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Ultrastructure of the Pulmonary Alveolar Cells of Rats Exposed to Arabian Mix Incense (Ma'amoul)

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Abstract: The ultrastructural pulmonary changes which can be induced by Arabian incense, Ma'amoul (mixing incense) exposure was investigated in the present study. Two groups of Wistar albino rats were used, one group (n=16) was exposed to 420 g of Ma'amoul for 14 weeks at the rate of 4 g/day in the exposure chamber. The second group of rats was of equal number and used as non-exposed control. At the end of the experimentation period lung tissues were removed from all experimental animals and processed for electron microscopy. Significant ultrastructural changes were noticed in alveolar pneumocytes of exposed animals. These fine changes involved the cell organelles and surfactant material of type II cells. Alveolar septal hypercellularity was due to hyperplasia of the alveolar cells in the affected lung tissue. Neutrophil cell infiltration in the alveolar lumina was accompanied with degenerative and necrotic changes of the alveolar lining cells. Many erythrocytes were extravasated from the distended alveolar capillaries. Alveolar walls revealed deposition of collagen fibrils which contributed in its thickening. It was concluded that exposure to Ma'amoul provokes ultrastructural pulmonary changes which may indicate impaired respiratory efficiency.

Key words: Incense, Ma'amoul, lung, ultrastructure, pneumocytes

INTRODUCTION

Over the last decades the impacts of air pollution on human health have been increasingly identified^[1]. Smokes are among the air pollutants which have deleterious effects on the respiratory system especially the lung tissue. Cigarette smoke is the most known hazard and its association with pulmonary diseases was extensively studied. However, another common activity which poses threats to the human health is the incense burning which is usually overlooked^[2]. It is a ceremonial practice in countries of East-South Asia where this ritual is performed regularly in an indoor environment. Regarding our localities, one of the common smoke sources to which human subjects are frequently exposed is the incense (Ma'amoul) in some Arabian countries. This is an oleoresin oozing from incision in the trunks and leaves of the genus *Boswellia* (*B. carterri* and *B. papyrifera*) native of Arabian area, Africa and India with a mix of some oriental oils and perfumes^[3,4]. According to the local customs the incense is burnt and as a result heavy smokes generated to which individuals are intimately exposed by inhalation. In Saudi Arabia, the non-smoking women who are exposed to such daily intensive indoor incense are facing respiratory risk^[5]. Also, considerable

number of the asthmatic cases among children in Qatar was attributed to exposure to the Arabian incense^[6]. There is a paucity of data on the direct *in vivo* biological effects of particulate material from ambient air^[1]. In this respect, there are no adequate studies to reveal the histological changes in lung tissue induced by exposure to Arabian incense smoke. Our previous study^[4] investigated these pulmonary histological changes using light microscope. A recent study^[7] identified the ultrastructural pulmonary changes induced by Bakhour inhalation.

For more definitive investigation, the present study was intended to elucidate the ultrastructural pulmonary changes induced by another Arabian incense material. The selected material was Ma'amoul which is in a common use in Saudi Arabia and Arabian Gulf countries.

MATERIALS AND METHODS

Experimental animals: Wistar albino male rats, *Rattus norvegicus*, weighing 95±10 g and of the same age were used. Animals were obtained from King Saud University colony and 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 two experimental groups (treated and untreated control) of 16 rats each. Rats of the treated group were exposed to 420 g of Ma'amoul for 14 weeks, at the rate of 4 g/day in an exposure chamber. Untreated animals were unexposed and served as control. At the end of experimentation period, all experimental rats were anesthetized, dissected and lungs were removed.

Electron microscopy: Immediately after removal of lungs from the dissected animals, tissues were diced into proper sized pieces (1 mm³) and fixed by immersion in 3% buffered glutaraldehyde (cacodylate buffer, pH 7.2) for 4 h at 4°C. Tissue specimens were then post-fixed in 1% osmium tetroxide (OsO₄), in cacodylate buffer pH 7.2, for 2 h 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 the pure resin (Epon/araldite mixture), tissue specimens were embedded in the same resin mixture. Semi-thin sections (1 µm thickness) were prepared for the purpose of tissue orientation and stained with toluidine blue. Accordingly, thin sections of silver-gold shades (70-80 nm) were cut on an ultramicrotome (Leica, UCT) with a diamond knife 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

In the exposed animals, alveolar pneumocytes were the most affected pulmonary structures. Pneumocytes type II were more altered than other alveolar cells.

Pneumocytes type II were usually separated from the alveolar basement membrane and seen desquamated within the alveolar lumen. The desquamated pneumocytes had only small number of intact surface microvilli and the majority was either lost or short and blunt (Fig. 1). Mitochondria of these cells were irregular and swollen. The surfactant containing vesicles in these cells were relatively larger but the concentration of the surfactant material was obviously less. Also, the surfactant material was deformed and of less lamellar nature. Profiles of Rough Endoplasmic Reticulum (RER) in the detached pneumocytes were markedly dilated.

The local alveolar capillaries were usually distended, each capillary contained several rows of erythrocytes. Frequently, lodged polymorphonuclear leukocytes (neutrophils) were recognized within these capillaries. Cytoplasm of the capillary endothelial cells had degenerated organelles. The desquamated pneumocytes

were occasionally found in a close contact (wall-wall adhesion) with the distended capillaries. In addition to the desquamated pneumocytes II, the alveolar lumena contained extravasated erythrocytes that exuded from the distended alveolar capillaries (Fig. 2).

Alveolar walls in such areas were obviously thickened due to pneumocytes proliferation (hyperplasia) as indicated from the crowded nuclei. Nuclei of the proliferated cells were of irregular shape (pleomorphic). Process of hyperplasia occurred on both sides of the alveolar basement membrane. In large number of alveolar lumena, there were infiltrated neutrophils beside the extravasated erythrocytes (Fig. 3). The alveolar lumena were filled with fine granular material which possibly represented a proteinaceous transudate.

In the vicinity of the degenerated pneumocytes and the leukocyte-lodged capillaries, collagen fibrils were discerned depositing in the alveolar walls (Fig. 4).

Considerable numbers of alveoli were occluded by detached intraluminal large reactive huge macrophages (Fig. 5 and 6). Such reactive alveolar macrophages contained numerous fat droplets of various sizes and much debris. Some of the phagocytosed particles within these alveolar macrophages showed similar density to that of the surfactant material in pneumocytes.

Pneumocytes II which were still attached to the alveolar basement membrane had considerable number of surfactant-containing vesicles; however the surfactant material was less dense and deformed. Also, these cells had markedly swollen mitochondria which revealed proliferated cristae (Fig. 7) compared to the much smaller mitochondria in pneumocytes of the non-exposed control animals (Fig. 8).

Concerning the state of pneumocyte I, they were hypertrophied as evidenced from the size of their nuclei. Some of these pneumocytes showed partially destructed cell membrane (Fig. 9). Most of pneumocytes type I revealed deterioration of their surface microvilli.

DISCUSSION

Significant ultrastructural changes in the lung tissue of animals exposed to the smoke of Ma'amoul were described in the present study. Affected pneumocytes were either detached from the alveolar basement membrane or degenerated. Pneumocytes II were the most altered and had deformed surfactant material and degenerated organelles. Leukocytes were lodged in the alveolar capillaries and also exuded to infiltrate the pulmonary tissues in association with large number of extravasated erythrocytes.

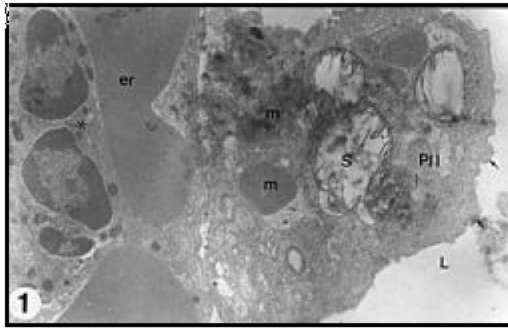


Fig. 1: Desquamated pneumocyte type II (PII) within the alveolar lumen (L). The remaining surface microvilli (arrows) of the desquamated pneumocyte are short and blunt. Mitochondria (m) are swollen and the surfactant material (S) is of less density and indistinct lamellation. Note the lodged polymorphnuclear leukocyte (*) within a distended capillary. Erythrocytes (er). Rat exposed for 14 weeks. X 10000

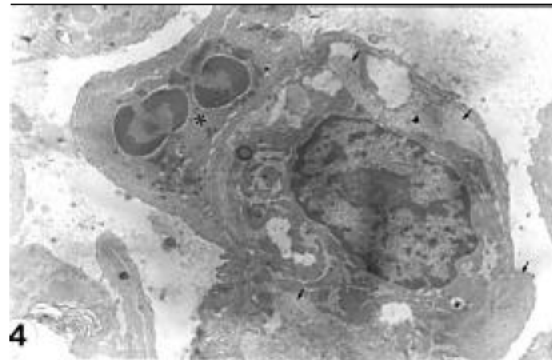


Fig. 4: Collagen fibrils (arrows) deposited within the alveolar walls. Note the leukocyte (*) lodged in the alveolar capillary. Rat exposed for 14 weeks. X 6700

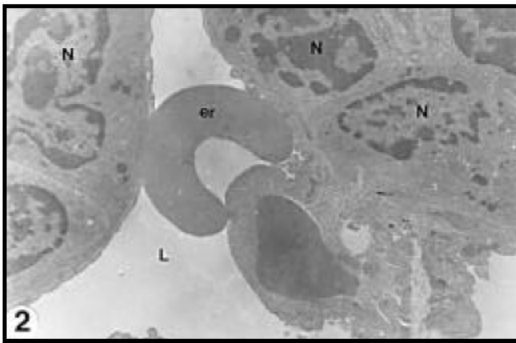


Fig. 2: Extravasated erythrocytes (er) within the alveolar lumen (L). Note the alveolar septal hypercellularity evidenced by the crowded pleomorphic nuclei (N) of the hyperplastic pneumocytes. Rat exposed for 14 weeks. X 6700

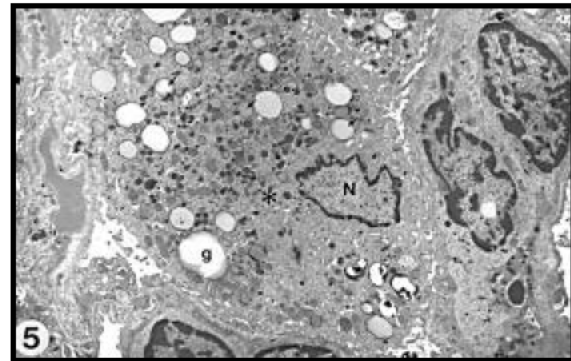


Fig. 5: Detached large reactive macrophage (*) containing numerous varied-sized lipid droplets (arrows) and much amount of debris. The large electron lucent globules (g) are probably deformed surfactant material phagocytosed by this macrophage. Rat exposed for 14 weeks. X 4000

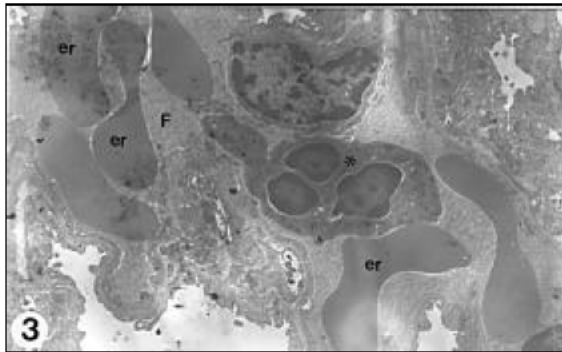


Fig. 3: Infiltrating polymorphnuclear leukocyte (*) associating large number of the extravasated erythrocytes (er). The alveolar lumen shows the presence of finely granular material (F) which is possibly proteinaceous material transuded from the damaged alveolar capillaries. Rat exposed for 14 weeks. X 4800

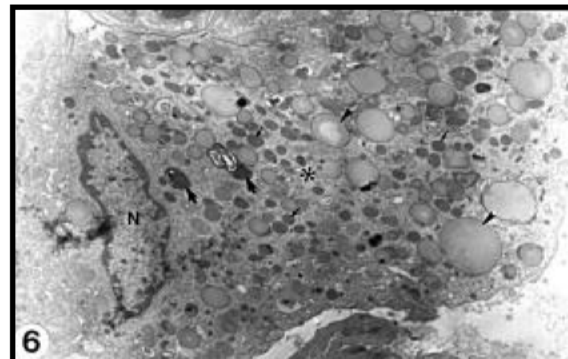


Fig. 6: Higer magnification for another reactive macrophage (*) filled with lipid droplets (thin arrows), deformed phagocytosed surfactant material (arrowheads) and debri (thick arrows). Nucleus of macrophage (N). Rat exposed for 14 weeks. X 6700

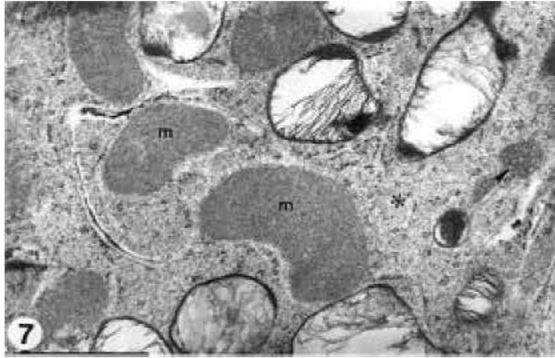


Fig. 7: Swollen and irregular-shaped mitochondria (m) in cytoplasm (*) of pneumocyte II. Cristae of these mitochondria are obviously proliferated. There is a multivesicular body (arrowhead). Rat exposed for 14 weeks. X 27000

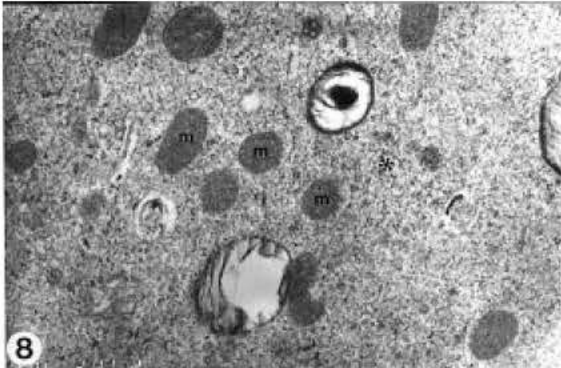


Fig. 8: Normal-sized mitochondria (m) in cytoplasm (*) of pneumocyte type II of unexposed control rat. X 27000

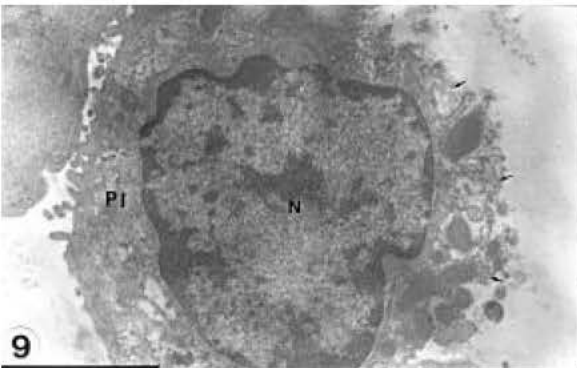


Fig. 9: Hypertrophied pneumocyte type I (PI) having enlarged nucleus (N) and partially destroyed cell membrane (arrows). Most of the surface microvilli are disintegrated. Rat exposed for 14 weeks. X 10000

The detected extravasation of erythrocytes was most possibly the sequel of endothelial cell damage in the alveolar capillaries. Erythrocytes extravasate through the resulted gaps between the damaged endothelial cells^[8,9].

Inhalant particles can get access to the distal alveolar surface area where the bioviable components released from these particles could affect the alveolar capillary beds^[10]. Extravascular localization (exudation) of leukocytes implies acute vascular injury which is a consistent feature of injury caused by most pulmonary toxicants^[11,12]. Exudation of leukocytes (mostly neutrophils) into the alveolar lumina of exposed animals was most probably attributable to increased permeability of the alveolar capillaries. Besides, inhalation of particulate materials and smoke was found to stimulate macrophages and pneumocytes to release chemoattractants for neutrophils^[13,14]. There is an experimental evidence that pneumocytes type II cells in response to smoke extract release cytokines which indirectly induce tissue damage^[14]. As the situation in cigarette smoking^[5], the used inhaled material provoked accumulation of inflammatory cells in the lung tissue. The infiltrating inflammatory cells may account for the damaging of the alveolar and interstitial pulmonary structures through the lytic effect of their enzymes^[14].

The observed detached alveolar macrophages which separated from its basement membrane into alveolar lumen were active cells participating in engulfing the particulate materials in the inhaled smoke. It is established that alveolar (resident) macrophages lie free in alveolar spaces to engulf foreign substances that get way to pulmonary alveoli^[6]. Also, these detached alveolar macrophages were likely activated to phagocytize the degenerate pneumocytes. Such macrophage state is reminiscent of the hypertrophied macrophages filled with large pleomorphic residual bodies in lungs of tobacco smokers^[17]. Furthermore, activated phagocytes could amplify and accentuate the primary lesions through elaboration of inflammatory chemotactic and mitogenic cytokines^[1].

The currently noticed pneumocyte hyperplasia is considered by some researchers^[18] as an early response of the alveolar wall to injury. Also, it is assumed that cellular hyperplasia occurred as a regenerative trial to replace the damaged alveolar cells. It is known that type II pneumocytes are the progenitors of type I cells (membranous pneumocytes), i.e., divide to replace the latter cells^[14].

The present study proved that inhalant smoke of Ma'amoul can exert a damaging effect on pneumocytes and also cause surfactant alteration. Little is known about

the effect of such inhalants on the ultrastructural alterations of the pulmonary surfactant^[19]. The latter authors reported that intracellular surfactant alterations are involved in some human respiratory diseases such as Adult Respiratory Distress Syndrome (ARDS). The fact that pulmonary surfactant is crucial in the prevention of alveolar collapse by reducing alveolar surface tension^[20,21] justifies the significance of any surfactant alterations. Also, surfactant is incorporated in the lung host defense mechanism through augmentation of alveolar macrophage migration^[22,23]. The noticed intracellular surfactant, in pneumocyte II, was of less lamellated pattern and also of low density which may indicate a disturbed surfactant synthesis or secretion. 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. Aracil *et al.*^[24] reported a similar suggestion for materials which can alter cell membrane properties of type II cells and subsequently reduce the release of intracellular surfactant. Moreover, it is hypothesized that plasma proteins in case of alveolar edema, as observed here, inhibits lung surfactant activity^[25].

Alveolar fibrosis in the presently exposed animals was evidenced by the deposited collagen fibrils in the alveolar walls. This was possibly initiated by the local hypoxia created at the area of damaged tissue and alternatively may reflect activation of fibrocytes due to the effect of mitogenic factors released from the reactive macrophages.

Currently, Alveolar walls seemed to be the target of the inhaled smoke since it showed the noticeable ultrastructural changes. This site of tissue changes implies impaired function of the blood-air barrier. In other words, a compromised capacity of the alveolar wall for gaseous exchange might resulted in animals exposed to the inhalant smoke^[26]. Thus, the total respiratory efficiency in the exposed animals is expected to be lowered. Low concentrations of respirable particles are known to be associated with respiratory outcomes^[1]. Also, it was found that bacterial infectivity can be enhanced by inhalant particles^[27].

The current study extends and confirms our previous one^[6] which showed at the level of light microscope that Arabian incense causes pulmonary histological changes. However, the presently used incense Ma'amoul appears to have more damaging effect on the pulmonary structures than the previously investigated incense Bakhour as indicated from the findings of electron microscopy. The present ultrastructural data together with previous report^[7] provide an evidence that inhalation of Bakhour and Ma'amoul, which is a common local daily

custom, can account for pulmonary tissue changes as revealed by electron microscopy.

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