Chapter 39

THE EFFECTS OF DIPALMITOYL LECITHIN COATED FRESH QUARTZ ON SUPEROXIDE RELEASE FROM ALVEOLAR MACROPHAGES IN VITRO

by

Eugene V. Cilento
Professor and Chairman
Department of Chemical Engineering
West Virginia University
Morgantown, WV 26506-6101

George B. Georgellis*
Graduate Research Assistant
Department of Chemical Engineering
West Virginia University
Morgantown, WV 26506-6101

ABSTRACT

The hypothesis that freshly-sheared quartz makes it more reactive in lung tissue than aged quartz was tested by its ability to stimulate superoxide anion (O_2^-) release from single adherent pulmonary alveolar macrophages (PAM). Fresh quartz was prepared by grinding for 30 min and then used within 2 hr, while stale quartz was from the same stock but aged 15 days prior to use. Also, dipalmitoyl lecithin (DPL), the major constituent of lung surfactant coating the alveoli surfaces, was coated onto the particles. Release of O_2^- was measured after 40 and 180 min of direct contact with quartz dust (225 μg/ml). Fresh quartz was more reactive than aged quartz, as indicated by the 78 and 63% increase after 40 and 180 min, respectively. A time delay and suppression of O_2^- release was observed in the ability of the DPL-coated fresh or stale dust to stimulate PAM. These results supported the hypothesis that the natural process of surfactant coating of inhaled particles in the alveoli may play a role in their phagocytic removal by PAM.

INTRODUCTION

Pulmonary alveolar macrophages (PAM) phagocytize foreign debris and bacteria [Brieland et al. 1987]. During the accompanying respiratory burst phagocytes consume more molecular oxygen to produce a superoxide anion radical (O_2^-) at the extracellular surface of the plasma membrane [Babior 1982]. The superoxide radical then dismutates to other more reactive oxygen metabolites which help detoxify inhaled microbes. However, the toxic effects of these reactive agents on surrounding lung tissue, as well as their overall effects on normal lung function, depends on the level of production but remains largely unknown [Babior 1982, Forman et al. 1986].

In this study, an electro-optical method developed previously in this laboratory was used to visualize and measure O_2^- production by single adherent PAM [DiGregorio et al. 1987]. PAM were stimulated by quartz particles and then the amount of O_2^- released per cell was measured by the reduction of nitroblue tetrazolium (NBT) to a diformazan precipitate ($NBTH_2$). Then, from video-recorded images the temporal changes in optical density (OD) of individual cells due to the $NBTH_2$ precipitate formed were measured. The total amount of diformazan produced was compared among cells under different exposure conditions.

While chronic and acute silicosis develops as a result of occupational exposure to crystalline silica [Vallyathan et al. 1988, Heppleston 1984, 1982], differences in disease manifestation can not be explained simply by the response of the lung to different amounts of silica. This suggests that perhaps at least part of the acute response is due to the different surface characteristics of the inhaled dust [Heppleston 1982, Wallace et al. 1985]. Therefore, the purpose of this study was to determine the effects of freshly sheared versus aged silica, and the effects of dipalmitoyl lecithin (DPL) surfactant coating of the particles, on the release of O_2^- by adherent PAM stimulated directly in vitro.

MATERIALS AND METHODS

PAM were obtained from male Long-Evans hooded rats by tracheal lavage. The preparation of cells, resuspension and lavage fluids as well the video imaging and recording procedures remained the same as reported previously [DiGregorio et al. 1987]. However, the procedures for preparing culture dishes were modified slightly to use adherent PAM that were cultured at 37°C with 2% serum for 2 hr prior to experiments. After incubation, PAM were washed thoroughly with resuspension fluid to remove any residual serum

and 3 ml of fresh, warmed NBT solution was added to the culture dish. Quartz was dispersed (as described below) at a concentration of 250 μg/ml into the culture dish and the dish placed on a heated stage (37°C) of an inverted microscope. After a period of 5 min to establish video baselines and for the dust to settle down, televised images of PAM (at 550 nm) were video-recorded for up to 180 min.

The average temporal changes in optical density (OD) for single PAM in contact with quartz particles were determined from the video-recorded images in order to calculate the mass of $NBTH_2$ precipitated as a function of time. Then, a previously developed phenomenological equation was used to calculate the maximum amount of $NBTH_2$ precipitated (MAX) by a single cell [DiGregorio et al. 1987]. MAX was used as a measure of the amount of O_2^- produced by each cell after direct in vitro stimulation with quartz.

Fresh quartz was prepared by grinding for 30 min in a centrifugal ball mill using procedures developed by Vallyathan et al. 1988. An agate mortar was used to minimize any trace impurities and then the dust was immediately sieved trough a 20 μm mesh filter just prior to use. Stale quartz was dust from the same stock that was aged at least 15 days prior to use.

DPL coated dusts were prepared as follows. DPL emulsions made using procedures developed by Wallace et al. 1985 were mixed with dry quartz, vortexed at a concentration of 100 mg DPL/g dust, and incubated for 1 hr at 37°C. Then, the mixture was centrifuged for 10 min at 990 g and the supernatant discarded. Quartz dusts not coated with DPL were treated similarly.

Data on MAX were statistically compared among treatment groups at different times after dust stimulation using analysis of variance (ANOVA). If ANOVA results were significant at the 95% confidence level ($p < 0.05$) then differences between means were determined using the Newman-Keuls test.

RESULTS

The preliminary results presented here are for cultured cells stimulated by direct in vitro exposure to stale or fresh quartz particles (250 μg/ml), either coated or uncoated with DPL. After 40 min, fresh and stale quartz induced significant increases ($p<0.05$) in $NBTH_2$ production compared to control cells. Fresh quartz induced a 381% increase while stale dust produced a 169% increase in MAX above control PAM; with fresh quartz producing a 78% greater increase compared to aged quartz (Table 1). Furthermore, $NBTH_2$ production continued to increase up to 180 min. Superoxide release for fresh quartz was 64% higher than stale quartz at 3 hr, and 68% more than fresh dust after 40 min.

The DPL-coating on the quartz particles (fresh or stale) delayed significant production of $NBTH_2$ until after 40 min. However, following PAM-quartz contact for 180 min, cells produced significantly more $NBTH_2$ than control cells (Table 1).

Table 1

Effects of direct contact of stale and fresh quartz (250 μg/ml), with or without a DPL-coating, on the maximum amount (MAX) of O_2^- released (fmol) by single PAM. Each value represents the mean (± SE) for 18 cells from 3 animals.

	QUARTZ PARTICLES	
Group	DPL-coating	no DPL-coating
	40 min exposure	
CONTROL	3.02 ± 0.39	2.52 ± 0.36
STALE QUARTZ	2.38 ± 0.67	6.79 ± 0.97
FRESH QUARTZ	4.94 ± 1.54	12.10 ± 1.46
	180 min exposure	
CONTROL	3.67 ± 0.44	2.37 ± 0.41
STALE QUARTZ	8.98 ± 0.91	12.45 ± 1.25
FRESH QUARTZ	13.53 ± 2.0	20.37 ± 2.15

Again, fresh quartz was more active biologically, increasing production by 269% compared to 145% for stale particles (Table 1). Also, the DPL-coating delayed O_2^- release compared to uncoated particles such that the 180 min response was about the same as seen for uncoated dust after 40 min. Longer-term time effects were not evaluated in this study.

Phagocytosis of quartz particles by PAM did not appear to be different whether the particles were coated or uncoated, or between fresh and stale quartz. This suggested there were no differences in the ability of these cells to phagocytize dust particles but only in the amount of superoxide released.

DISCUSSION

Occupational exposure to crystalline, respirable silica is known to be associated with chronic pulmonary disease [Brieland et al. 1987, Heppleston 1984, 1982, Cantin et al. 1988]. Chronic silicosis is demonstrated a long time after the first exposure and is characterized by development of concentric nodular lesions in the lung. Although much is known about the full term conditions related to chronic silicosis, very little is known about the early development of acute silicosis or the etiology of the disease. Clinical differences in the manifestation of the pulmonary disease can not be explained simply by

the response of the lung to different amounts of silica, suggesting that at least part of the acute response might be due to the surface characteristics of the inhaled dust [Vallyathan et al. 1988, Wallace et al. 1985].

In this study the toxicity of quartz was monitored by its ability to stimulate O_2^- production from adherent PAM that had been cul-tured for 2 hr in 2% serum. A high concentration of stale quartz (250 μg/ml) resulted in a two-fold or more increase in O_2^- release after 40 min of contact and an even greater increase after 180 min. Freshly sheared quartz was more reactive than aged quartz under the same conditions of direct in vitro exposure supporting the hypothesis that it is more reactive biologically.

The higher reactivity most likely occurs because of different surface characteristics induced during the process of shearing, so that the greater biologic activity might be due to increased silicon radicals on the particle surface that react with aqueous media to produce OH· radicals [Vallyathan et al. 1988, Wallace et al. 1985]. This is significant because OH· radicals have been shown to be particularly harmful because they can peroxidize lipids in the membranes of living systems [Singh et al. 1987]. Another explanation might be that silanol groups (SiOH) on the dust surface, formed by the hydrolysis of silicon-based radicals, increase interactions between particles and the cell membrane [Vallyathan et al. 1988, Heppleston 1984, 1982]. For example, hydrogen bonding may bring the particle and cell closer together, facilitating the initiation of lipid peroxidation and/or activation of the cell membrane.

Although lung tissue is equipped with normal defense mechanisms, such as scavengers of reactive oxygen intermediates that protect the tissue under normal conditions, an increased burden of free-radicals may exceed the normal defense capacity of the lung. Under such conditions, biological damage may occur. The coating of particles with dipalmitoyl lecithin (DPL), the major constituent of lung surfactant, delayed release of O_2^- back to control levels for stale and fresh dusts. Wallace et al. 1985 have shown that the time dependency might be explained by partial removal (using phospholipase A2 and C) of the DPL prophylactic cover from the dust surface during enzymatic digestion in the phagolysozome. The present results support this hypothesis and suggest that as the native dust surface is slowly re-exposed inside the phagolysozome it stimulates release of O_2^-.

This hypothesis is being tested further with studies designed to look more closely at individual cell-particle interactions occurring during phagocytosis of fresh and stale quartz (or other dusts) by the alveolar macrophage, as well as some of the mechanisms involved in mediating this inflammatory response.

ACKNOWLEDGMENTS

The authors wish to gratefully acknowledge the support of the Department of the Interior's Mineral Institute program administered by the Bureau of Mines through the Generic Mineral Technology Center for Respirable Dust (G1135142) for this work.

* - The new address for Mr. George Georgellis is: Exxon Chemical Group, Polymers Group - Tech Polymers, 5200 Bayway Drive, P. O. Box 5200, Baytown, TX 77522-5200.

REFERENCES

Brieland, J. K., R. G. Kunkel, and J. C. Fantone, 1987, Pulmonary Alveolar Macrophage Function During Acute Inflammatory Lung Injury, Am. Rev. Respir. Dis., 135: 1300-1306.

Babior, B. M., 1982, The Enzymatic Basis For O_2^- Production By Human Neutrophils, Can. J. Physiol. Pharmacol., 60: 1353-1358.

Forman, H. G., and M. J. Thomas, 1986, Oxidant Production And Bactericidal Activity Of Phagocytes, Ann. Rev. Physiol., 48: 669-680.

DiGregorio, K. A., E. V. Cilento, and R. C. Lantz, 1987, Measurement Of Superoxide Release From Single Pulmonary Alveolar Macrophages, Am. J. Physiol., 252: C677-C683.

Vallyathan, V., X. Shi, N. S. Dalal, W. Irr, and V. Castranova, 1988, Generation Of Free Radicals From Freshly Fractured Silica Dust, Potential Role In Acute Silica-induced Lung Injury, Am. Rev. Respir. Dis., 138: 1213-1219.

Heppleston, A. G., 1984, Pulmonary Toxicology Of Silica, Coal And Asbestos, Env. Hlth. Persp., 55: 11-127.

Heppleston, A. G., 1982, Silicotic Fibrogenesis: A Concept Of Pulmonary Fibrosis, Ann. Occup. Hygen., 26: 449-462.

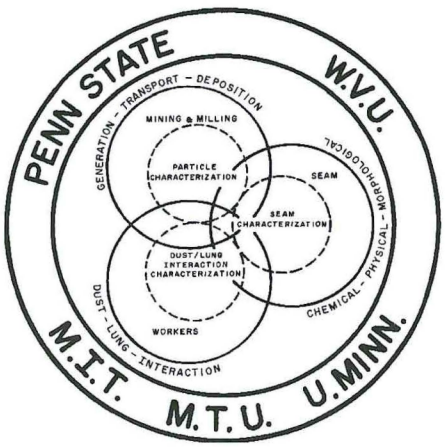
Wallace, W. E., V. Vallyathan, M. J. Keane, and V. Robinson, 1985, In Vitro Biologic Toxicity Of Native And Surface-modified Silica And Kaolin. J. Tox. Env. Hlth, 16: 415-424.

Cantin , A., F. Dubois, and R. Begin, 1988, Lung Exposure To Mineral Dusts Enhances The Capacity Of Lung Inflammatory Cells To Release Superoxide, J. Leuk. Biol., 43: 299-303.

Singh, V. S., P. N. Viswanathan, and Q. Rahman, 1987, Interaction Between Erythrocyte Plasma Membrane And Silicate Dusts, J. Appl. Toxicol., 7: 91-96.

3rd SYMPOSIUM ON RESPIRABLE DUST IN THE MINERAL INDUSTRIES

Edited by
ROBERT L. FRANTZ
and
RAJA V. RAMANI



Published by
Society for Mining, Metallurgy, and Exploration, Inc.
Littleton, Colorado • 1991

Printed in the United States of America by
Cushing-Malloy, Inc., Ann Arbor, MI

Library of Congress Catalog Card Number 91-66952
ISBN 0-87335-098-7

RESPIRABLE DUST IN THE MINERAL INDUSTRIES

Proceedings of the 3rd Symposium on Respirable Dust
in the Mineral Industries
October 17-19, 1990
Pittsburgh, PA

Sponsored by

The Generic Mineral Technology Center for Respirable Dust
The Pennsylvania State University
West Virginia University
University of Minnesota
Massachusetts Institute of Technology
United States Bureau of Mines (USBM)
Mine Safety and Health Administration (MSHA)
National Institute for Occupational Safety and Health (NIOSH)
American Conference of Governmental Industrial Hygienists (ACGIH)