Journal of Hyperbaric Medicine, Vol. 7, No. 2, 1992

Evaluation of Subcutaneous Oxygen Tensions at Varied Ambient Oxygen Pressures in Mice

D. W. Criswell and W. J. Mehm

Division of Altitude and Hyperbaric Physiology, Armed Forces Institute of Pathology, Washington, DC 20306-6000

Criswell DW, Mehm WJ. Evaluation of subcutaneous oxygen tensions at varied ambient oxygen pressures in mice. J Hyperbaric Med 1992; 7(2):81–87.—The objective of this study was to ascertain changes in subcutaneous (s.c.) tissue oxygen tensions in mice used in an experimental model of wound healing. We altered tissue oxygen tensions by varying the ambient oxygen pressure to which the mice were exposed. Anesthetized mice were exposed to 12, 21, and 100% oxygen at an ambient pressure of 100 kPa and to 100% oxygen at 250 kPa. Oxygen tension measurements were made with a polarographic combination oxygen electrode encased in a hypodermic needle. The needle electrode was inserted through the skin into the s.c. tissue in the back of the mouse. Readings were recorded 10 min after transfer from a normoxic to an experimental environment. Subcutaneous oxygen tension rose with increasing ambient oxygen pressure. To determine if conditioning mice to a hypoxic environment (12% oxygen) resulted in any difference in tissue oxygen tension compared to acute exposure, we exposed mice to 12% oxygen for 12 days. No adaptive response was seen to the conditioning since tissue oxygen levels were not significantly different between mice conditioned to hypoxia (17.4 ± 1.7 mmHg) compared to the animals acutely exposed to the hypoxia (19.0 ± 1.4 mmHg). Using a simple experimental technique, we quantified the oxygen tensions achieved by the alteration of oxygen pressures.

hyperoxia, hypoxia, oxygen electrode, wound healing

Introduction

Hypoxia and hyperoxia can have detrimental effects on tissue and organ function. Many experimental models have examined the effects of both low and high tissue oxygen tensions on biological processes (1, 2). To achieve hypoxic and hyperoxic states, experimental animals may be exposed to altered ambient oxygen pressure. This exposure is an attempt to change the oxygen tension in the tissue or organ system being studied. To assess the effects of hypoxia and hyperoxia in various tissues it is necessary to measure the changes in oxygen tension.

Our laboratory is interested in the role of oxygen in wound healing. We are interested in this because nonhealing wounds in humans often occur in areas of low oxygen tension (3). A commonly used treatment for these nonhealing wounds is to relieve the tissue hypoxia by increasing the oxygen tension in the area of the wound. This is accomplished by elevating ambient

oxygen pressures in the hope that tissue oxygen tensions will be raised to optimal levels for wound healing. A recent experiment in our laboratory examined the effects of hypoxia and hyperoxia on an experimental model of wound healing (4). This experimental model measured the growth of granulation tissue in wounds, the precursor of scar, into specially prepared polyvinyl alcohol sponges. To produce tissue hypoxia and hyperoxia we exposed mice to hypoxic and hyperoxic ambient oxygen pressures in an attempt to manipulate the subcutaneous (s. c.) oxygen tension and thus affect the growth of tissue into the sponge. For this evaluation it was critical to know how s.c. oxygen tension changed as a function of altering ambient oxygen pressure.

To verify that changing the ambient oxygen pressure alters s.c. oxygen tension, investigators require a stable and reliable method of evaluation. Many of the methods currently employed for measuring tissue oxygen tension suffer from a number of disadvantages. Some methods, such as mass spectrometry, consume oxygen and thus affect the accuracy of the measurement (5). Others such as the use of isotopic oxygen and fluorescent probes are exceedingly complex, requiring the use of elaborate and often unreliable equipment (6, 7). Most of the methods for measuring tissue oxygen tension use polarographic oxygen electrodes which are very sensitive to damage from biological molecules in the tissues. To prevent this damage, the electrodes are often implanted in some type of oxygen-permeable sheath or are covered by an oxygen-permeable membrane. These modifications to the electrodes separate them from the tissue being studied and decrease the accuracy of the reading. For example, the tissue tonometry method, involving chronic implantation of polarographic electrodes in silastic tubing underneath the skin (8), induces an inflammatory response in the tissue where the tubing is implanted. This inflammatory response inherently alters the normal physiologic state of the tissue at the measurement site, and hence the tissue oxygen tension (6). Another technique, transcutaneous oximetry, uses polarographic electrodes in electrolytic solution placed behind a polyethylene membrane that is placed on the skin. This transcutaneous method requires heating the skin, which alters tissue metabolism (9) and affects oxygen tension.

In this study we examined the effect of a reduction of ambient oxygen pressure on oxygen tension in the s.c. tissue of mice. A Ag/AgCl polarographic electrode placed in a hypodermic needle was used to measure s.c. oxygen tension. This method permits measurement of tissue oxygen tension in s.c. tissue without many of the problems previously encountered with polarographic electrodes. Advantages and disadvantages of this method are discussed.

Materials and Methods

Subcutaneous Tissue Oxygen Measurement

A Ag/AgCl combination Po_2 needle electrode with Ag/AgCl reference electrode (product no. 768-20R, needle combination oxygen electrode, Diamond

Electro-Tech Inc, Ann Arbor, MI) was used to measure tissue oxygen tension. Measurements of oxygen tension derived from electrical potentials of the electrode were read with a chemical microsensor system (Diamond General Corp, Ann Arbor, MI). These electrodes were placed in a hypodermic needle which was inserted through the skin in the back of an anesthetized mouse into the s.c. tissue. The electrode was positioned at the site the sponge was implanted in mice used in the wound-healing studies. For oxygen tension measurement, readings were taken 10 min after electrode insertion or the animal being placed in an altered gas mixture. If erratic changes in oxygen tension were observed, the animal was examined for misplacement of the electrode, and if the electrode had changed position the data were excluded. Readings were obtained from 5 animals in each of the following experimental groups:

1. acute exposure to 12% oxygen, 100 kPa absolute pressure;
2. chronic exposure to 12% oxygen (12 days), 100 kPa absolute pressure;
3. control exposure to 21% oxygen, 100 kPa absolute pressure;
4. acute exposure to 100% oxygen, 100 kPa absoulte pressure; and
5. acute exposure to 100% oxygen, 250 kPa absolute pressure.

Results were analyzed by one-way analysis of variance. Level for statistical significance was set at $P < 0.05$.

Control of Ambient Oxygen Pressure

For exposure to the designated gas environment, animals were placed in cylindrical polyvinyl chloride hyperbaric chambers (0.33 × 0.75 m) which were continuously ventilated at a rate of 5 liters · min^{-1} with the respective gas mixture. The hypoxic mixture (12% oxygen, 78% nitrogen) was produced by blending streams of nitrogen and air with a flowmeter. The boxes were ventilated at 1 liter · min^{-1} and gas tensions were monitored to ensure that carbon dioxide levels did not exceed 1.0%. Oxygen pressures of the air being exhausted from the chambers were monitored with a blood gas analyzer (model 178, Ciba-Corning, Medfield, MA) to ensure that correct gas composition was maintained. Monitoring of the oxygen pressures in the box revealed readings of 78–83 mmHg (slightly lower than the 91 mmHg calculated for 12% oxygen, due to metabolic consumption of oxygen by the mice).

The hyperbaric oxygen exposure required pressurization of the chamber, for which we used medical grade oxygen. Descent of the chamber was performed at a rate of 50 kPa · min^{-1}, which is a rate comparable to those used for human exposures. No untoward effect on the animals was noticed from the pressurization. The electrode was inserted before pressurization of the chamber with the animal in a 21% oxygen environment. The lead from the electrode inserted in the animals was passed through the hyperbaric chamber door. A seal was readily achieved by the use of putty around the electrical lead exiting at the door of the chamber.

Chronic Exposure to Hypoxia

For chronic exposure of the animals to a hypoxic environment the animals were kept in animal cages that were placed in ventilated clear plastic boxes (0.5 × 0.5 × 0.5 m) for control of atmospheric gases. Animals were maintained in this environment for 12 days with the only exposure to ambient air being the time required for opening the boxes for animal care, and for electrode insertion before s.c. oxygen tension measurement.

Animals

Female mice, 25–30 g (CF1, Harlan Sprague Dawley Co, Ind, MN) were used in all experiments to allow the placement of multiple animals in a single cage without cannibalism. The mice were kept in 29 × 19 × 13-cm animal cages, given food and water ad libitum, and were maintained on a 12 h light/12 h dark cycle. Mice were anesthetized before oxygen tension measurement using a s.c. injection of ketamine (50 $mg \cdot kg^{-1}$) and xylazine (20 $mg \cdot kg^{-1}$). Euthanasia was preceded by anesthetic injection and accomplished by cervical dislocation.

Results

Subcutaneous tissue oxygen tension rose in mice acutely exposed to high ambient oxygen pressures and fell in mice exposed to low ambient oxygen pressures, as depicted in Fig. 1. Mice exposed to the lowest ambient oxygen pressure (80 mmHg) had s.c. oxygen tensions of 17.4 ± 1.7 mmHg compared to normoxic controls with oxygen tensions of 34.8 ± 1.7 mmHg. Subcutaneous oxygen tension of mice in a 100% environment rose to 93.8 mmHg at normal ambient pressure (100 kPa), whereas hyperbaric pressurization (250 kPa) resulted in s.c. oxygen tensions as high as 589.0 ± 75.1 mmHg. A comparison of ambient oxygen pressure to s.c. oxygen levels shows that while increases in s.c. oxygen tensions coincided with an increase of ambient oxygen pressure (Table 1) this increase was not in direct proportion to the increase in ambient oxygen pressure. All s.c. oxygen tensions were significantly different from each other at all different ambient oxygen pressures. The chronic conditioning of mice at 12% oxygen for 12 days before measurement also did not alter s.c. oxygen tension (17.4 ± 1.7 mmHg) when compared to acute exposure of normoxically conditioned animals (19.0 ± 1.4 mmHg).

The readings of tissue oxygen tension were consistent because we measured normoxic mice at 3 separate times and the means ($n = 5$) of the three readings were within 1.2 mmHg of each other (34.6 ± 2.0, 34.8 ± 2.5, 35.8 ± 2.6). Immediately after inserting the electrode, tissue oxygen tension readings were erratic for approximately the first 5 min, then stabilized. Sometimes the readings became erratic again after stabilization. These erratic values were always associated with either movement of the animal and subsequent displacement of the electrode, or bleeding at the tip of the electrode. With

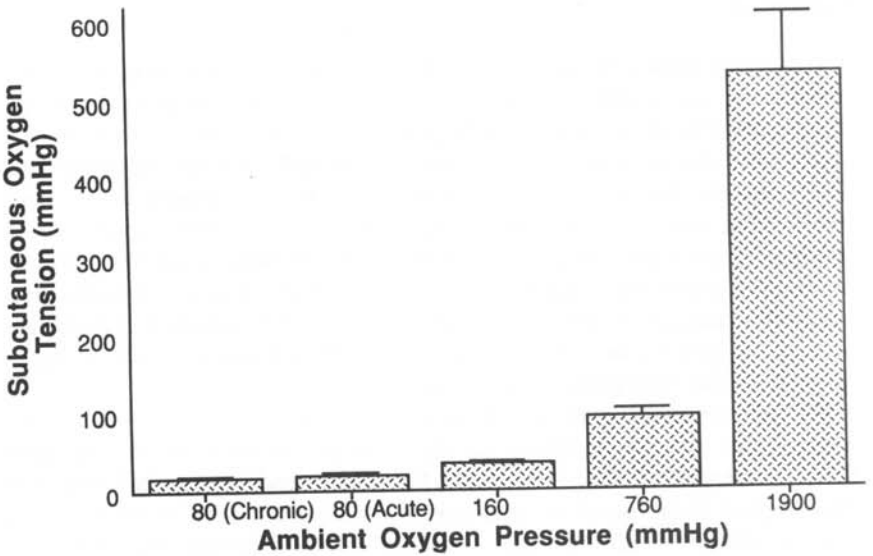

FIG. 1—Effect of different ambient oxygen pressures on s.c. oxygen tension in mice. *Vertical bars* represent the mean ± the standard error of the mean of the s.c. oxygen tension for 5 ambient oxygen treatment groups. Subcutaneous oxygen tension was significantly different at different ambient oxygen pressures, with the exception of the 80 mmHg chronic and acute exposure groups, which were not different from each other.

Table 1: Subcutaneous Oxygen Tension Expressed as a Function of Ambient Oxygen Pressure After Exposure

Ambient Oxygen		Subcutaneous Oxygen Tension, mmHg	Subcutaneous Oxygen Tension/Ambient Oxygen Pressure
Percentage of Oxygen	Pressure of Oxygen, mmHg		
12	80	19.0 ± 1.4	0.238
21	150	34.8 ± 1.8	0.218
100	760	93.8 ± 9.8	0.123
100	1900	589.0 ± 75.1	0.278

the anesthetic level employed in this study, movement of the animal was invariably noticed at least 30 min after electrode insertion. To determine if the readings we were measuring were real and not artifactual, we measured tissue oxygen tensions in several animals killed immediately after electrode insertion. Tissue oxygen tension fell immediately after death and remained at approximately 5–10 mmHg even when the animal was exposed to 100% oxygen.

Discussion

This study demonstrated that s.c. oxygen tensions can be repeatedly measured using special polarographic oxygen electrodes placed in a hypodermic needle. We found consistent readings for s.c. oxygen tension in normoxic mice. Values for s.c. oxygen tension in mice exposed to hypoxic and hyperoxic environments change with changing ambient oxygen pressure, as has been observed in rats (10) and humans (11). Since the s.c. tissue oxygen tension was not the same percentage of ambient oxygen pressure at different ambient oxygen pressures, we conclude that s.c. tissue oxygen was not directly correlated with changes in ambient oxygen pressure. This indicates that changes occurred in blood flow or tissue metabolism with alteration of ambient oxygen pressure at the hyperoxic tissue oxygen tensions.

We did not observe an adaptation to tissue hypoxia as seen in previous studies with rats (10) because s.c. tissue oxygen tension was not different between mice conditioned to hypoxia for 12 days and those acutely exposed. The adaptive response of s.c. tissue to systemic hypoxia seen in rats was most likely mediated by either changes in cutaneous blood flow or tissue metabolic rates. This adaptation was transitory, occurring between 5 and 15 days after insertion of the silastic tubing containing the polarographic electrodes, and could have been an inflammatory response to the tubing. Since we were unable to chronically measure s.c. oxygen tension, we cannot rule out some type of adaptive response to systemic hypoxia in mice.

Techniques for measuring tissue oxygen tension have been reviewed by Sheffield (6) and Hunt et al. (7). All have their disadvantages; many of the methods to study normal physiologic oxygen tension involve techniques that induce an inflammatory response. This alteration of the physiology of the area being measured may produce results that do not correspond to the true physiologic state of the tissue. The simple technique presented in this study requires minimal invasion of the tissue, is not excessively complicated or expensive, and gives a fairly accurate measurement of the actual tissue oxygen tension because it requires minimal disruption of the tissue.

This method has several disadvantages. It requires the animal to be anesthetized, which may alter physiologic and metabolic parameters affecting tissue oxygen tension measurement. Also, muscular activity by the animal may displace the electrode and affect measurement. This displacement may cause the needle to penetrate muscle or s.c. tissue and induce bleeding, which will affect electrode function and result in erratic values. This tendency for the animal to move limits the period of time in which valuable readings can be obtained to approximately 10–20 min after electrode insertion. Thus, this technique is not suitable for anything but a one-time measurement of tissue oxygen tension. Although the electrodes are protected by the hypodermic needle, these electrodes are fragile and frequently cannot be recalibrated after several uses.

The opinions or assertions are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Air Force or of the Department of Defense. This work was funded by a grant from the Air Force Office of Scientific Research #89-0543. The experiments reported herein were conducted according to the principles described in "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, U.S. Department of Health and Human Services Publication No. (NIH) 85-23.

Correspondence should be addressed to Major William J. Mehm.

References

1. Marsala M, Danieisova V, Chavko M, Hornakova A, Marsala J. Improvement of energy state and basic modifications of neuropathological damage in rabbits as a result of graded postischemic spinal cord reoxygenation. Exp Neurol 1989; 105:93–103.
2. Thom SR. Hyperbaric oxygen therapy. J Intensive Care Med 1989; 4:58–74.
3. Hunt TK. The physiology of wound healing. Ann Emerg Med 1988; 17:1256–1273.
4. Fajardo LF, Kowalski J, Kwan HH, Prionas SD, Allison AC. Methods in laboratory investigation: the disc angiogenesis system. Lab Invest 1988; 58:718–724.
5. Woldring S, Owens G, Erecinska M. Continuous in vivo recording of partial pressures by mass spectrography. Science 1966; 153:885–887.
6. Sheffield PJ. Tissue oxygen measurements. In: Davis JC, Hunt TK, eds. Problem wounds: the role of oxygen. New York: Elsevier Science Publishing Co, 1988:17–51.
7. Hunt TK, Rabkin J, Jensen JA, Jonsson K, Smitten K, Goodson WH. Tissue oximetry: an interim report. World J Surg 1987; 11:126–132.
8. Hunt TK. A new method of determining tissue oxygen tension. Lancet 1964; 2:1370–1371.
9. Sheffield PJ, Workman WT. Transcutaneous tissue oxygen monitoring in patients undergoing hyperbaric oxygen therapy. In: Huch R, Huch A, eds. Continuous transcutaneous blood gas monitoring. New York: Marcel Dekker, 1988:655–660.
10. Kivissairi J, Niinikoski J. Effects of hyperbaric oxygenation and prolonged hypoxia on the healing of open wounds. Acta Chir Scand 1975; 141:14–19.
11. Sheffield PJ. Tissue oxygen measurements with respect to soft-tissue wound healing with normobaric and hyperbaric oxygen. Hyperbaric Oxygen Rev 1985; 6:18–46.