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Superoxide dismutase and tolerance to pulmonary oxygen toxicity

JD Crapo

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A M E R I C A N C O L L E G E O F



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Electron microscopy was performed by Dr. I.Y.R. Adamson, Department of Pathology, University of Manitoba.

Discussion

Dr. Lynn: Have you measured the amount of the drug left in the tumor at the end of five months?

Dr. Klass: No, we have no means of measuring urethane. We don't know if the drug is directly carcinogenic or whether it is carcinogenic by some secondary route such as impairment of cellular immunity or induction of a virus.

Dr. Massaro: I wonder if in fact you did on a cellular basis find increased incorporation because you expressed it per wet weight. Perhaps if you had measured incorporation differently, such as DNA, the rates may have been reduced.

Dr. Klass: We were reluctant to use DNA as a basis for the incorporation studies because we found there was a change in the DNA in these tumors.

Dr. Tierney: Have you been able to isolate a surface active material from these tumors which do contain inclusion bodies similar to type 2 cells?

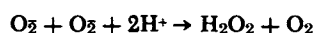
Dr. Klass: Unfortunately, we do not have enough tumor tissue to do this.

SESSION 7: EFFECTS OF EXPOSURE TO OXYGEN

Superoxide Dismutase and Tolerance to Pulmonary Oxygen Toxicity*

James D. Crapo, M.D.

In order to both tolerate and utilize oxygen in the environment, animals had to develop defenses against the toxic products of aerobic metabolism. One such product is superoxide (O_2^-), produced by the univalent reduction of oxygen by many biologic reactions. Even low levels of this very reactive free radical are presumed to be inimical to the integrity of the living cell. The primary defense against superoxide appears to be an enzyme which catalyzes the reaction



and which has been given the name superoxide dismutase (SOD).¹ It is an enormously efficient catalyst which operates at rates approaching the theoretic limits of diffusion. The important role of this enzyme in protecting against the toxic effects of oxygen is suggested by the fact that it is ubiquitous among oxygen metabolizing cells and is lacking in obligate anaerobes.² Exposure to high oxygen tensions leads to production of increased amounts of O_2^- by some enzymatic reactions *in vitro*;³ a similar response may occur in lungs of animals. If this increase in superoxide production were enough to overwhelm the natural defense mechanisms of the cell it

would undoubtedly contribute to the development of oxygen toxicity.

It has been shown that large rats exposed to 100 percent oxygen almost all die within 72 hours. If rats are first exposed to 85 percent oxygen for seven days they develop "tolerance" and can then survive in 100 percent oxygen for prolonged periods. It has been shown that at the same time tolerance develops, the activity of pulmonary SOD increases about 50 percent. The rate at which rats exposed to 85 percent oxygen acquire tolerance is parallel to the time course for increased SOD activity. The rate at which tolerance is lost when these rats are returned to air correlates closely with the decline of SOD activity to normal levels.⁴

Two structurally different forms of SOD exist: the mitochondrial and the cytosol forms.⁵ While the cytosol SOD is present in the largest amounts, either one or both of these enzymes could be responsible for the change in SOD activity in lungs of oxygen exposed rats. Also, the change in SOD activity could have been due to the presence of increased amounts of the enzyme or to an enhanced specific activity.

MATERIALS AND METHODS

To study these questions, the cytosol SOD was purified from rat liver and rabbit antisera specific for rat cytosol SOD was prepared. Rats weighing 300 gm were exposed to 85 percent oxygen and antibody titrations were performed on the supernatant fraction of lung homogenates. The end point of the titrations was determined by electrophoresis on agarose gels which were then stained for SOD with nitroblue tetra-

*National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Table 1—SOD, Tolerance, and Alveolar Type 2 Cell Changes During Exposure to Oxygen

	Days of exposure to 85% oxygen			
	0	3	5	7
SOD activity assay (Units/whole lung)*	811 ± 105	890 ± 121	1246 ± 76	1230 ± 307
Antibody titration for cytosol SOD (μg/whole lung)	109 ± 7			155 ± 13
Percentage of animals tolerant to 100% O ₂	0	70	100	100
No. alveolar type 2 cells/0.46 mm ²	29 ± 11	22 ± 9	79 ± 12	90 ± 3

All values are ± SD

*1 unit = 50% inhibition of a 2.5 ml epinephrine assay⁶

zolium. Total SOD activity determined by the epinephrine-adrenochrome assay⁶ increased 52 percent after seven days of exposure (from 811 ± 105 to 1230 ± 307 units/whole lung) (Table 1). Antibody titrations for the cytosol form of SOD showed a 42 percent increase (from 109 ± 7 to 155 ± 13 μg/whole lung). Therefore, continuous exposure of rats to 85 percent oxygen leads to an increased activity of SOD and this can at least in part be explained by an increase in the absolute amount of the cytosol form of SOD.

The increased amounts of SOD produced during exposure to hyperoxia may be due to induction of the enzyme or to proliferation of a SOD rich cell type. Ultrastructure studies on mammals exposed to hyperoxia have shown proliferation of alveolar type 2 cells.⁷ This histology of rats exposed to 85 percent oxygen was studied by both light and electron microscopy. The lungs were fixed by intratracheal infusion with glutaraldehyde and were embedded in epon. They were sectioned at 0.5 μ intervals and stained with toluidine blue for light microscopy. Using an eyepiece with a calibrated grid, the number of alveolar type 2 cells were counted in an area of .46 mm² in each section. A 300 percent increase in alveolar type 2 cells that could be identified by light microscopy was found to occur at the same time that tolerance developed and that SOD was shown to increase (Table 1). Electron microscopy was used to confirm the quantitative measurements made by light microscopy and its findings suggested that the light microscopy techniques may underestimate the true increase in the number of alveolar type 2 cells occurring in this experimental model. The increased viability of alveolar type 2 cells during exposure to oxygen may be related either to a greater initial content of SOD or to induction of increased amounts of SOD.

RESULTS

These studies have shown that during exposure of rats to sublethal doses of oxygen SOD activity is increased and this increase closely correlates with the development of tolerance to 100 percent oxygen. A significant portion of the increase in SOD has been shown to be due to an increase in the absolute amount of the cytosol form of SOD. Alveolar type 2 cells have been shown to proliferate at the same time that tolerance develops and that SOD is increased, which is consistent with the postulate that this adaptive response to oxygen may be specifically located in the alveolar type 2 cell. The inferred relationship of SOD and the alveolar type 2 cells now requires further experimental elucidation.

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Discussion

Dr. Massaro: Do you see a difference between young and old rats?

Dr. Crapo: No, there is not.

Dr. Caughey: There is a great debate going on in the hemoglobin business these days concerning the fact that oxygen is really O₂⁻ when it's bound to hemoglobin. What I would like to know is: is it clear that superoxide itself is really so extremely toxic?

Dr. Crapo: You're right that we don't have good conclusive evidence for what it does. It's a brand new species and techniques for study have only been available for three or four years. It has been shown that superoxide and hydrogen peroxide together can produce the peroxy free radical. This free radical is the most potent free radical known and it may be that most of the toxic effects of superoxide are in fact not due to superoxide itself but due to the peroxy radical.

Dr. Kuepper: Does your antibody inhibit the SOD activity?

Dr. Crapo: It does to a small degree but antibody-enzyme complexes are enzymatically active.

Enzymatic Activity in Rat Lungs. Some Changes with Exposure to 1 ATM Oxygen*

Donald F. Tierney M.D.,** Josephine Yang, M.D., and Larry Ayers, M.D.

Currently, changes of lung metabolism with oxygen "tolerance" are being determined with the goal of relating them to the mechanisms of pulmonary oxygen

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