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Breathing 100% Oxygen After Global Brain Ischemia in Mongolian Gerbils Results in Increased Lipid Peroxidation and Increased Mortality

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Joseph F. Weiss, and Giora Feuerstein

Exposure of Mongolian gerbils to a 100% oxygen atmosphere after 15 minutes of global brain ischemia resulted in a marked increase in the production of pentane, an in vivo product of lipid peroxidation. Much less pentane production occurred in animals subjected to global brain ischemia then exposed to an air atmosphere and in animals exposed to a 100% oxygen atmosphere without ischemia. Gerbils placed in 100% oxygen for 3–6 hours after 15 minutes of ischemia also had a threefold increase in 14-day mortality compared with gerbils subjected to ischemia and then placed in an air atmosphere. These findings raise a serious question about the use of oxygen-enriched atmospheres during reperfusion following ischemia. (Stroke 1987;18:426–430)

BREATHING oxygen-enriched atmospheres is a usual and customary emergency procedure for patients suffering from an acute ischemic injury to the brain. Clinicians show little concern that the resulting arterial PO_2 is elevated. Instead, concern is focused on whether hypoxia is occurring as a consequence of respiratory complications.

Production of free radicals of oxygen or reactive oxygen species occurs during reperfusion following ischemia.[1] Lipid peroxidation also occurs in the brain during ischemic injury,[2] possibly related to the presence of endogenous iron.[3] In postischemic brain, iron ion delocalization is thought to promote lipid peroxidation by the production of free radicals of oxygen.[4-6] Xanthine oxidase is also a source of reactive oxygen species damage in ischemia.[7-9] Allopurinol, a xanthine oxidase inhibitor, has been shown to prevent free-radical-induced reperfusion injury.[10] However, following ischemia, mitochondrial respiration is impaired[11] so that an increase in substrate, i.e., molecular oxygen, could result in a dramatic increase in reaction product, i.e., oxygen free radicals. The increase in reactive oxygen species as a consequence of increased tissue PO_2 could result in the tissue injury and lipid peroxidation.[12]

There are many products of lipid peroxidation,[13] including those that result from cleavage of the hydrocarbon chain of polyunsaturated fatty acids. Among these products are the alkanes pentane and ethane.[14,15] Pentane is produced from peroxidative cleavage of the *cis* double bond 6 carbons from the hydrocarbon end of ω-6 fatty acids such as linoleic or arachidonic acids. Ethane is produced in a similar manner from ω-3 fatty acids such as linolenic or eicosapentaenoic acids. These volatile hydrocarbons are eliminated into the expired air and reflect in vivo peroxidation. When collected and measured, alkane production is more indicative of lipid peroxidation than the measurement of thiobarbituric-acid-reactive substances, which is most often used.[16]

To our knowledge, no studies measuring pentane production in relation to brain ischemia have been published.

Materials and Methods

Male Mongolian gerbils (Tumblebrook Farms), weighing 55–65 g were subjected to 15 minutes of global brain ischemia by bilateral carotid occlusion under anesthesia with 2% halothane (2-bromo-2-chloro-1,1,1-trifluoroethane, Halocarbon Laboratories, Hackensack, N.J.). Control animals were subjected to anesthesia and sham surgery but not ischemia. At the end of 15 minutes of ischemia and/or anesthesia, each animal was placed in an atmosphere of either pure 100% oxygen (UN 1072, Air Products) or breathing quality air (Air Products). To reduce the amount of halothane present in the closed atmosphere, each animal breathed the atmosphere flowing through a closed container measuring 20 × 14 × 6 in. for 30 minutes. At the end of 30 minutes, the animal was placed in a closed system to measure pentane produc-

tion. To decrease environmental hydrocarbon contamination of the expired atmospheric sample, a 10-minute washout period of the closed system was required. The experiments were conducted according to the principles set forth in the "Guide for Care and Use of Laboratory Animals," Institute of Laboratory Animal Medicine, National Research Council, Department of Health, Education, and Welfare, publication no. (NIH) 80-23, 1980.

The animal holding chamber used for sampling volatile compounds was similar to that described by Lawrence and Cohen[17] and Hafeman and Hoekstra,[18] with some modifications. Figure 1 represents the closed system used in these studies. A 2-liter Nalgene vacuum-type dessicator was used as the animal chamber. Oxygen or air was introduced through a polypropylene Y tube into the air or O_2 reservoir. The reservoir was connected with another polypropylene Y tube to the inlet of the chamber to allow oxygen or air to enter from the reservoir. A water seal maintained the closed system at ambient pressure. The expired chamber air was cycled through a series of 3 traps: 5% H_2SO_4 to remove NH_3, 10% KOH to remove CO_2, and a cold finger ice bath to condense water vapors. As NH_3 and CO_2 were removed from the chamber air, equal volumes of oxygen or air entered the chamber through a stopcock to maintain a constant atmospheric pressure. Before sampling, the pump was turned off. Forty-ml samples were removed with a gastight syringe at a three-way stopcock, and an equal volume of oxygen or air entered the chamber through another stopcock to maintain constant atmospheric pressure.

The sample was passed through a stainless steel cartridge (3.2 × 70 mm) filled with activated alumina, kept at −32° C during filling and before analysis. A Varian 3700 gas chromatograph was equipped with a Chemical Data System Model 310 concentrator. The cartridge was inserted in the desorber probe, and the desorber was flash-heated to 210° C for 3 minutes to desorb and transfer the hydrocarbons from the alumina cartridge to the concentrator trap (3.2 mm × 60 cm, leading half filled with charcoal, the other half with Tenax[12]). On flash-heating the trap desorbed the hydrocarbons onto the gas chromatographic column (3.2 mm × 2 m filled with Porasil B). The column was heated at 60° C for 3 minutes, and then the temperature was increased to 75° C at 1°/min. The injection port was maintained at 220° C and the flame ionization detector at 250° C.

Areas of hydrocarbon peaks and standard samples were determined by triangulation, and the quantity of pentane evolved was calculated.[12]

Activated charcoal (nutshell type SK-4, 60/80 mesh), activated alumina (Alcoa type F-1, 60/80 mesh), Porasil B (80/100 mesh), Tenax (2,6-diphenyl-p-phenyleneoxide polymer, 80/100 mesh) were all purchased from Applied Science Laboratories, State College, Penn. Hydrocarbon standards (approximately 100 ppm in helium) were obtained from Scott Specialty Gases, Plumsteadville, Penn. Air, helium, hydrogen, and oxygen (scientific grade) for gas chromatography were purchased from MG Industries, Valley Forge, Penn. Teflon tubing with Luer adapters was obtained from Hamilton, Reno, Nev., and Tygon tubing, type R-3603, from Fisher Scientific Company.

A sample of the atmosphere was taken at the time the system was closed. This became the zero time (30 minutes postischemia) sample. Subsequent samples were taken at 60, 90, 120, and 180 minutes after ischemia or anesthesia. There were 4 groups, each with 7 animals: 2 ischemic groups (air and oxygen) and 2 control groups (air and oxygen).

Mortality was assessed for 14 days after ischemia in gerbils exposed to oxygen for 3 or 6 hours.

The results were analyzed by analysis of variance (ANOVA) followed by the Student-Newman-Keuls test for multiple comparisons using an HP-85 microcomputer. Fisher's exact test was used to analyze mortality.

Results

After 3 hours of oxygen exposure following ischemia, a total of 581.3 ± 77.1 (mean ± SEM) pmol of
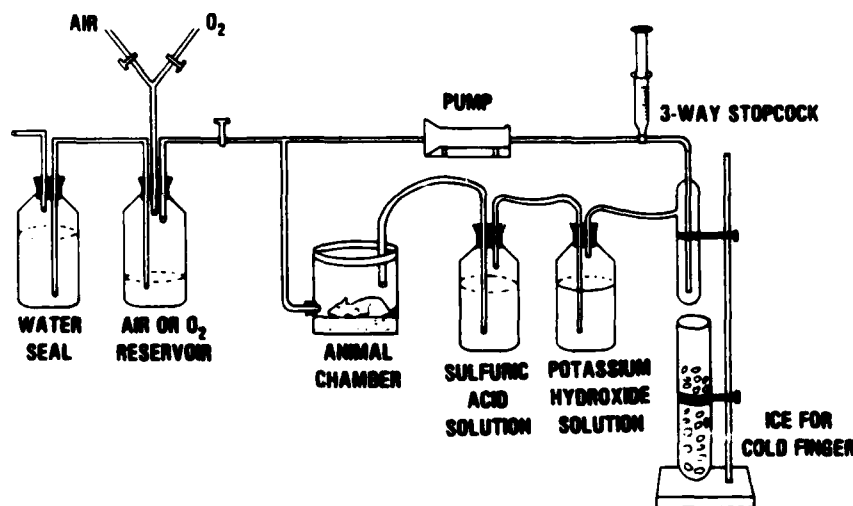

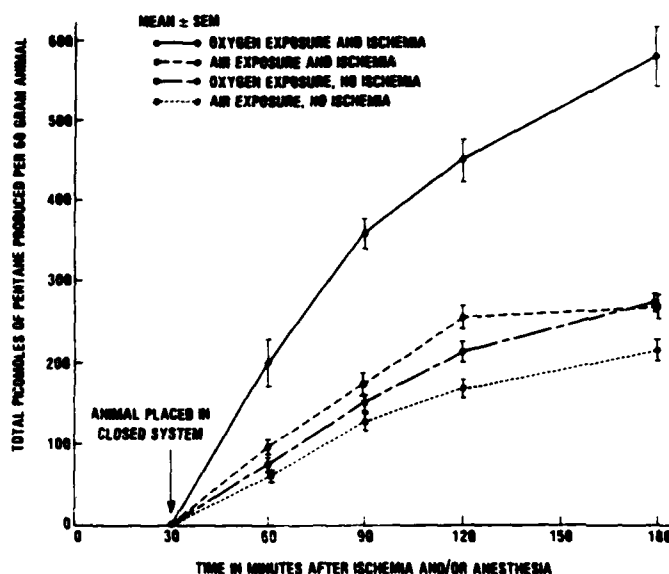FIGURE 1. *Diagram of closed system used.*

FIGURE 2. *Production of pentane by Mongolian gerbils after brain ischemia and/or anesthesia with reperfusion in air or a 100% oxygen atmosphere.*

pentane per 60-g animal was collected during a 150-minute collection period. Ischemic animals placed in air produced only 271.5 ± 26.2 pmol of pentane per 60-g animal. Control animals placed in 100% oxygen produced 276.7 ± 15.7 pmol of pentane per 60-g animal, and control animals placed in air produced 213.2 ± 28.4 pmol of pentane per 60-g animal. The total amount of pentane produced by the ischemic animals exposed to oxygen was about twice that produced by the other groups and is significantly different from each of the other groups $(p < 0.01)$. Although differences in pentane production were noted between the ischemic animals placed in air and the control groups, these differences were not significant at all times sampled (Figure 2). Although the values for 60 minutes were marginally significant, the increases in the amount of pentane formed by ischemic animals exposed to oxygen at 90, 120, and 180 minutes were significant $(p < 0.01)$ by the Student-Newman-Keuls test. The amount of peroxidation measured by pentane production is essentially 3 times as much as that expected at 180 minutes by the sum of the individual contributions of ischemia and of oxygen alone over the values for control gerbils exposed to air. Since the only difference between animals exposed to oxygen after anesthesia and animals exposed to oxygen after occlusion is a 15-minute period of brain ischemia, the increased tissue oxygen content from breathing 100% oxygen during brain reperfusion is assumed to result in a marked increase in lipid peroxidation. However, at this time we have no direct experimental evidence demonstrating that the increase in pentane production occurs only in the brain.

Furthermore, exposure to 100% oxygen for 3–6 hours following global brain ischemia results in a threefold increase in 14-day mortality when compared with animals exposed to air following ischemia. In fact, oxygen-treated animals displayed significantly higher mortality $(p < 0.001)$ throughout the 14 days

following the ischemic insult. [In a subsequent experiment, exposure of ischemic animals to 100% oxygen for 30 minutes resulted in no difference in mortality compared with ischemic animals exposed to air $(n = 16$ in each group).] There was no significant difference between exposure to 100% oxygen for 3 or 6 hours (Figure 3).

Discussion

Pentane is a reliable parameter for measuring in vivo lipid peroxidation.[19] Pentane is also a more sensitive index for ethanol-induced lipoperoxidation than ethane.[20] A problem in measuring pentane production in a closed system is that pentane is metabolized by the monooxygenase cytochrome P-450 at a rate 5–10 times faster than ethane,[21] resulting in a decrease in the total amount of pentane recovered due to metabolic elimination.[22] For that reason, the actual amount of pentane produced is probably higher than that measured in these studies. However, the total volume of our system is larger than of systems used by others, and perhaps there is a lesser proportion of pentane metabolized.

Since all factors were the same for our control and ischemic animals except the occurrence of ischemia, it is most unlikely that a gastrointestinal or bacterial origin for the measured pentane is of any consequence in this study,[23] and, most likely, the brain is the site of the increased pentane production during oxygen exposure following global brain ischemia.

The basal rate of pentane production in control animals exposed to air was 24 pmol pentane/kg body wt/min. This compares with 16 pmol pentane/kg body wt/min in human infants.[24] Pentane production in control animals breathing 100% oxygen was 31 pmol pentane/kg body wt/min. If one assumes that the increase in production of pentane by animals subjected to brain ischemia and then exposed to 100% oxygen is derived from the brain itself, it is possible to derive the follow-
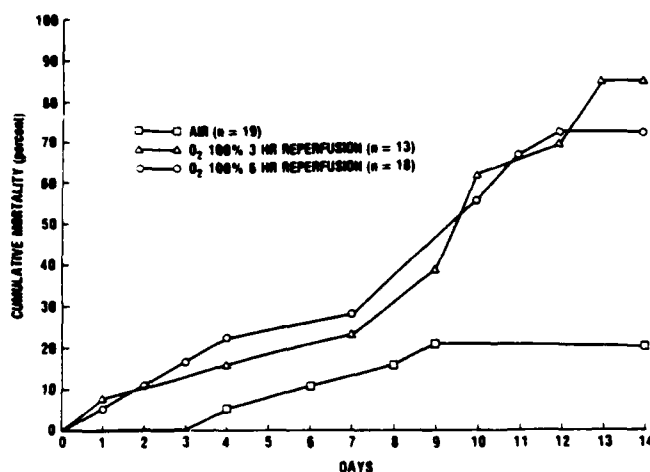
FIGURE 3. *Cumulative mortality in gerbils following 15-minute bilateral carotid occlusion.*

ing calculations. Assuming that the brain of a gerbil weighs 1 g and that the increase in pentane production following global brain ischemia represents lipid peroxidation in the brain, there is a production of pentane in the brain of ischemic animals exposed to air of 389 pmol/min/kg brain wt. Calculated from the difference between the ischemic and control animals exposed to 100% oxygen, pentane production from the brain of ischemic animals exposed to oxygen, however, is 2,031 pmol/min/kg brain wt, or 2 pmol/min from the gerbil brain. This is almost 100 times the basal rate of pentane production occurring in the control gerbil breathing air. However, we cannot state that this dramatic increase in lipid peroxidation caused the marked increase in mortality occurring in these animals.

The fact that giving supplemental oxygen is an unquestioned standard practice in clinical medicine for ischemia affecting the brain or heart makes the questions created by our observations of major importance. Whether lipid peroxidation is the only pathogenetic mechanism that is operative in the oxygen effect on reperfusion was not demonstrated in this study. The threshold of oxygen concentration and the duration of exposure needed to produce the deleterious oxygen effect have not yet been determined. Our findings also suggest the possibility that drugs which block peroxidation of lipids might have a potential beneficial effect in the reperfusion injury following ischemia.

Since supplementation with 100% oxygen is a common standard practice in the clinical management of acute myocardial ischemia and stroke, this study suggests that its value should be reassessed, especially in cases where no obvious respiratory difficulties occur and where no deficiency of oxygen is present in perfused tissue.

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