

TITLE: ELECTRON MICROSCOPIC LOCALIZATION OF ANTIOXIDANT ENZYMES IN RAT LUNG.
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Introduction. Antioxidant enzymes appear to be critical to the adaptive response of the lung to oxidant stress. Antioxidant enzymes may also play pivotal roles in the pulmonary development of tolerance to lethal hyperoxia that occurs following various pretreatments with agents such as sublethal hyperoxia, hypoxia, or endotoxin. Furthermore, supplementation therapies are currently being developed to blunt oxidant damage using liposome- or PEG-entrapped antioxidant enzymes. These points emphasize the importance of defining the tissues and cells of the lung in terms of their constituent antioxidant enzyme content. We report the results of experiments to develop optimal techniques for light (LM) and electron microscopic (EM) labeling of lung tissue from normoxic rats using polyclonal antibodies to Mn and CuZn superoxide dismutases (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST) and catalase. GST, while technically not an antioxidant enzyme, is significant to glutathione metabolism and, therefore, maintenance of cellular redox states.

Materials and Methods. Following anesthesia, lung tissue from adult male Holtzman or Sprague-Dawley SPF rats was fixed for 30 or 60 minutes using a variety of fixatives. Samples were processed for cryoultramicrotomy (cryo) or embedding in paraffin, epoxy resin, or Lowicryl K4M. Multiple EM and LM tissue sections were incubated overnight with rabbit antisera for each antigen and then further processed for immunogold (EM) or horseradish peroxidase (LM)

secondary labeling. The antisera used have been characterized previously in terms of antigen specificity by Western blot analysis.

Results. Paraffin sections demonstrated best labeling intensity in tissue fixed with neutral buffered formalin (NBF), as compared to glutaraldehyde or paraformaldehyde (PF) fixation. At the LM level of parenchymal alveolar tissue, all antisera showed predominant labeling of the epithelium of small bronchioles and slight labeling of septal wall structures. For EM, variable results were obtained depending on the enzyme studied. It was found that short NBF fixation provided good histological preservation of cellular detail at the EM level. Mn SOD and catalase gave best labeling intensity with either PF or NBF fixation and embedding in K4M, comparable to fixed tissue sectioned by cryo. Mn SOD label was largely confined to mitochondria, predominantly in Type II pneumocytes. Catalase was also found mainly in Type II cells, generally enclosed in small vesicles. CuZn SOD was best labeled using PF fixed tissue sectioned by cryo; substantially less label was apparent in tissue embedded in epoxy resin and even less was observed in K4M. This SOD was mainly found in Type I and II cell heterochromatin and Type II and endothelial cell cytoplasm. The glutathione enzymes were best demonstrated with PF fixation and embedding in K4M. Both GPx and GST were predominantly found associated with elastin in the connective tissue of the alveolus.

Discussion. This is the first description of antioxidant enzyme localization in the lung at the subcellular level. A variety of fixation and embedding techniques was necessary for optimal demonstration of antioxidant enzymes. EM localization of the antioxidant enzymes demonstrated significant levels undetectable by LM methods. The description of the antioxidant enzyme content of specific pulmonary cell types may be critical to a better understanding of the mechanism of oxidant stress damage, hyperoxic adaptation and tolerance, or possible protective effects with parenteral treatments.

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TITLE: THE EFFECT OF EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO) ON CEREBRAL BLOOD FLOW (CBF) AUTOREGULATION IN THE NEWBORN LAMB.
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ECMO is used to support neonatal patients with profound cardiopulmonary failure. The most significant morbidity associated with ECMO is intracranial hemorrhage. Impairment of CBF autoregulation may be a factor in this devastating event. We therefore, undertook this study to examine the effects of ECMO on CBF autoregulation in a newborn lamb model. Lambs (1-7 days) were anesthetized with pentobarbital and ventilated. Catheters were placed in the left facial artery (reference catheter), sagittal sinus, lateral ventricle, and femoral artery and vein. CBF was measured by the radioactive microsphere technique. In the ECMO animals (n=7), the right carotid artery (CA) and jugular vein (JV) were ligated and ECMO

cannulae placed. In the control group (n=5), the CA and JV were ligated and a left ventricular catheter inserted. The ECMO animals were placed on 120-150 cc/kg/min of ECMO flow over 30 min. After 1 hr of stabilization, baseline measurements were made (mean arterial pressure, intracranial pressure, sagittal sinus pressure, arteriovenous O₂ contents) and microspheres injected. The cerebral perfusion pressure (CPP) was lowered in a stepwise fashion by ventricular infusion of artificial cerebrospinal fluid and studies obtained 5 min after a change. In the control group, measurements were begun 90 min after the completion of surgery. Data were analyzed using one way ANOVA. All values were expressed as mean \pm SEM.

CPP was lowered from 77 \pm 5 to 21 \pm 6 mmHg in the control group. CBF did not significantly change over this range of CPP. Blood flow to the right hemisphere (ligated CA) was not significantly different from blood flow to the left hemisphere (no ligation). In the ECMO group, CPP ranged from 64 \pm 14 to 24 \pm 4 mmHg. In contrast, CBF fell linearly as CPP decreased and blood flow to the ligated side fell faster than the non-ligated side. We conclude that ECMO alters CBF autoregulation. Once CBF autoregulation is lost CA and JV ligation may further impair CBF.