 ml of the mixture into 5 separate intradermal sites on their shaved backs. After 10 days, the injected animals showed neurological signs indicative of EAE. Histological sections of nervous tissue prepared between 14–20 days revealed extensive demyelination and perivascular infiltation of monocytes.

OHP Therapy

Inoculated Hartley guinea pigs were randomly divided into groups of 12. Two groups of Strain 13 guinea pigs contained 5 animals each. Each experiment consisted of one control group and one or two OHP treatment groups. Treatments were conducted in a 13 cu ft hyperbaric chamber beginning 5 days after animal inoculation. Liquid O_2 of 99.995% purity (Airco Industrial Gas Division, Birmingham, AL) was employed to compress and decompress the chamber at rates of 1 lb/sq in/min. During OHP exposure, food and water were withheld from all treated and control animals. The chamber was flushed continuously with 100% O_2 at pressure to prevent CO_2 accumulation. Chamber and ambient temperatures differed by less than 2°C.

Control and OHP treated guinea pigs afflicted with EAE are designated C and T, respectively, followed by a number which represents a particular group (e.g., C10, T11). Preliminary studies on both normal and EAE animals determined guinea pig tolerance to both O_2 and hyperbaric conditions. The number of OHP therapy days, the number of exposure hr/day, and the hyperbaric conditions (atmospheres absolute = ATA) eventually selected are cited in the text.

Measurement of Clinical Signs and Survival

All animals were weighed and were examined for neurological signs associated with EAE. The severity of EAE was quantitated by 2 parameters. The time for an animal to right itself after being placed supine was scored as follows: 0 = normal, immediate righting; 1 = righting <0.5 sec; 2 = 0.5–1 sec; 3 = 1–2 sec; 4 = 2–6 sec; and 5 = no righting. Limb paralysis and response to a pain stimulus were estimated as follows: 0 = normal, no limb weakness, rapid limb movement in response to pain; 1 = hind limbs exhibited slow response to a pain stimulus; 2 = hind limbs weak with sluggish response to pain but animals not ataxic; 3 = hind limbs very weak and animals ataxic; 4 = hind limbs paralyzed, forelimbs weak; and 5 = quadriplegia, with only the ability to stand weakly on forelimbs for several sec. These parameters were assessed daily. The numbers assigned to righting ability and to the degree of paralysis were added daily to establish a clinical index for each guinea pig. Summed values established the cumulative clinical index. Mean cumulative clinical indices (±SE) were calculated for groups of animals surviving either to or beyond the median day of death in controls. In these, survival was sufficient to ascertain whether OHP therapy would delay the onset and/or reduce the severity of EAE. The mean cumulative clinical indices were calculated until fewer than three animals remained in any one group. The Student's *t*-test (two-tailed) was employed to determine whether the mean cumulative clinical indices and mean survival times for the OHP treated animals differed significantly from that observed in controls.

RESULTS

In preliminary control studies, EAE did not occur when guinea pigs were injected with the adjuvant mixture lacking the lyophilized spinal cord antigen. All animals inoculated with the adjuvant-antigen mixture developed clinically apparent EAE which was uniformly fatal in Strain 13 animals and was fatal in 94% of Hartley animals. Animals that developed the attenuated non-fatal form of EAE were not used for data analysis because they would falsely distort the statistics concerning survival. When Hartley

TABLE 1

SURVIVAL OF CONTROL AND OHP-TREATED HARTLEY GUINEA PIGS AFFLICTED WITH EAE

Experiment Number	Animal Groups	100% O_2 Days Treated	Hr/Day	ATA	N[a]	Days Survival[b]
1	C7	–	–	–	10	17.3 ± 1.4
	T6	5–14	2	2.5	11	18.6 ± 1.1[c]
2	C8	–	–	–	12	17.8 ± 1.2
	T7	5–14/15–19	2/1	2.5	11	21.0 ± 1.6[d]
3	C6	–	–	–	11	15.7 ± 0.7
	T4	5–19	2	2.0	11	18.3 ± 1.7[d]
	T5	5–19	2	2.5	11	19.1 ± 1.6[e]
1 + 2 + 3	C6 + C7 + C8				33	16.6 ± 0.8
	T4 + T5 + T6 + T7				44	19.2 ± 0.8[f]

[a]N = number of animals/group that died of EAE [b]Mean ± SE [c]$p>0.100$ [d]$p<0.100$ [e]$p<0.050$ [f]$p<0.025$

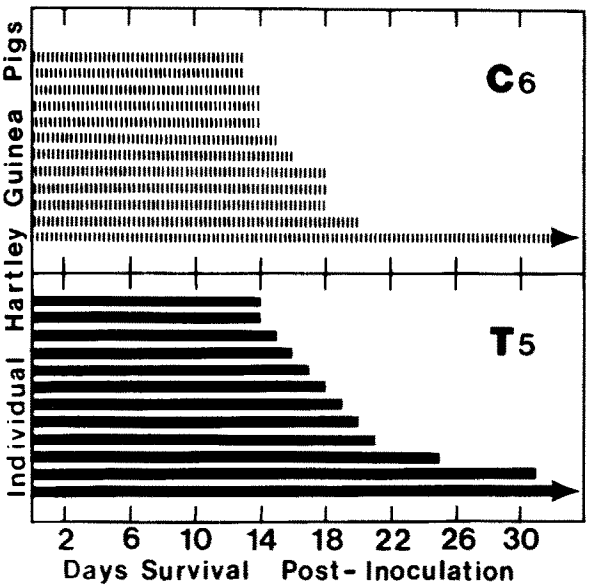

FIG. 1. Survival of individual Hartley guinea pigs in control Group C6 and OHP treated Group T5. The single surviving animal in each group as represented by the arrow was omitted from the mean survival time calculations in Table 1.

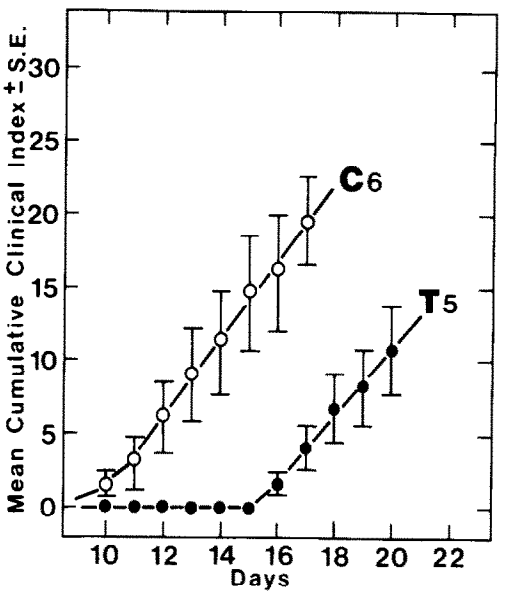

FIG. 2. Delayed onset of EAE clinical signs in Hartley animals treated with OHP. Only animals that survived beyond the median day of death in the control group are represented. Curve C6 (○): Control; N = 6 from Days 10–15; N = 5 on Days 16 and 17. Curve T5 (●): Treatment with 100% O_2 at 2.5 ATA for 2 hr/day from Days 5–19; N = 8 from Days 10–16; N = 7 on Day 17; N = 6 on Day 18; N = 5 on Day 19; N = 4 on Day 20; $p<0.050$ from Days 12–17.

animals were treated from 5–15 days with either air or 100% O_2 at 1 ATA, mean survival times and clinical sign measurements did not differ significantly from controls. Similar results were obtained when air at 2 ATA was substituted for 100% O_2. Animals exposed to 3 ATA 100% O_2 for more than 2 hr developed rapid respirations. In some cases, pulmonary edema and death occurred at this elevated pressure.

Hartley Guinea Pig Experiments

Groups of EAE-afflicted animals were treated with 100% O_2 at elevated pressures for varying periods. Three representative experiments are shown in Table 1. When EAE animals were exposed to 100% O_2 at 2.5 ATA for 2 hr/day over a 10 day period, with or without an additional 5-day period for 1 hr/day, survival was extended relative to controls (Experiment 1 and 2). Similar results were observed when 100% O_2 at 2 ATA was administered for 2 hr/day over a 15-day period (Experiment 3). However, increasing the pressure of 100% O_2 to 2.5 ATA during treatment extended survival in a statistically significant manner (Experiment 3). Figure 1 depicts the survival times of the individual animals in Groups C6 and T5 from Experiment 3. Table 1 also shows that survival is increased significantly when all controls were compared to OHP treated animals in Experiments 1–3. The mean survival days of all control Hartley animals (N = 38) used in this study was 16.5 ± 0.7. For all OHP treated guinea pigs (N = 49), survival was extended to 19.5 ± 0.7. Thus, these results indicate that OHP therapy can increase the mean survival times of guinea pigs afflicted with EAE. Furthermore, the data in Table 1 suggest that increased survival time is proportional to increased 100% O_2 pressure and prolongation of treatment.

The mean cumulative clinical indices for Groups C6 and T5 are shown in Fig. 2. In the control group, clinical signs associated with EAE appeared 10 days after inoculation among the long-term survivors. In contrast, clinical signs of EAE appeared 16 days after inoculation of the treated animals. The almost parallel C6 and T5 curves suggest that once clinical signs began to appear in the treated group, the course of EAE was similar to that observed in controls.

Strain 13 Guinea Pig Experiments

EAE occurs more uniformly in Strain 13 than in Hartley guinea pigs. In addition, the disease is more severe and its course more predictable. Mean survival times and cumulative clinical indices were measured in EAE Strain 13 animals in control and OHP exposure circumstances.

Table 2 clearly demonstrates that OHP therapy extended significantly the mean survival time in the experimental group relative to controls, 21.6 ± 0.6 days compared to 16.0 ± 0.4 days. The mean cumulative clinical indices for control and OHP treated Strain 13 animals is represented in Fig. 3. Clinical signs associated with EAE appeared in control animals after 10 days. In contrast, clinical signs were initially observed in the OHP treated animals after 13 days. The reduced slope of the linear portion of Curve T11 relative to Curve C10 suggests that the disease was less severe in the OHP treated group prior to death relative to controls.

DISCUSSION

These results indicate that OHP therapy can prolong survival and delay the onset of EAE clinical signs in two different varieties of afflicted guinea pigs. The most pronounced increase in survival times occur when Strain 13 animals with EAE are exposed to 100% O_2 at 2 ATA for 4 hr/day over a 12-day period; the survival time increases 31% relative to controls (Table 2). EAE clinical signs in these treated animals begin to appear at the same time that

TABLE 2

SURVIVAL OF EAE-AFFLICTED STRAIN 13 GUINEA PIGS WITH AND WITHOUT OHP EXPOSURE

Animal Groups	100% O_2 Days Treated	Hr/Day	ATA	N[a]	Days Survival[b]
C10	–	–	–	5	16.0 ± 0.4
T11	5–16	4	2	5	21.6 ± 0.6[c]

[a]All animals died of EAE [b]Mean ± SE [c]$p<0.001$

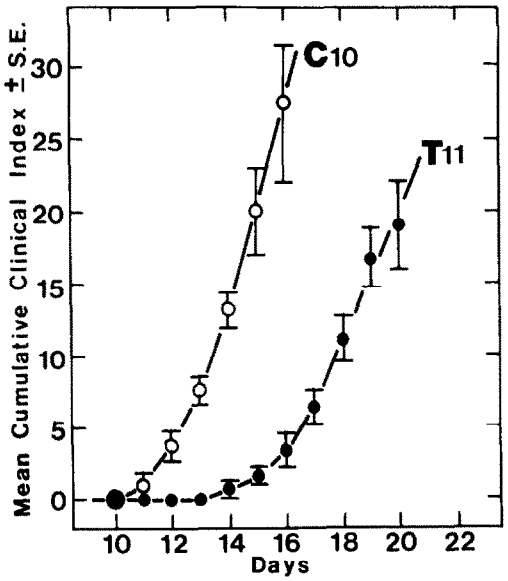

FIG. 3. Delayed onset of clinical signs with reduced severity of EAE in Strain 13 guinea pigs treated with OHP. Only animals that survived to and beyond the median day of death in the control group are represented. Curve C10 (○): Control; N = 5 from Days 10–14; N = 3 on Days 15 and 16. Curve T11 (●): Treatment with 100% O_2 at 2.0 ATA for 4 hr/day from Days 5–16; N = 5 from Days 10–19; N = 4 on Day 20; $p<0.005$ on Day 12; $p<0.001$ from Days 13–16.

all control animals, which manifest severe clinical signs, die of the disease (Fig. 3).

General physical appearance, a criterion of illness which could not be quantitated, also declines more rapidly in control animals relative to the OHP-treated animals of both varieties. When EAE clinical signs become detectable in either control or OHP-treated afflicted animals, weight loss occurs at the same time. However, weight loss was not used as a clinical sign of EAE in this study. The contribution of weight loss due to the cachexia of illness could not be separated from that due to weakness that interferes with feeding.

Under the experimental conditions employed in this study, the beneficial effects of OHP therapy in ameliorating EAE clinical signs and extending survival are minimal at best. In future studies designed to enhance these effects, increasing the pressure of 100% O_2 beyond 2.5 ATA is not feasible since 3 ATA is life-threatening to normal animals. Therefore, extending the number of treatment days or increasing the number of OHP therapy hr/day may conceivably extend survival times and retard even further the progression of the disease. In this regard, it should be noted that none of the mildly-afflicted Strain 13 animals die while receiving 100% O_2 at 2 ATA for 4 hr/day but die 5 days after OHP therapy is withheld. In contrast, some afflicted Hartley guinea pigs die when 100% O_2 at 2.5 ATA is administered for 2 hr/day (Fig. 1). Whether this discrepancy is due to strain differences in their sensitivities to OHP therapy or is related to the number of OHP therapy hr/day remains to be established.

Possible mechanisms that underlie the amelioration of EAE by OHP therapy may include the reduction of cerebral edema together with intracranial pressure [5,13], modulation of altered myelin metabolism associated with EAE [12], changes in glucose metabolism produced by hyperoxygenation [1,2], and the suppression of T-lymphocyte functions. Whatever the mechanism, the results reported in this communication represent the first observations that OHP therapy can prolong survival and does delay the onset of clinical signs in guinea pigs suffering from a demyelinating disease.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Arthur J. Grizzle, Ms. Linda Weinland and Dr. Theresa Lee for technical assistance. We also thank Mr. Keith Hawkins and Dr. N. Kandawalla for preparing and examining histological sections of guinea pig brain. This work was supported by a grant from the Mellen Foundation.

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