exposure to halothane. The ciliary activity after exposure to increasing concentrations of halothane showed that at 1 percent and 2 percent halothane there was no impairment, while 3 percent halothane caused some ciliostasis. Dramatic reduction in the ciliary activity occurred at halothane concentrations of 4 percent and 5 percent. Results of recovery of ciliary activity of tracheal rings exposed to 4 percent halothane concentrations showed that there was immediate and complete recovery for as long as three days after the removal of the anesthetic. After four days of exposure, recovery was incomplete, and after one week no recovery of ciliary activity was noted with death of respiratory epithelial cells.

**DISCUSSION**

Pulmonary alveolar macrophages (PAM) play a key role in the antibacterial defenses of the lung. Their activity is modulated by both humoral and cellular immunity. Phagocytosis and intracellular killing of bacteria by the PAM is an active energy-utilizing process, dependent entirely on aerobic oxidation. Halothane is an agent known to depress oxidative phosphorylation. Also, the microtubular structures of cells are distorted by halothane and their integrity is essential for phagocytosis. Therefore, it could be argued from a theoretic point of view that halothane will depress pulmonary macrophage function.

However, a previous study by Goldstein et al. concluded that halothane failed to depress PAM, whereas methoxyflurane and cyclopropane did depress their activity. Our results are in variance with these data, and suggest halothane does cause significant depression of lung antibacterial activity. This finding is consistent with the anesthetic action of halothane and methoxyflurane at a sub-cellular level. The in vitro organ culture model of tracheal rings has the advantage of assessing the effects of prolonged exposure to halothane on ciliary activity. Our studies showed that halothane concentrations of 4 percent or more impaired ciliary activity and prolonged exposure was cytotoxic after several days. Halothane exposure of less than four days was followed by complete recovery of ciliary activity. Since in clinical practice 4.0 percent halothane concentrations are rarely used, and then only briefly for induction, we conclude that at the usual concentrations of halothane there is minimal depression of ciliary activity, which is reversible after termination of the exposure.

**REFERENCES**


**DISCUSSION**

Dr. Rylander: Have you considered that the effect of anesthesia on the incidence of pneumonia may be caused by the inhalation of mucus? There were some papers about 15 years ago which showed that instillation of pure mucus down into the alveolar region very severely depressed bacterial action.

Dr. Manawadu: I do not know whether anyone has done any work on the effects of anesthetic agents on the mucus.

Dr. Repine: Does halothane or its products remain in the lung and later the multiplication rate of the bacteria independently of host defense mechanisms?

Dr. Manawadu: Yes, it might have an effect because halothane per se inhibits bacterial multiplication.

**Impairment of Human Alveolar Macrophage Oxygen Consumption, and Superoxide Anion Production by Local Anesthetics Used in Bronchoscopy**

John R. Hoidal, M.D.; James G. White, M.D.; and John E. Repine, M.D.*

Bronchoscopic subsegmental lavage is used extensively to obtain human alveolar macrophages (AM) for immunologic studies. These cells are large phagocytic cells which closely resemble bone marrow macrophages in morphology, size, and functions. They can be obtained by using a bronchoscope to wash the lung tissue with saline solution. The AM are then separated from the lungs by centrifugation and washed. This procedure results in the removal of inflammatory cells and red blood cells. The AM are then resuspended in a tissue culture medium and studied in vitro for their immune functions.

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study. Two cationic local anesthetics, lidocaine (LDC) or tetracaine (TRC), are commonly used during bronchoscopic procedures to anesthetize the airways. Recent investigations have shown that these drugs alter membrane-dependent responses of a variety of cells. Therefore, it would be important to determine the concentration of these agents in the lavage effluents and their potential effect on the metabolic activities and morphology of lavaged AM. In the present investigation, we measured the concentration of LDC present in the lavage effluents, and determined the effect of these agents in vitro on the structure and function of human AM. The results indicate that appreciable amounts of LDC are present in bronchopulmonary lavage effluents and comparable pharmacologic doses of LDC or TRC in vitro inhibit the oxidative metabolism of human AM and cause striking surface changes in human AM.

MATERIALS AND METHOD

Human alveolar macrophages were obtained by subsegmental sterile saline solution lavage of normal volunteers (21), as well as nonsmokers (9), or smokers (26) undergoing diagnostic fiberoptic bronchoscopic procedures. Topical anesthesia of the airways was achieved by spraying a maximum of 7 ml of 4 percent LDC into the upper airways and by flushing 2-3 ml boluses of 1 percent LDC through the inner channel of the bronchoscope to anesthetize below the vocal cords. A maximum of 30 ml of 1 percent LDC was used for the procedure. Lavage was performed by injecting 60 ml aliquots of sterile saline solution through the bronchoscope which was recovered by gentle suction. AM were then separated by centrifugation, resuspended in Hank's balanced salt solution (HBSS) and counted. LDC concentrations in the lavage effluents were determined by sensitive gas chromatographic analysis.4 Oxygen uptake (biologic O2 probe technique) and superoxide anion (O2\textsuperscript{-}) release (superoxide dismutase inhibitable reduction of cytochrome C methodology) were measured for unstimulated AM or AM stimulated by 100 opsonized heat-killed S aureus (HKB) AM [100 opsonized heat-killed bacteria/AM] or the membrane-active chemical, phorbol myristate (PMA).4 In some experiments, HBSS containing various concentrations of LDC or TRC was substituted for HBSS. Morphology of washed AM was evaluated in the presence or absence of LDC or TRC using standard techniques of scanning electron microscopy (SEM) or transmission electron microscopy (TEM).7

RESULTS AND DISCUSSION

Our first goal was to determine the range of concentrations of LDC to which AM were potentially exposed in the lavage fluid. Measurable levels of LDC were uniformly present in lavage returns, but the concentrations varied widely. The mean concentration of LDC present in the fluid recovered from the initial 60 ml aliquot from eight subjects was 5.5 mM (range 0.5 mM to 11.5 mM). Significantly (P < 0.01) lower concentrations of LDC were present in subsequent lavage effluents. The mean concentration present in the second lavage effluent was 0.6 mM and the mean concentration present in the remainder of the saline solution recovered was 0.4 mM.

Our second goal was to determine the effect of LDC or TRC on O2 consumption and O2\textsuperscript{-} release by unstimulated or stimulated AM. LDC (2-16 mM) or TRC (0.5-16 mM) in a dose-dependent manner rapidly reduced O2 uptake and O2\textsuperscript{-} release by unstimulated or stimulated human AM (Fig 1). The effects were produced by concentrations of LDC which were present in the lavage effluents, but were more marked at higher concentrations. LDC (16 mM) decreased O2 consumption by unstimulated or stimulated AM by 67 percent or 74 percent respectively (P < 0.001). Similarly, LDC inhibited O2\textsuperscript{-} release by unstimulated or HKB-stimulated AM by approximately 80 percent (P < 0.01). In a comparable pattern, TRC in a dose-dependent manner progressively reduced O2 uptake and O2\textsuperscript{-} release by human AM. The degree of inhibition of metabolism by 2 mM TRC was similar to that by 16 mM LDC. Additional experiments showed that in concentrations used LDC or TRC were not cytotoxic to human AM since their inhibitory effect on O2 consumption was reversed by washing AM in HBSS.

Our third goal was to assess the influence of cationic anesthetics on the ultrastructure of human AM. Examination of control AM and samples of AM which had been incubated with TRC (0.5-2 mM) revealed striking differences in surface morphology. Human AM exposed...
shown that the cationic local anesthetics, LDC or TRC, are potent inhibitors of oxidative metabolism of AM and cause marked alterations of their surface membranes with rounding of the cells. The potential influence of these agents must be carefully considered in design and interpretation of all studies evaluating human cells obtained by bronchopulmonary lavage. While this effect must be considered, these cationic anesthetics may be valuable agents for evaluating membrane related events of human AM.

REFERENCES


DISCUSSION

Dr. Davis: I congratulate you on a very complete and multi-faceted study. Just to give you the reassurance that it gave me, your values for oxygen uptake by human alveolar macrophages, both stimulated and unstimulated, virtually match ours to the decimal point. I wonder if you would comment on the fact that oxygen uptake and superoxide generation appear to be matched quite closely when you inhibit them with lidocaine. Since oxygen uptake is much easier to do and requires fewer cells, is it necessary to look at both in order to say anything about killing potential?
Dr. Hoidal: To take the last part first, I think it's necessary to look at both. Using a very special inhibitor of mitochondrial respiration antimycin A, which inhibits the electron transfer from cytochrome B to C, we were able to inhibit approximately two-thirds of alveolar macrophage oxygen uptake, but not superoxide release. Subsequently, we have been interested in comparing alveolar macrophages from smokers and nonsmokers.

Our initial studies compared a population of older patients undergoing diagnostic fiberoptic bronchoscopy. The O₂ uptake by smokers was approximately 50 percent greater than the O₂ uptake by nonsmokers. We then evaluated alveolar macrophages from young smoking volunteers and age-matched nonsmokers. The O₂ uptake was approximately the same in two groups, but the pathways of oxygen utilization appeared different. Specifically, the smokers' alveolar macrophages when stimulated had a greater superoxide release than the nonsmokers. So I think that O₂ uptake above doesn't tell you how the oxygen is utilized by the cell.

Dr. Brody: Where do you think that the lidocaine that you applied to the vocal cords ends up? Do you think that the effect you are seeing on the macrophage occurs in the small airspaces or subsequent to the removal of the cells?

Dr. Hoidal: I think that a good part of the anesthetic which produced an effect on the cells came from the smaller airways. In the study all tubing was changed after anesthetization of lower airways and prior to recovering any fluid during the lavage.

Dr. Lynn: Did you happen to do any of your studies in the absence of protein or serum?

Dr. Hoidal: Yes, we sedimented the cells and washed with balanced salt solution.

Dr. Reiss: Do you wish to speculate as to the mechanism of action of lidocaine in the light of the fact that it's highly soluble in lipid.

Dr. Hoidal: We've tried to determine the mechanism and action of the lidocaine on these cells. Lidocaine has been reported to have numerous actions. It removes the adsorbed calcium from the cell membrane. Secondly, it probably blocks the sodium and potassium channels in the cell membrane. And thirdly, it fluidizes the phospholipid bylayers of the cell membrane. To date, the study has been directed at determining whether removal of adsorbed calcium from the cell membrane was the mechanism of lidocaine action. We have not been able to establish this. We've made two types of preliminary studies. First we've replaced the calcium and tried to override the block, without success. Secondly we've tried to utilize ionophores to transport calcium across the membrane. Again this did not prevent the effect of lidocaine.

Dr. Musson: Dr. Hoidal, were you looking at production of superoxide and the consumption of oxygen with adherent cell cultures or were those cultures in suspension?

Dr. Hoidal: The biochemical tests were done on recently harvested cells in suspension.

Local and Systemic Immunity following Localized Deposition of Antigen in the Lung*

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The results of previous studies using dogs indicated that intrapulmonary immunization resulted in an increase in antigen-specific antibody-forming cells in the population of cells removed by lung lavage. However, it remains unclear whether these cells were produced by antigen deposition and stimulation of cells in airway-associated lymphoid tissues, or in regional and distant lymphoid tissues followed by a migration and accumulation of antibody-forming cells in the lung. To obtain additional data concerning the role of local and systemic immune responses following instillation of antigen into the lung, dogs were immunized in specific airways and the resulting immune response was measured in immunized as well as control airways, in regional lymphoid tissues and in blood and spleen.

MATERIALS AND METHODS

Beagle dogs two and three years of age were immunized by instillation of 10¹⁰ sheep red blood cells (SRBC) in 1.0 ml saline solution into airways of individual lung lobes. A fiberoptic bronchoscope was used to locate specific airways and the SRBC suspension was delivered through 1.6 mm diameter polyethylene tubing into airways approximately 2 mm in diameter. The SRBC were instilled into the airways of the right or left apical and right or left diaphragmatic lung lobes of 16 dogs. These dogs were sacrificed five days after immunization and cell suspensions were prepared from individual lung-associated lymph nodes and the spleen. The Jerné plaque assay as modified by Cunningham was used to determine the number of anti-SRBC plaque-forming cells (PFC). To evaluate the local response in immunized and control airways, nine additional dogs were immunized in airways of either the left or right apical lung lobes. The immunized airway and a control airway in the opposite apical lobe were lavaged with five saline solution washes of 10 ml each using the fiberoptic bronchoscope on days 3, 5, 7, 10, 12, 14 and 21 after SRBC exposure. An average volume of 42.2 ml lavage fluid was recovered. The cells recovered were counted and the percentage of macrophages, lymphocytes and granulocytes was determined. Blood samples were also taken and the number of direct (IgM) and indirect (IgG) PFC in the blood, and in the cells from immunized and control airways was determined.

RESULTS

The number of plaque-forming cells in lung-associated

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