2000-06-01

Identifying a site for maximum delivery of oxygen to transplanted cells

Shubjeet Kaur
University of Massachusetts Medical School

Et al.

TISSUE ENGINEERING
Volume 6, Number 3, 2000
Mary Ann Liebert, Inc.

Identifying a Site for Maximum Delivery of Oxygen to Transplanted Cells*

SHUBJEET KAUR, M.D., JOAQUIN CORTIELLA, M.D., and CHARLES A. VACANTI, M.D.

ABSTRACT

For *in vivo* cell implantation techniques to be successful, the energy and metabolic substrate requirement of the cells being grown must be met. Certain cells with high-energy requirements (*e.g.,* hepatocytes, pancreatic island cells) experience a high degree of cell death after implantation due to a limited supply of oxygen. We proposed that the pleural cavity might be an oxygen-rich environment and hence an excellent site for cell implantation. To test the hypothesis that the delivery of oxygen to the pleural cavity is directly proportional to the inspired oxygen concentration we measured the pO_2 of saline instilled in the pleural cavity as compared to that of the peritoneal cavity. We postulated that the physiologic basis for any difference was the result of direct diffusion of oxygen into the pleural space across the alveoli. The study was conducted on sheep ($n = 6$), after induction of general anesthesia, in two phases, control and experimental. Saline was instilled into the peritoneal and pleural cavities via catheters. after equilibration at given FiO_2, the pO_2 of the paline aspirated from the two cavities was compared. In the experimental group, animals were sacrificed (no circulation) and ventilated. The same sequence of steps as in the control phase were repeated. In the control group, the pO_2 of saline aspirated from the pleural cavity approached the arterial pO_2 at all FiO_2 levels. The pO_2 of the peritoneal saline aspirate fell over time. In the experimental phase (no circulation), the pO_2 of the pleural cavity saline rose to >400 mmHg. We conclude that this is a result of direct diffusion and is a potential source of unlimited oxygen supply not dependent on vascular supply.

INTRODUCTION

As applications for engineered tissues develop, one barrier to cell survival is optimization of the supply of oxygen to cells immediately upon implantation. Some cell types have a very low metabolic rate and will survive in a relatively hypoxic environment until a vascular supply can be established. Other cell types with high-energy requirements (*e.g.,* hepatocyte, pancreatic islet cells) may have a better survival if they are immediately implanted into an oxygen-rich environment. These cell types may experience a high degree of cell death if this oxygen requirement is not met. We tested the hypothesis that the pleural cavity

Department of Anesthesiology, UMass Memorial Medical Center, Worcester, Massachusetts.

*Presented in part at the Annual Meeting of the Association of University Anesthesiologists, Washington DC, April 17–20, 1997.

Table 1. Gas Analysis of Saline Samples Aspirated from the Abdominal Cavity as a Function of Time (FiO_2 0.70) in Control Group Animals

Time	*0 (mean ± SEM)*	*5 min (mean ± SEM)*	*25 min (mean ± SEM)*	*35 min (mean ± SEM)*	*45 min (mean ± SEM)*
pH	6.85 ± 0.070	6.8 ± 0.071	6.58 ± 0.047	6.51 ± 0.040	6.47 ± 0.028
pCO_2	20.16 ± 4.96	22.33 ± 5.68	74.33 ± 3.78	118.66 ± 5.23	154.83 ± 3.4
pO_2	102.83 ± 12.26	130.16 ± 17.74	9.66 ± 2.20	3.16 ± 0.401	2.66 ± 0.422

pO_2 and pCO_2 values reported are in mm of Hg.

may provide such an oxygen-rich milieu. Furthermore, it was previously believed that gas delivery to any body cavity is a function of its vascular supply.[1,2] We proposed that this is not the case, that instead oxygen diffuses directly through the lung tissue into the pleural space and its delivery is independent of vascular supply. If indeed this were true, the concentration of oxygen delivered to cell implants in the chest cavity (pleural space) could (1) be easily manipulated by adjusting the concentration of inspired oxygen, and (2) its supply would be virtually unlimited, similar to the environment in an incubator, with oxygen diffusing down a concentration gradient to the cells, as it is metabolized, rather than being dependent on blood supply that is disrupted and seriously impaired when the cells are implanted elsewhere in the body.

MATERIAL AND METHODS

The study design was reviewed and approved by our Institutional Animal Studies Review Committee in accordance with APS/NIH guidelines. A total of six sheep (average weight 50 kg) were studied in two phases; a control group was studied as described below. The same animals were then allowed to reequilibrate and then sacrificed and studied in an identical manner as the experimental group. In the control group, general anesthesia was induced with intramuscular xylazine and ketamine and the animals paralyzed with pancuronium. After endotracheal intubation (9.0 i.d.), the animals were placed on controlled ventilation (Ohmeda 7000 Ventilator) and anesthesia was maintained with isoflurane. A 14-gauge catheter was placed in the femoral artery and baseline arterial blood gas analysis was performed (Instrumentation Laboratories 16/40 pH, Blood Gas, Electrolytes Analyzer, Lexington, MA) while the animals were ventilated with 100% oxygen. A 20-gauge catheter (PerFix Epidural Anesthesia Set, Braun Medical Inc., Bethlehem, PA) was then advanced into the peritoneal cavity just lateral to the umbilicus. A similar catheter was placed in the right pleural cavity in the fifth intercostal space in the midaxillary line. The blood gas analysis was repeated to document that there was no change from baseline reflecting lung injury secondary to pleural catheter placement. Animals were then ventilated with an FiO_2 of 0.25%, and 50 cc of normal saline was injected via the previously placed catheters into each cavity. After allowing 10 min for equilibration (based on prior preliminary experiment data, not reported), saline samples (0.5 to 1 cc) were aspirated from each body cavity as well as the arterial catheter for analysis of pH, pO_2, and pCO_2. The sheep were then ventilated with increasing inspired oxygen concentrations (0.25, 0.45, 0.70, 100%) in a stepwise manner (control phase),

Table 2. Gas Analysis of Saline Aspirated from Pleural Space as a Function of Time (FiO_2 0.70)—Control Phase

Time	*0 (mean ± SEM)*	*5 min (mean ± SEM)*	*25 min (mean ± SEM)*	*35 min (mean ± SEM)*	*45 min (mean ± SEM)*
pH	6.9 ± 0.121	6.91 ± 0.034	6.88 ± 0.03	6.91 ± 0.047	6.91 ± 0.016
pCO_2	19.25 ± 1.662	20 ± 4.139	35.66 ± 2.34	40.66 ± 0.98	43 ± 0.955
pO_2	180.16 ± 17.98	157 ± 7.47	202 ± 2.37	349 ± 6.6	431.50 ± 3.46

pCO_2 and pO_2 values reported are in mm of Hg.

Table 3. Comparison of pO_2 (mmHg) of Pleural Versus Peritoneal Aspirates at Varying FiO_2 During Control Phase (Ventilation plus Circulation)

FiO2	*100% (mean ± SEM)*	*70% (mean ± SEM)*	*45% (mean ± SEM)*	*25% (mean ± SEM)*
Pleural aspirate				
pO_2	217.66 ± 20.36	209.00 ± 19.37	170.00 ± 13.69	188.60 ± 19.09
Peritoneal aspirate				
pO_2	62.50 ± 7.93	57.20 ± 8.93	77.40 ± 19.10	68.00 ± 13.30
p Value	$<0.001^*$	$<0.001^*$	$p = 0.004^*$	$<0.001^*$

$^*p$ value statistically significant.

allowing 5–10 min at each stage for reequilibration. The analysis of pH, pO_2, and pCO_2 was repeated at each inspired oxygen concentration. At an FiO_2 of 70% the pH, pO_2, and pCO_2 of both the pleural and peritoneal saline aspirate was analyzed at time points of 5, 25, 35, and 45 min. The animals were ventilated on room air with an FiO_2 of 0.25% until equilibration.

At this time the experimental phase of the study began. The animals were sacrificed by intravenous pentobarbital injection. Ventilation was continued with a stepwise increase in inspired oxygen concentration after cardiac arrest (no circulation). Saline samples were aspirated from each body cavity and analyzed for pH, pCO_2, and pO_2 for this phase of the study.

A detailed statistical analysis and comparison using Student's *t*-test was performed between the mean value of the pO_2 of the saline aspirate from the pleural versus the peritoneal cavity at four different inspired oxygen concentrations (for 0.25%, 0.45%, 0.75%, 100%).

RESULTS

The baseline values for arterial blood were a pH of 7.6 pCO_2 of 26 and a pO_2 of 148. For the pleural cavity saline aspirate, the pH was 7.15 pCO_2 22.9 and pO_2 was 226. The peritoneal saline aspirate showed a pH 7.18, pCO_2 18 pO_2 of 44.

In the control group (*i.e.,* animals with circulation and ventilation), the peritoneal saline aspirate analysis on an inspired FiO_2 of 70% was analyzed sequentially over a period of 45 min. The results are demonstrated in Table 1 and show that the pO_2 of the saline aspirated from the peritoneal cavity, during the control phase, decreased from a peak of 156 mmHg to 2 mmHg over 45 min. During the same time period of the control phase of the experiment, the pO_2 of saline aspirated from pleural space rose from 165 mmHg to 433 mmHg, as shown in Table 2. The pO_2 of the pleural cavity saline aspirate approximated or was greater than the arterial pO_2 at all FiO_2 levels. Comparison of the pO_2 of the saline aspirate from the pleural versus peritoneal cavity at various inspired oxygen concentrations during the control phase of the experiment is summarized in Table 3. The pleural saline pO_2 was consistently higher than the peritoneal aspirate and this difference was statistically significant at all levels of FiO_2 ($p < 0.001$).

In the experimental phase (ventilation without circulation) the pO_2 of the pleural aspirate, at an FiO_2 of .70% is shown (Table 4) in comparison to the pleural saline aspirate pO_2 at the same FiO_2 in the control phase (animal alive). The two values were comparable.

Table 4. Comparison of the pO_2 of Pleural Cavity Saline Aspirate at an FiO_2 of 70% in the Control Versus Experimental Phase ($p = 0.001$)[a]

	(mean ± SEM)
Control phase	209.00 ± 19.37
Experimental phase	367.00 ± 25.57

[a]Statistically significant.

DISCUSSION

It has been previously believed that gas delivery to any body cavity is dependent on its vascular supply.[1,2] Our findings refute this long-held belief. Our results demonstrate that the partial pressure of oxygen in the pleural space is a function of the inspired oxygen concentration and independent of circulation (vascular delivery). The pO_2 of the saline aspirated from the pleural cavity approached the arterial pO_2 at all FiO_2 levels. Increasing the inspired oxygen concentration can easily increase the concentration of oxygen in the pleural cavity. This can result in a virtually unlimited supply of oxygen in the pleural space. The pleural space may mimic the environment in an incubator and provide the ideal *in vivo* environment for growth of cells with a high metabolic need. This can result in a significantly higher survival ratio for cells implanted in this cavity.

In contrast to the pleural space, the pO_2 of the saline aspirate from the peritoneal cavity fell over time (possibly secondary to local consumption or diffusion into the intestines). Furthermore, in the experimental phase, even after the animal was sacrificed (*i.e.,* no circulation/vascular delivery, but ongoing ventilation), the pO_2 of the pleural cavity aspirate increased with increasing FiO_2. The high pO_2 of the pleural cavity aspirate in the second phase of the experiment can only be accounted for by direct diffusion of oxygen from the alveoli to the pleural cavity across a concentration gradient.

In conclusion, our study has identified a high oxygen availability site for *in vivo* cell growth and established direct diffusion to be the mechanism responsible for transfer of oxygen from the alveoli to the pleural space. The size of the pleural space (in the clinical setting of an expanded lung) may be a limiting factor for the cell mass that may be grown in it. Further experiments are needed to define this issue. Cells may be grown in the pleural space for subsequent transplantation in other body sites or as a permanent implant, *e.g.,* hepatocytes in the right pleural space. This opens areas for further investigation and research.

REFERENCES

1. Tenney, S.M., Carpenter, F.G., and Rahn, M. Gas transfers in a sulfur hexafluoride pneumoperitoneu m. J. Appl. Physiol. **6,** 201, 1953.
2. Eger, E.I., and Saidman, L.J. Hazards of nitrous oxide anesthesia in bowel obstruction and pneumothorax. Anesthesiology **26,** 61, 1965.

Address reprint requests to:
Shubjeet Kaur, M.D.
Department of Anesthesiology
UMass Memorial Medical Center
55 Lake Avenue, North
Worcester, MA 01655

E-mail: shubjeet.Kaur@umassmed.edu