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Effect of Inhalation Anesthetics on the Mitochondrial Structure and Secretory Activity of Human Alveolar Cells

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During administration, anaesthetics can affect physiology of the lung and can provoke oxidative injury applied even in a small concentration. This may cause post-operative disturbances and inflammation and as a consequence programmed cell death. Our previous investigations on the impact of inhalation anesthetics halothane and penthrane on human bronchial and alveolar epithelial cell lines showed strong *in vitro* cyto- and genotoxic effect, after treatment with concentrations close to clinically relevant.

In this study we focused our attention on changes in structure and distribution of mitochondria and in organization of lamellar bodies after treatment of A549 cells with inhalation anesthetics halothane and penthrane. The experiments were performed with a lung-derived human carcinoma cell line A549. Specific *in vivo* staining with Yanus green B was used to assess cytoplasmic localization of mitochondria. Analysis of ultrastructural changes was performed with electron microscopy observations.

Key words: halothane, penthrane, A549 cells, stress-induced apoptosis, necrosis.

Introduction

Recently inhalation anesthesia is widely used in medical practice. During adminisration, anesthetics can affect physiology of the lung applied even in small concentrations and can provoke oxidative damage [1]. This may cause post-operative disturbances and inflammation and as a consequence programmed cell death. Many severe lung diseases, such as adult respiratory distress syndrome and idiopathic pulmonary fibrosis, are associated with increased apoptotic characteristics in pneumocytes type II [2]. This process is accompanied with changes in surfactant production as well [3]. The alveolar surfactant is a phospholypide-protein complex, which prevents alveolar collapse during breath and has protective and antioxidant effect [4]. It is produced by alveolar pneumocytes type II in the form of special organelles — lamellar bodies. Considering the surfactant production, these cells are progenitor for alveoli and have central role in tissue turnover and repair.

Our previous investigations on the impact of inhalation anesthetics halothane and penthrane on human bronchial and alveolar epithelial cell lines (16HBE14o-

and A549, respectively) showed strong *in vitro* cyto- and genotoxic effect after treatment with concentrations close to clinically relevant [5, 6]. We found time- and dose-dependent genotoxic effect of halothane expressed as fragmentation of nuclear DNA and chromosome aberrations [7]. Furthermore we observed changes in nuclear morphology, nuclear and cellular fragmentation during post-treatment period [8].

In accordance with the degree of injury, cells can avoid or undergo cell death (stress-induced apoptosis). The mitochondria considered to be the structures participating in regulation of apoptosis. Signals of pro-apoptotic proteins can activate caspases and hence to induce apoptosis released cytochrom c as a result of injury. All these data focused our attention regarding alterations of mitochondrial structure and distribution in A549 cells and changes in organization of lamellar bodies after treatment with inhalation anesthetics halothane and penthrane.

Material and Methods

Cell culture

The experiments were performed with A549 cells, a lung-derived human carcinoma cell line, which maintains many of the morphological and biochemical characteristics of pneumocytes type II [9] (kindly provided by Anna Segerman, Umeå University, Sweden). Cells were grown in DMEM, supplied with 10 % FBS (HiBond), at 37°C, 5 % CO_2 until they reached 80 % confluence.

Cells were treated for 4 hours in a culture medium, pre-saturated with 3 mM halothane (Narcotane, Leciva) [10] or 0.5 % penthrane (Abbott, USA) at 37 °C. After treatment the cells were washed with phosphate buffer saline (PBS), pH 7.4, the medium was changed and the cells were maintained up to 3 days at optimal conditions without anesthetic.

Analysis of redistribution of mitochondria by light microscopy observations

Specific *in vivo* staining with Yanus green B was used to assess cytoplasmic distribution of mitochondria. The cells were grown on sterile cover slides. One to four days after treatment, cells were washed with PBS, pH 7.4 and incubated with 0.0025 % stain solution for 5-10 min, at 37°C. Redistribution of mitochondria was assessed under the Fluoval light microscope.

Analysis of ultrastructural changes by electron microscopy observations

On the first and third day after treatment, the cells were washed twice with PBS, pH 7.4 and fixed with 1.6 % glutaraldehide in 0.1 M cacodilate buffer (CB), for 1 hour at 4°C in darkness. The dishes were washed threefold with 0.1M CB (each 5 min) and monolayers were detached by scraping, transferred in Eppendorf tubes and pelleted by centrifugation. Post-fixation with 1 % osmium tetraoxide in 0.1 M CB for 1 hour at 4°C (in darkness) was performed. Cells were repeatedly washed with 0.1 M CB, dehydrated following a standard procedure and counterstained with uranyl acetate. The samples were embedded in Epon 812. Ultrathin sections were obtained by Reichert-Jung Ultracut E ultramicrotome and observed under the transmission electron microscope EM "Hitachi H-500".

Results and Discussion

In the control untreated cells generally mitochondria are localized into cytoplasm and after staining they form network-like structure (Fig. 1. — *A*). When the cells undergo apoptosis, mitochondria are accumulated around the nucleus, which probably due to the disturbances of cytoskeletal structures and kinesin-mediated transport [11]. We detected significant mitochondrial displacement after treatment of A 549 cells with 1.5 mM and 2.1 mM halothane. They are gathered in the central part of cytoplasm, mainly around the nucleus (Fig. 1. — *B*). Mitochondrial redistribution maintained to the end of studied period and this may serve as indication of apoptosis induction. If the anaesthetics caused reversible damages in the cells, mitochondrial redistribution could persist only in earlier stages of post-treatment period. Observed mitochondrial behaviour suggested irreversible injury in the A549 cells.

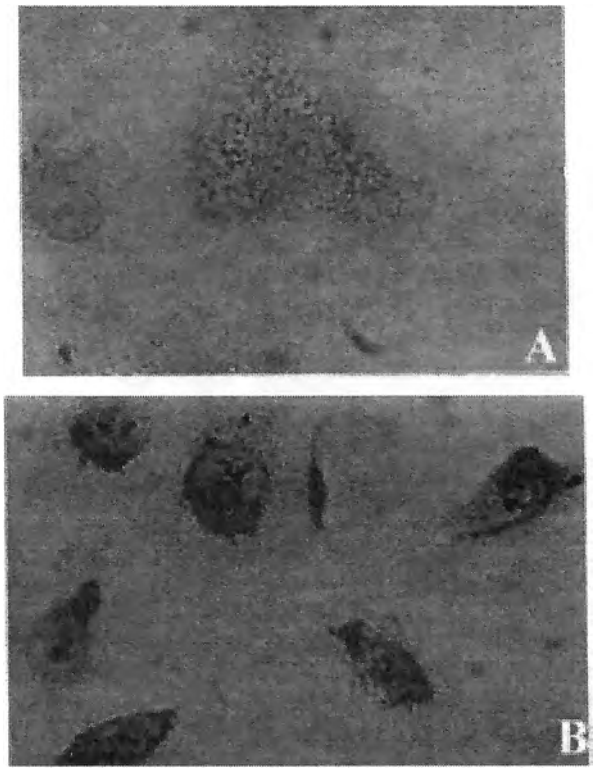

Fig. 1. Redistribution of mitochondria in A 549 cells after 2 hours treatment with 1.5 mM halothane
A — Localization of mitochondria in control (untreated) cell; *B* — Cells on the second day after treatment. In vivo staining with Yanus Green B, original magnification (×400)

The alterations mentioned above were confirmed on the ultrastructural level by electron microscopy observations. Normally A 549 cells have well structured cytoplasm with the typical membrane organelles such as endoplasmic reticulum, Golgi apparatus and lysosomes; moderate number of mitochondria; a large nucleus with small amount of heterochromatin component and one or two nucleoli. Specific organelles for pneumocytes type II as well as for A549 cells are lamellar bodies, which are storage structures for alveolar surfactant (Fig. 2. — *A*).

After treatment with halothane we established significant injuries of mitochondria, which related with swelling, lightening of mitochondrial matrix and kristae de-

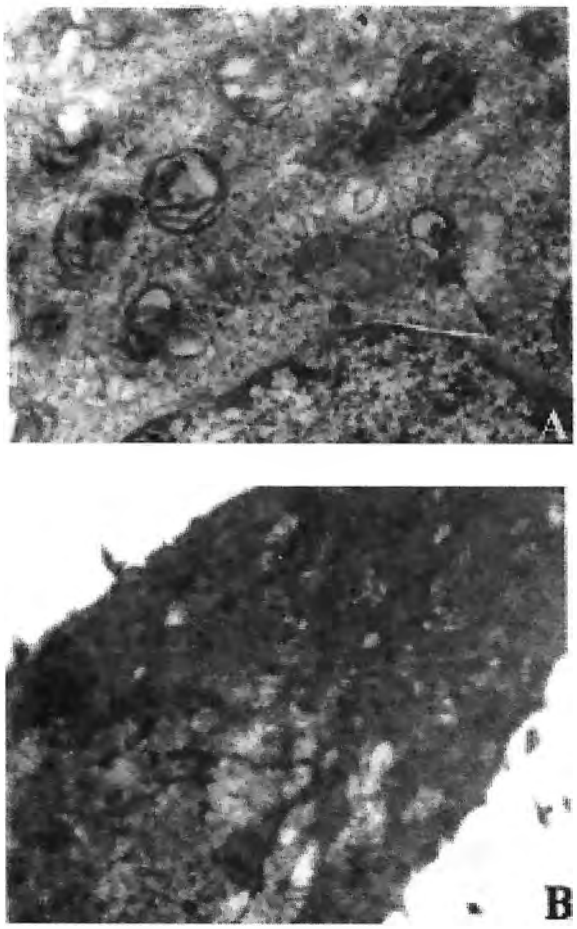

Fig. 2. Ultrastructural changes in mitochondria after halothane treatment. *A* — Ultrastructure of an untreated A 549 cell. Note the lamellar bodies in cytoplasm (×15 000); *B* — On the third day after treatment highly damaged mitochondria are localized around the nucleus and lamellar bodies are disappeared (×12 000)

struction (Fig. 2. — *B*). These changes continued during all studied period. At the same time we observed redistribution of mitochondria around the nucleus; often they form dense clumps close to the outer nuclear membrane. Cells in all samples were with smooth periphery and without lamellar bodies in cytoplasm which indicated inhibition of surfactant synthesis (Fig. 2. — *B*).

Treatment with penthrane caused necrosis in a part of cells (not shown), while other part managed to adapt and possessed relatively preserved morphology (Fig. 3). The cytoplasm was well structured, with many lamellar bodies, endoplasmic reticulum and mitochondria (Fig. 3. — *A*). The cell periphery was relatively smooth but we do not observed secretion (Fig. 3. — *B*). Some mitochondria look dense and with reduced volume.

Conclusions

The ultrastructural changes observed after treatment with 3 mM halothane allowed us to suggest induction of apoptosis-like cell death. The halothane applied at this concentration completely suppressed surfactant production and mitochondria are

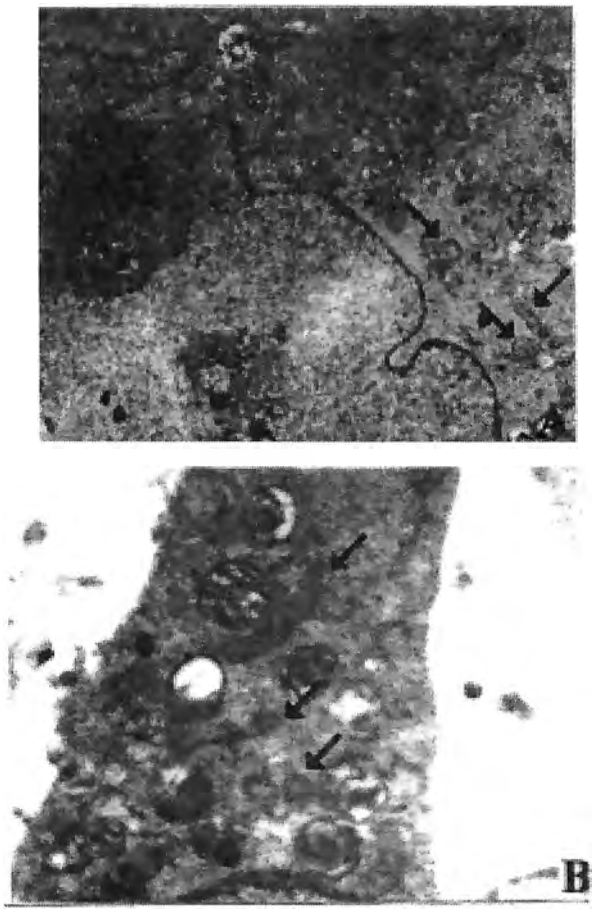

Fig. 3. Survived A 549 cells after treatment with penthrane
A — A 549 cell on the first day after treatment. Large nucleus with two nucleoli and many membrane structures in cytoplasm are seen (×6 400); *B* — A 549 cell on the third day after treatment. The lamellar bodies still persist but cell periphery is relatively smooth (×12 000). Some mitochondria look dense and with reduced volume (marked with arrows)

subjected to ultrastructural injuries, which were observed up to the third day after treatment. We established two groups of cell populations in dependence of the susceptibility to penthrane — more sensitive, which undergo necrosis, and more resistant, which preserve the morphology relatively unchanged.

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