The Effect of Prior Pulmonary Injury on the Rate of Development of Fatal Oxygen Toxicity

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The oxygen tolerance limits of normal man and experimental animals are largely known. However, toxic oxygen concentrations are seldom administered to patients without prior pulmonary injury, since the primary utility of high oxygen concentrations in clinical medicine is to overcome the systemic hypoxia arising from acute respiratory insufficiency. Virtually nothing is known about the pulmonary response to toxic oxygen concentrations in the presence of prior pulmonary damage. It would seem logical that such forms of injury would be at least additive. However, it is clinically evident that some patients in acute respiratory failure appear to be resistant to potentially toxic concentrations of oxygen. Furthermore, experimental work indicates that phosgene gas and oxygen (prior intermittent exposure to hyperoxia) both cause structural changes in the lung and delay the rate of development of fatal oxygen toxicity at 1 atm.

Methods

This study was designed to investigate the oxygen tolerance of rabbits with prior pulmonary injury induced by the intravenous injection of oleic acid. Investigations were carried out on male, adult New Zealand rabbits who were exposed to 100 percent O₂ in a large, double-lock exposure chamber. Oxygen, carbon dioxide, humidity and temperature were monitored and controlled. Food and water were available ad lib. A marginally sublethal dose of oleic acid was determined to be 0.25 ml/kg. This insult-induced blood gas derangement is indicated in Table 1.

Comparisons were made of survival times in oxygen following various intervals between injection and exposure, pulmonary pathology and histology. Each animal was removed from oxygen immediately after death, and the lungs fixed with 10 percent neutral buffered formalin. Slides were stained with H&E, osmic acid, PTAH, VVG, and Gomori’s trichrome.

Animals were exposed to oxygen under five experimental conditions: (1) control, no oleic acid injection; (2) immediately (within 2 hr) following 0.25 ml/kg oleic acid; (3) 24 hr following 0.12 ml/kg oleic acid; (4) 24 hr following 0.25 ml/kg oleic acid; and (5) 7 days following 0.25 ml/kg oleic acid.

Results

Survival data are depicted in Table 2 and Figures 1, 2, and 3.

Table 1—Arterial blood gas levels in ten rabbits spontaneously breathing air before and 24 hours after injection of oleic acid and comparison of blood gas levels in two groups of rabbits spontaneously breathing 100% oxygen (means ± SE)

<table>
<thead>
<tr>
<th>Rabbits, No.</th>
<th>Air</th>
<th>Oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>preinjection</td>
<td>24 hr after</td>
</tr>
<tr>
<td></td>
<td>oleic acid IV</td>
<td></td>
</tr>
<tr>
<td>P₁O₂, torr</td>
<td>79.8 ± 1.0</td>
<td>48.8 ± 3.5*</td>
</tr>
<tr>
<td>P₁CO₂, torr</td>
<td>29.8 ± 0.9</td>
<td>30.5 ± 1.4</td>
</tr>
<tr>
<td>pH, units</td>
<td>7.43 ± 0.01</td>
<td>7.4 ± 0.01</td>
</tr>
<tr>
<td>*p &lt; 0.001.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2—Survival Data of Rats Placed in Oxygen

<table>
<thead>
<tr>
<th>Oleic acid dosage, ml/kg</th>
<th>Control</th>
<th>Test</th>
<th>Test</th>
<th>Test</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time lapse before exposure to O₂</td>
<td>—</td>
<td>0.12</td>
<td>0.25</td>
<td>0.25</td>
<td>—</td>
</tr>
<tr>
<td>Placed into O₂</td>
<td>20</td>
<td>24 hr</td>
<td>0</td>
<td>24 hr</td>
<td>7 days</td>
</tr>
<tr>
<td>Survival in O₂ hr</td>
<td>82.7 ± 5.4</td>
<td>106.8 ± 16.7</td>
<td>143.7 ± 21.9*</td>
<td>164.4 ± 17.6*</td>
<td>92.4 ± 4.6</td>
</tr>
</tbody>
</table>

*Significantly different from control value p < 0.001 (means ± SE)

A brief summary of the histologic findings indicates:
(1) Injected (0.25 ml/kg), air exposed (24 hr delay)—lesions were predominantly peripheral and subpleural in location. There was striking proliferation of granular pneumocytes lining alveolar septa. Also seen was early proliferation of fibrous tissue and collagenization. (2) Uninjected, oxygen exposed—changes were generalized, and showed predominant pink amorphous membranes lining alveoli and bronchioles, interstitial and intra-alveolar edema and hemorrhage were present as were mononuclear cells. Fibrous proliferation and collagenization were absent. (3) Injected, oxygen exposed (24 hr delay)—histologic changes seen in this group were a composite of the previous two. Superimposed on the general exudative features of hyaline membranes, edema and hemorrhage, were type 2 cell hyperplasia and fibroproliferation. (5) Injected, oxygen exposed (seven-day delay)—exudative changes were essentially absent. Type 2 cell hyperplasia was present but not more dominant than at 24 hr. Fibroproliferative changes were more marked and of a higher degree of organization.

DISCUSSION

It is apparent from the data that tolerance to toxic levels of oxygen is not reduced by prior pulmonary injury. Indeed, there is evidence that there is a dose-dependent temporal protection against death from oxygen toxicity by such prior damage. The interval between injury and the onset of oxygen exposure would seem to be of prime importance in the degree of such protection. If exposure to oxygen is initiated very shortly after injury, protection is markedly present. A 24-hr delay between injection and exposure increases the degree of protection, but not significantly. If one week is allowed to elapse between injection and exposure, all evidence of protection has vanished.

We predicted that the observed protection would be found, and have hypothesized that it was due to the alteration in pulmonary morphology found in response to virtually any diffuse injury. Specifically, we presumed that granular (type 2) pneumocytes would be induced by initial injury and would, subsequently, be more re-

Rabbits breathing 100% O₂ at 1 ATM

![Graph](http://example.com/graph.png)

FIGURE 1. Prior injury of lungs of rabbits by injection of oleic acid increases, not decreases survival time in 100 percent O₂ at atmospheric pressure. Increase in tolerance appears to be dosage related, with the greatest protection afforded by a marginally sublethal dose, which doubles survival time.

Rabbits breathing 100% O₂ at 1 ATM

![Graph](http://example.com/graph2.png)

FIGURE 2. Marked and significant increase in oxygen survival time is seen if oxygen exposure is begun almost immediately after injury. Slightly greater protection is afforded if 24-hr delay is allowed between injury and exposure, but difference is not statistically significant.
Dr. Kilburn: Did you look at mortality of control rabbits at arterial oxygen tensions which had been produced by the oleic acid induced lung damage (namely 320 torr) in your experimental rabbits? You are really comparing two levels of oxygen. What is the mortality in control rabbits at 320 torr as compared to oleic and treated rabbits at 320 torr?

Dr. Winter: Are you referring to arterial or alveolar oxygen?

Dr. Kilburn: Arterial.

Dr. Winter: They are all breathing the same oxygen level.

Dr. Kilburn: That's the point! I don't think you have a control for the oleic acid exposed group. You are assuming that what is important is alveolar oxygen tension, but you could just as well assume what is important is arterial oxygen tension.

Dr. Winter: I presume that the work of Ashbaugh and the work of Miller had eliminated the arterial oxygen level as an etiologic factor in the development of pulmonary toxicity.

Dr. Kilburn: You are dealing with death as an important index of oxygen toxicity and you are making an awfully broad assumption that death is due to pulmonary oxygen toxicity. I don’t see that you have any evidence for that.

Dr. Winter: The pulmonary changes are classic for oxygen toxicity changes.

Dr. Weibel: This is a very interesting study and could shed some light on some of the mechanisms that are involved in normal oxygen toxicity. I think you have used too large an interval (seven days) in your last experiment.

Dr. Winter: I was hoping you would say that.

Dr. Weibel: I would suggest that you should analyze the subsequent changes of the epithelium and the endothelium at an earlier time point. In the rat I think you would get peak changes after approximately two or three days; after this time you should have a nice new population of epithelial cells. This could show whether repair of tissue is an important protective effect against oxygen toxicity.

Have you looked to see if there was any effect of oleic acid on the red cells? I don’t know if you remember the work in which we proposed the hypothesis that the primary damage of endothelial cells in rat lung from oxygen toxicity might be due to an interaction of the primary damage to red cells and the high oxygen tension there. Then Dr. Kilburn’s argument would be pertinent.

Dr. Winter: We did not follow that line of reasoning as it had been demonstrated to our satisfaction that at one atmosphere the level of arterial oxygen tension played no role in the rate of development of pulmonary damage. Therefore, we didn’t investigate that further.

Dr. Evans: I’m interested in knowing at what time the proliferative response occurs during exposure. In our studies we found that during continuous exposure to NO2, tolerance to the NO2 developed. Initially there was injury, followed by division of cells and repair of the damaged tissues. Despite continuous exposure there was no further injury to the tissue and the labeling indexes.
returned to control levels. We also did an experiment involving a very brief exposure to NO₃. The same pattern of events was seen, injury followed by cell division and repair in about the same time sequence as during continuous exposure. We have been working on re-exposure of these animals to see if tolerance would develop with this brief exposure and if the tolerance is associated with new cells that repaired the damaged tissues.

Dr. Ashbaugh: Somewhere in the literature on oleic acid, and I really don’t remember where I read this, it is stated that by repeatedly injecting small doses of oleic acid you can build up tolerance to oleic acid damage in the lung. You then can give progressively larger and larger doses without killing the animal.

In some experiments we did several years ago on chronic oxygen toxicity in dogs, we found that a few of our animals (and it was impossible to pick these animals out beforehand) would reach a state where they could then be kept at 100 percent oxygen (which at Denver is 575 torr) for many days. They reached a certain state of chronic exposure where they did not get worse. We were taking serial lung biopsies for light and electronmicroscopy in these animals. The pathologic changes reached a peak in seven to ten days and then persisted at that same level throughout their exposure. After return to room air breathing, it took about six months before the animals that survived the oxygen exposure reverted to their normal lung anatomy. Evidently acute changes develop which are partially protective, and then a chronic change occurs which may be protective to an even greater extent.

Dr. Winter: I think that Dr. Boatman would attest to the fact that a similar phenomenon occurs with ozone; when you injure the lung with sublethal doses of ozone, then challenge such animals with what usually would be a lethal dose of ozone, they survive longer than “normal” animals. Isn’t that correct?

Dr. Boatman: We really have not used lethal doses. What we have shown is that tolerance develops. You see certain morphologic changes if you give a medium dose of ozone, say 1 ppm for three hours. If you give several consecutive doses, which in their totality would be substantially higher than 1 ppm, you don’t see any increase in cellular damage, so the first dose amounts to imparting tolerance to subsequent doses.

Effect of 48- and 72-Hour Oxygen Exposure on the Rabbit Alveolar Macrophage*

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Patients exposed to high concentrations of oxygen for prolonged periods may be at increased risk for superimposed bacterial pneumonia. Recently, McCarthy et al. showed delayed bacterial clearance from mouse lungs after oxygen exposure. In the present experiments, we studied alveolar macrophage function and structure to determine whether O₂ damage to these cells might be responsible for the delayed clearance.

**METHODS AND RESULTS**

Rabbits, treated 12 days previously with intravenously administered BCG on two successive days, were placed in a chamber filled with 95 to 100 percent O₂ for 48 (nine experiments) or 72 (ten experiments) hours. The Pco₂ in the box was <4 mm Hg. Drinking water was provided ad lib, but food was not given in order to prevent fluctuation in dietary conditions between oxygen toxic and control animals. In 14 control experiments, BCG-injected rabbits were placed in the exposure chamber which was left open to the atmosphere.

Of 37 rabbits exposed to 100 percent oxygen, six died at varying intervals during the exposure (Fig 1). Many of the animals surviving the 72-hour exposure showed “air hunger” following removal from the oxygen chamber. The cumulative 72-hour mortality for O₂ exposure was 26. There were no spontaneous deaths among the 23 rabbits in the 14 control experiments.

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At the end of the exposure period, the animals were killed, and cells were obtained from the lung by modification of the technique of Myrvik et al. Cells were not lavaged from animals that died spontaneously. The cell yield varied between 1 x 10⁶ and 3 x 10⁶ cells per rabbit. With both oxygen exposed and control rabbits, more than 93 percent of the cells obtained had morphologic characteristics of alveolar macrophages (AM) by light microscopy of Wright-stained smears. More than 90 percent of the cells obtained from all rabbits were viable based on exclusion of eosin. Electronmicrographs of glutaraldehyde-fixed cells showed no differences between macrophages from control, 48-hour, and 72-hour O₂-exposed rabbits.

Rates of glucose utilization and lactate, pyruvate, and

**FIGURE 1. Mortality for 37 rabbits exposed to 100 percent oxygen. Bars represent percentage of rabbits at risk that died during each exposure period. Zero to 72-hour value is sum of mortality for other three periods.**

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