Ultrastructural changes of pneumocytes of rat exposed to Arabian incense (Bakhour)

Saud A. Alarifi, BSc, PhD, Mohammed M. Mubarak, MSc, PhD, Majed S. Alokail, MSc, PhD.

ABSTRACT

Objective: Impacts of air pollution on the human health have been recognized over the last decades. Smokes, in particular, have deleterious effects on the respiratory system. According to a local tradition, incense "Bakhour" is burnt and the resultant heavy smokes are inhaled. The objective of the present study is to investigate the ultrastructural pulmonary changes which can be induced by Arabian incense, Bakhour, exposure.

Methods: The study was conducted from September through to December 2003, at the Animal House, College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia. Two groups of Wister albino rats, Rattus norvegicus, were used. One group (n=16) was exposed to 420 grams of Bakhour for 14-weeks at the rate of 4 grams/day in the exposure chamber. Additional group of rats, of equal number, was used as non-exposed control. At the end of the exposure period, lung tissues were removed from all experimental animals and processed for electron microscopy.

Results: Alveolar pneumocytes of exposed animals revealed significant ultrastructural changes which involved the cell organelles and surfactant material of type II cells. Hyperplasia of alveolar cells was a feature in the affected lung tissue. Neutrophils were recognized infiltrating pulmonary alveoli and accompanied with degenerative and necrotic changes of the alveolar cells. Deposition of collagen fibrils in the alveolar walls was also observed.

Conclusion: Basing upon the results of electron microscopy, it was concluded that exposure to Bakhour can induce ultrastructural pulmonary changes which may imply compromised respiratory efficiency.

Saudi Med J 2004; Vol. 25 (11): 1689-1693

Impacts of air pollution on human health have been recognized for over a century.[1] Smokes, in general, have deleterious effects on the respiratory system, particularly the lung tissue. Cigarette smoke is the most common, and its relationship with pulmonary diseases was extensively studied. One of the common smoke sources to which individuals are frequently exposed in Arabian countries is the incense "Bakhour" which is also called "Aoud" in some Arabian countries. This is an oleoresin that oozes from incision in the trunks and leaves of the genus *Boswellia (B.carterri and B.papyrifera)* native of Arabia, Africa and India.[2] Incense is burnt according to the local tradition and as a result, heavy smoke raised to which individuals are intimately exposed by inhalation. Thus, a suspected airborne risk develops which may threatens the human health. In the Kingdom of Saudi Arabia (KSA), such intensive indoor incense exposure was found to bear respiratory risk factors for non-smoking women.[3] Also, exposure to the Arabian incense accounts for a considerable proportion of the asthmatic cases among children in Qatar.[4] However, no adequate studies have been conducted to reveal the histological changes in lung tissue due to exposure to Arabian incense smoke. Our previous study[5] investigated these pulmonary histological changes using light microscope. For

From the Department of Zoology (Alarifi, Mubarak), Department of Biochemistry (Alokail), College of Science, King Saud University, Riyadh, *Kingdom of Saudi Arabia*.

Received 14th April 2004. Accepted for publication in final form 21st June 2004.

Address correspondence and reprint request to: Dr. Saud A. Alarifi, Zoology Department, College of Science, King Saud University, PO Box 2455, Riyadh 11451, *Kingdom of Saudi Arabia*. Tel. +966 (1) 4675779. Fax. +966 (1) 4678514. E-mail: alarifi66@yahoo.com

more definitive investigation, the present study was intended to elucidate the ultrastructural pulmonary changes induced by incense exposure. The selected material was Bakhour which is a commonly used type of Arabian incense in KSA.

Methods. ***Experimental animals.*** Wister albino male rats, rattus norvegicus, weighing 95 ± 10 g and of the same age were used. Animals were obtained from King Saud University colony. Rats were maintained under standard laboratory conditions including diet and temperature (25°C). Water and feed were available ad libitum.

Experimental design. Animals were divided randomly into 2 experimental groups (treated and untreated control) of 16 rats each. Treated rats were exposed to 420 grams of Bakhour for 14 weeks, at the rate of 4 grams/day in an exposure chamber. Untreated animals were unexposed and served as control. At the end of treatment period, all experimental rats were anesthetized, dissected and lungs were removed.

Electron microscopy. Immediately after removal of lungs, tissues were diced into proper sized pieces (1 mm^3) and fixed by immersion in buffered 3% glutaraldehyde (cacodylate buffer, pH 7.2) for at least 4 hours at 4°C. Tissue specimens were then post-fixed in 1% osmium tetroxide (Oso4), in cacodylate buffer pH 7.2, for 2 hours at 4°C. Dehydration of the fixed tissues was performed using ascending grades of ethanol, and then tissues were transferred to epoxy resin via propylene oxide. After impregnation with pure resin (Epon/araldite mixture), tissue specimens were embedded in the same resin mixture. Semi-thin sections (1um thickness) were prepared for the purpose of tissue orientation and stained with toluidine blue. Accordingly, thin sections (70-80 nm) were cut with an ultramicrotome (Leica, UCT) and double stained with uranyl acetate and lead citrate. Stained tissue sections were observed with a transmission electron microscope (JEOL, 100 CX) operating at 80 kv.

Results. Pneumocytes of both types (I and II) were degenerated and had surface cytoplasmic blebbings. Pneumocytes type II was more affected and had degenerated organelles, their mitochondria were swollen and Rough Endoplasmic Reticulum (RER) was dilated. Degenerated type II cells contained surfactant material of indistinct lamellated pattern and low density **(Figure 1)**. Nuclei of type II cells were obviously irregular in shape and only little heterochromatin was marginated on the nuclear membrane. Pneumocytes type I in a close proximity to the leukocyte-obliterated capillaries had also irregular nuclei, but in contrast these nuclei showed dense heterochromatin clumped on the nuclear membrane. As a general observation, mitochondria of pneumocyte II were swollen or elongated and had proliferated cristae **(Figure 2)**. Proliferation of the cristae increased the matrical density of mitochondria. Elongated mitochondrial profiles possibly represented a tendency for mitochondrial proliferation. Also, cisternae of RER were dilated and the vesiculated appearance of RER was a frequent finding. Dilated RER was partially degranulated due to loss of the surface ribosomes. Also, Golgi complex was well-developed at the juxtanuclear position. The degenerated type I cells contained scarce organelles. Some areas of the affected lung tissue revealed marked hypercellularity as evidenced by the crowds of nuclei of the proliferated cells. This cellular hyperplasia included pneumocytes, capillary endothelial cells and fibroblasts. The proliferated pneumocyte II were recognized by little deformed surfactant material. Hyperplastic pneumocytes were identified by the irregularly shaped nuclei which had condensed chromatin. Proliferating fibroblasts were marked by the abundant RER in their cytoplasm and the deposited collagen fibrils in their vicinity. Hyperplasia of the latter cells were noticed in a close proximity to the degenerated alveolar cells. Cellular hyperplasia caused thickening of the alveolar walls or even occasional collapse of the alveolar lumina. Concerning the detached (sloughed) intra-alveolar degenerated pneumocytes type II, they lost their surface microvilli and their organelles, including mitochondria and RER, were degenerated. Little surfactant material in these cells was of the lamellated pattern, however, the rest was abnormal and appeared as dense unlamellated bodies of various shapes and sizes **(Figure 3)**. Also, these cells contained amorphous lipoid material. Some of the desquamated pneumocytes type II contained only moderately dense amorphous structures. Nuclei of these cells were small and their chromatin was dense compared with that attached to the alveolar basement membrane. Detached alveolar macrophages were also discerned in association with the sloughed pneumocytes and the exuded neutrophils. Macrophages were filled with lipoid material, probably as a consequence of phagocytosis of the sloughed pneumocytes. In the examined exposed cases, many alveolar capillaries were distended and contained several raws of erythrocytes reflecting the hyperemia of the pulmonary vasculature. Cytoplasm of the capillary endothelial cells showed surface blebbings and had degenerated organelles. Lodged leukocytes, obliterating considerable number of alveolar capillaries, were an outstanding feature. These leukocytes were mostly neutrophils which had dense secondary lysosomal structures. Heterochromatin in nuclei of these cells was noticeably condensed. Edema surrounding the obliterated capillaries was obvious. In a large

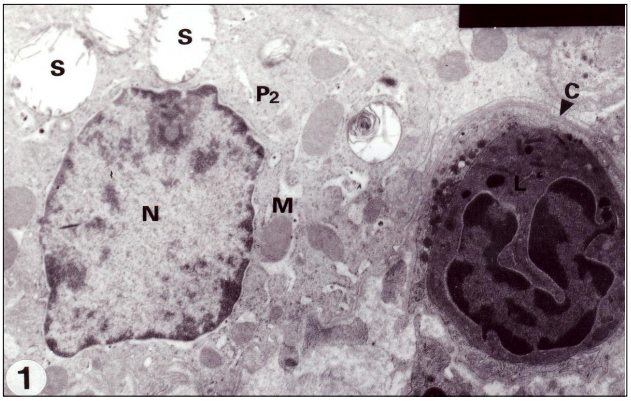

Figure 1 - Leukocyte neutrophil (L) obliterating a pulmonary alveolar capillary (C). Neutrophil has dense secondary lysosomal structures and its nuclear heterochromatin is noticeably condensed. The adjacent pneumocyte type II (P2) contains swollen mitochondria (M) and dilated rough endoplasmic reticulum. Nucleus (N) is irregular and has little heterochromatin. Surfactant material (S) is of low density and indistinct lamellation. Transmission electron micrograph x 7200.

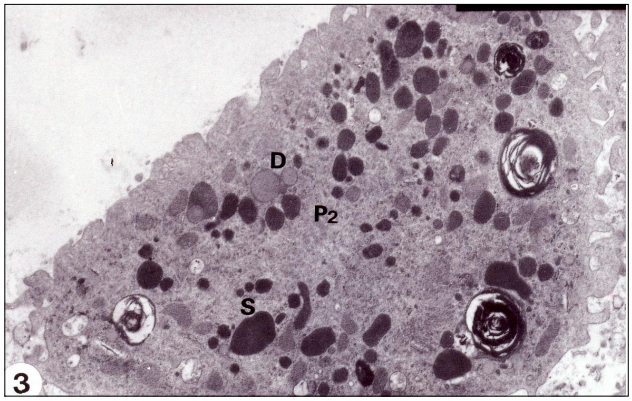

Figure 3 - Transmission electron micrograph showing a sloughed pneumocyte type II (P2) which lost most of its microvilli. Most of the surfactant material (S) is seen as amorphous dense unlamellated bodies. Lipid droplets (D) are also discerned X7200.

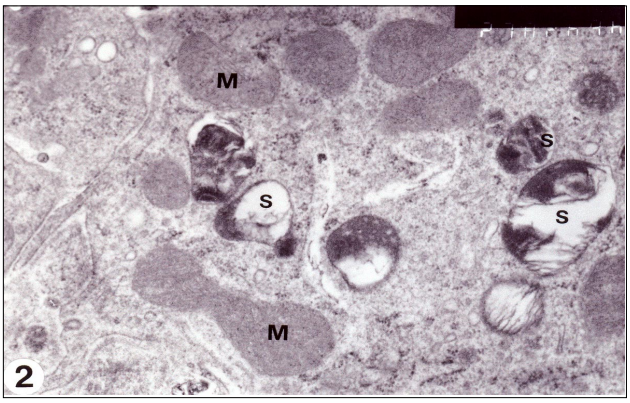

Figure 2 - Electron micrograph showing swollen and elongated mitochondria (M) in pneumocyte type II. The proliferated cristae increased the matrical density of mitochondria. Surfactant material (S) is dense and of poor lamellation X27000.

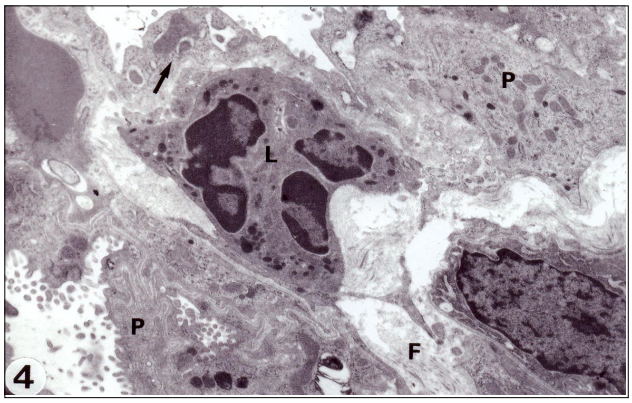

Figure 4 - Exuded leukocyte neutrophil (L) in the alveolar lumen. Cytoplasmic contents (arrow) released from necrosed pneumocytes are seen. Degenerated pneumocytes (P) are seen in the vicinity of the exuded neutrophils. Collagen fibrils (F) are deposited within the alveolar walls. Transmission electron micrograph X5800.

number of alveoli, neutrophils that exuded from the distended alveolar capillaries were found free in the alveolar lumina **(Figure 4)**. All the alveolar lining cells in such areas were degenerated and some were necrosed with a resultant release of the cytoplasmic contents of these cells. In addition to the exuded neutrophils and cell debris, alveolar lumina contained precipitated material of possible protein nature. Affected alveoli, since their lumina were not aerated and instead filled with necrosed sloughed pneumocytes and infiltrated neutrophils, were in a state of collapse. Deposited collagen fibrils were readily discerned at the site of the alveolar necrosed cells, such fibrils were running along the alveolar walls.

Discussion. In the present study, animals exposed to the smoke of Bakhour revealed significant ultrastructure changes in their lung tissue. Pneumocytes were either degenerated or detached from the alveolar basement membrane. Pneumocytes II had deformed surfactant and degenerated organelles. Leukocytes appeared lodged in alveolar capillaries and also infiltrated the affected pulmonary tissues. The current study presented an evidence that inhalant smoke of Bakhour can exert a damaging effect on pneumocytes and also induce surfactant alteration. In this respect, little is known regarding the effect of such inhalants on the alterations of the pulmonary surfactant at the ultrastructure level.[6] According to the latter authors intracellular surfactant alterations,

as observed here, are involved in some human respiratory diseases such as adult respiratory distress syndrome (ARDS). The importance of surfactant alterations comes from the fact that pulmonary surfactant plays a crucial role in prevention of alveolar collapse by reducing alveolar surface tension[7,8] Also, surfactant is an active component of the lung host defense mechanism.[9] The noticed intracellular surfactant, in pneumocyte II, was of decreased amount, less lamellated nature or high density which may indicate a disturbed surfactant secretion. Decreased rate of surfactant secretion can lead to increased density of the surfactant material.[6] It is supposed that some active particulates in the smoke has bound to the cell membrane of type II pneumocytes and affected its capability of surfactant secretion. It has been reported a similar suggestion for materials which can alter cell membrane properties of type II cells and subsequently reduce the release of intracellular surfactant.[10] The observed pneumocyte hyperplasia is considered as an early response of the alveolar wall to injury.[11] It is suggested that cellular hyperplasia has taken place as a regenerative trial to replace the damaged alveolar cells. Type II pneumocytes are known to be the progenitors of type I cells (membranous pneumocytes).[12] Detachment of alveolar macrophages from its basement membrane into alveolar lumen indicates activity of these cells in engulfing the particulate materials in the inhaled smoke. Alveolar macrophages lie free in alveolar spaces to phagocyte foreign substances that enter pulmonary alveoli and digest it.[13] Also, the observed detached alveolar macrophages seemed to be activated to phagocytose the degenerate pneumocytes. This macrophage state is reminiscent of the enlarged macrophages filled with large pleomorphic residual bodies in lungs of tobacco smokers.[14] Exudation of leukocytes (mostly neutrophils) into the alveolar lumina in lung tissue of exposed animals was most probably related to increased permeability of the alveolar capillaries. Extravascular localization of leukocytes points to acute vascular injury which is an important component of injury caused by most pulmonary toxicants.[15,16] Besides, inhalation of particulate material and smoke was found to stimulate macrophages and pneumocytes to release chemotactic factors for neutrophils.[1,12,17] Similar to cigarette smoking[18] the used inhaled material caused accumulation of inflammatory cells in the lung tissue. The exuded inflammatory cells may contribute in damaging of the alveolar and interstitial pulmonary structures through elaboration of lytic enzymes.[12] In the present exposed animals, the deposited collagen fibrils in the alveolar walls was an evidence of alveolar fibrosis. This was possibly initiated by the local hypoxia created at the area of damaged tissue. The described pulmonary ultrastructural changes were concentrated at the alveolar wall which means impaired function of the blood-air barrier. This implies a compromised capacity of the alveolar wall for gaseous exchange in animals exposed to the inhalant smoke.[19] This finally can lead to compromised respiratory efficiency in the exposed animals.

The current study is confirm to our previous one[5] which revealed by the light microscopy that Arabian incense (Bakhour), can provoke pulmonary histological changes. The present data can be considered as an evidence that inhalation of Bakhour, which is a common local tradition, can induce changes in lung tissue as evidenced by electron microscopy.

References

1. Bouthillier L, Vincent R, Goegan P, Adamson IYR, Bjarnason S, Stewart M et al. Acute effects of inhaled urban particles and ozone lung morphology, macrophage activity, and plasma endothelin-1. ***Am J Pathol*** 1998; 153: 1873-1884.
2. Basto AS, Azenha A. Contact dermatitis due to incense. ***Contact Dermatitis*** 1991; 24: 312-313.
3. Dossing M, Khan J, Al-Rabiah F. Risk factors for chronic obstructive lung disease in Saudi Arabia. ***Respir Med*** 1994; 88: 519-522.
4. Dawod ST, Hussain AA. Childhood asthma in Qatar. ***Ann Allergy Asthma Immunol*** 1995; 75: 360-364.
5. Alokail MS and Alarifi S A. Histological changes in lung of wister albino rats (Rattus norvegicus) after exposure to the Arabian incense (Genus Boswellia). ***Annals of Saudi Medicine*** 2004; 24: 217-219.
6. Fehrenbach H, Brasch F, Uhlig S, Weisser M, Stamme C, Wendel et al. Early alterations in intracellular and alveolar surfactant of the rat lung in response to endotoxin. A***m J Respir Crit Care Med*** 1998; 157: 1630-1639.
7. Hawgood S. Surfactant: composition, structure, and metabolism. In: The lung: Scientific Foundations. RG Crystal RG, West JB, Barens PJ, Weibel ER, editors. 2nd ed. Philadelphia (PA): Lippincott-Raven; 1997. p. 557-571.
8. Günther A, Schmidt R, Feustel A, Meier U, Pucker C, Ermert M et al. Surfactant subtype conversion is related to loss of surfactant apoprotein B and surface activity in large surfactant aggregates. Experimental and clinical studies. ***Am J Respir Care Med*** 1999; 159: 244-251.
9. Ochs M, Nenadic I, Fehrenbach A, Albes JM, Wahlers T, Richter J et al. Ultrastructural alterations in intraalveolar surfactant subtypes after experimental ischemia and reperfusion. ***Am J Respir Crit Med*** 1999; 160: 718-724.
10. Aracil FM, Bosch MA , Municio AM. Influence of *E.coli* lipopolysaccharide binding to rat alveolar type II cells on their functional properties. ***Mol Cell Biochem*** 1985; 68: 59-66.
11. Adamson IYR, Bowden DH. Role of monocytes and interstitial cells in the generation of alveolar macrophages. ***Lab Invest*** 1980; 42: 518-524.
12. Masubuchi T, Koyama S, Sato E, Takamizawa A, Kubo K, Sekiguchi M et al. Smoke extract stimulates lung epithelial cells to release neutrophil and monocyte chemotactic activity. ***Am J Pathol*** 1998; 153: 1903-1912.
13. Bils RF, Christie BR. The experimental pathology of oxidant and air pollutant inhalation. ***Int Rev Exp Pathol*** 1980; 21: 195-293.

14. Pratt SA. The ultrastructure of active macrophages from human cigarette smokers and nonsmokers. ***Lab Invest*** 1971; 24: 331-335.
15. Shaw JO. Leukocytes in chemotactic-fragment-induced lung inflammation. Vascular emigration and alveolar surface migration. ***Am J Pathol*** 1980; 101: 283-302.
16. Atwal OS, Persofsky MS. Ultrastructural changes in intraacinar pulmonary veins. ***Am J Pathol*** 1984; 14: 472-486.
17. Driscoll KE, Maurer JK, Higgins J, Poynter J. Alveolar macrophage cytokine and growth factor production in a rat model of crocidolite-induced pulmonary inflammation and fibrosis. ***J Toxicol Environ Health*** 1995; 46: 155-169.
18. Snider GL, Lucey EC, Stone PJ. Animal models of emphysema. ***Am Rev Respir Dis*** 1986; 133: 149-169.
19. Nickerson PA, Matalon S, Farhi LE. An ultrastructural study of alveolar permeability to cytochrome C in the rabbit lung: effect of exposure to 100% oxygen at one atmosphere. ***Am J Pathol*** 1981; 102: 1-9.