Institute of Experimental Morphology and Anthropology

Acta morphologica et anthropologica, 10
Sofia • 2005

Changes in A 549 Cells Morphology in Response to the Toxic Effect of Halothane

R. Valtcheva, E. Stephanova*, Z. Lalchev**, G. Altankov***, R. Pankov*, S. Kalenderova*****

* *Department of Cytology, Histology and Embryology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia*
** *Department of Biochemistry, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia*
*** *Institute of Biophisics, Bulgarian Academy of Sciences, Sofia*
**** *Department of Chemistry and Biochemistry, Medical University–Sofia*

Cell adhesion, motility and cellular response to different external stimuli are regulated by the dynamic interaction of the cytoskeletal elements. The integrins are transmembrane receptors that interact with actin cytoskeleton and with the extracellular matrix proteins. This interaction induces clustering of signal proteins, forming focal and fibrilar adhesions.
The goal of the present study was to determine the influence of the volatile anaesthetic halothane on actin cytoskeleton and focal adhesion contacts in A 549 cells. The results from immunofluorescence staining with RITC-phalloidin, revealed that the F-actin is disrupted by 1.5 and 2 mM halothane. Indirect immunofluorescence staining of paxillin in the focal adhesion complexes, showed the same effects of anaesthetic. These changes were absent when cells were treated with the non-toxic halothane concentration of 0.9 mM, although cell spreading was delayed.

Key words: A 549 cells, focal adhesions, cytoskeleton, anaesthetics.

Introduction

The structural organization of eukaryotic cells depends on the dynamic interactions among the cytoskeletal elements. The cytoskeletal systems induce changes in cell morphology and motility in response to external stimuli [1, 2]. Actin cytoskeleton interacts with the transmembrane receptors called integrins, which are responsible for the cell adhesive properties and signal transduction between cellular interior and the extracellular matrix [3-5]. The interactions between integrins and their ligands (extracellular matrix proteins, such as fibronectin, laminin, collagen etc.) induce clustering of a various signal transduction proteins and result in the formation of focal and fibrilar adhesions complexes [6, 7].

In our study we used lung cell line A 549 as a model system, which possesses the typical property of type II pneumocytes, to synthesize and release some of the

alveolar surfactant components [8]. Lung tissue is the first one, that interacts with inhaled agents, such as anaesthetics. The anaesthetics could induce immunosuppression, due to the reduction of the activity of NK cells and macrophages [9]. There are also data, demonstrating that the volatile halogenated hydrocarbones could directly interact with the components of the alveolar surfactant, leading to its dysfunction [10]. All these negative effects of the anaesthetics result in metabolic, cardiovascular and respiratory problems in the post-operative period. These anaesthetic agents interact with the phospholipid bilayer, which results in an increased fluidity and changes in the activity of the ion-channels [10, 11]. There are data, showing that the anaesthetics influence the organization MHC molecules on the cell surface and the cell surface receptors, responsible for cell adhesion [12, 13, 14].

The goal of the present study was to determine whether the volatile anaesthetic halothane, applied in concentrations that induce membrane damages, causes also disorganization of the actin cytoskeleton and changes in the focal adhesion contacts in A 549 cells. The results from direct immunofluorescence analyses (staining with RITC-phalloidin), revealed that the F-actin is disrupted by 1.5 and 2 mM halothane. Indirect immunofluorescence staining of paxillin in the focal adhesion complexes, showed the same negative effects of the halothane doses mentioned above. These morphological changes were absent when cells were treated with the non-toxic halothane concentration of 0.9 mM, although cell spreading was delayed.

Material and Methods

Cell culture: The human lung carcinoma cell line A 549 (ATCC No CCL-185) was grown in DMEM supplemented with 10 % fetal bovine serum (FBS) and antibiotic mixture Ampicilline/Streptomycine/Fungizone. Cells were cultured at 37°C in the presence of 5 % CO_2 and were subcultured 24 hours before each experiment.

Halothane dilution and treatment: The culture medium DMEM, supplemented with FBS was saturated with halothane to the final concentration of the anaesthetic agent of 3mM [13].

Immunofluorescence. For visualization of F-actin cytoskeleton and the focal adhesion complexes, A 549 were cultured for 5 hours on pre-coated cover slips with 10 μg/ml collagen IV, until most of the cells adhere, then cells were treated with indicated halothane concentrations for 2 hours [13]. After treatment, cells were fixted in 4 % paraphormaldehyde and stained for 30 minutes at 37°C with 20 U/ml phalloidin conjugated with the fluorochrome RITC, or with mouse monoclonal anti-human paxilline antibody and secondary anti-mouse antibody, conjugated with CY3™.

Results and Discussion

Our previous studies on the effect of halothane on A 549 cells showed, that these cells reach maximal adhesion after 5 hours of culturing. The adhesive properties of the cells were reduced after treatment with halothane concentrations equal or higher than 2.1 mM. The present study revealed that the lower halothane dose of 1.5 mM destroyed F-actin in some of the cells (Fig. 1. — *C*), and after 2 hours of treatment with concentration of 2 mM there were a complete disorganization of the actin cytoskeleton (Fig. 1. — *D*). Similar results were observed in the focal adhesion complexes (Fig. 2). Paxillin level has decreased evidently as a result of the treatment with anaesthetic, applied at concentration of 1.5 mM (Fig. 2. — *C*) and there was a com-

plete loss of the focal adhesions in cells affected by 2.1 mM (Fig. 2. — *D*). Although the treatment with the non-toxic dose of 0.9 mM halothane didn't seem to influence F-actin and the morphology of the focal adhesion complexes, it's clear from Fig. 2. — *A* and *B* that A 549, treated with non-toxic halothane concentration spread two times slower, than non-treated cells. These results show that the non-toxic halothane doses, however affect cell morphology, resulting in delayed and/or inhibited cellular spreading.

We could summarized that the volatile anaesthetics belonging to the halogenated hydrocarbon family negatively influenced the A549 lung cells. The doses, we used for cell treatment correspond to the clinically relevant concentrations cited in the literature [15]. Our results, concerning whole cell morphology, cytoskeleton and the focal adhesions, showed that the cell line A 549 could be used as a useful in vitro model system for studying the dose-dependent effect of the volatile anaesthetics on the cellular level.

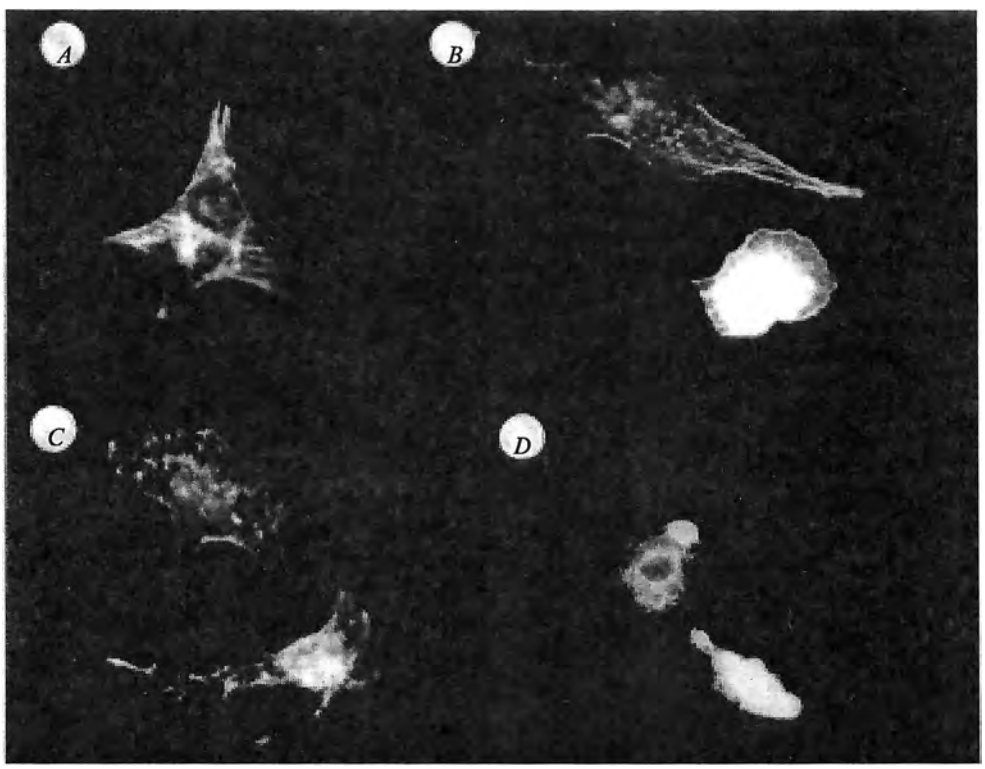

Fig. 1. Direct immunofluorescence staining with RITC-phalloidin. F-actin in A 549 cells is visualized, after 5 hours culturing on collagen IV covered glasses.
A — non-treated cells; *B* — cell treaed 2 hours with 0.9 mM halothane; *C* — cell treaed 2 hours with 1.5 mM halothane; *B* — cell treated 2 hours with 2.1 mM halothane (all photos were taken at magnification 100×)

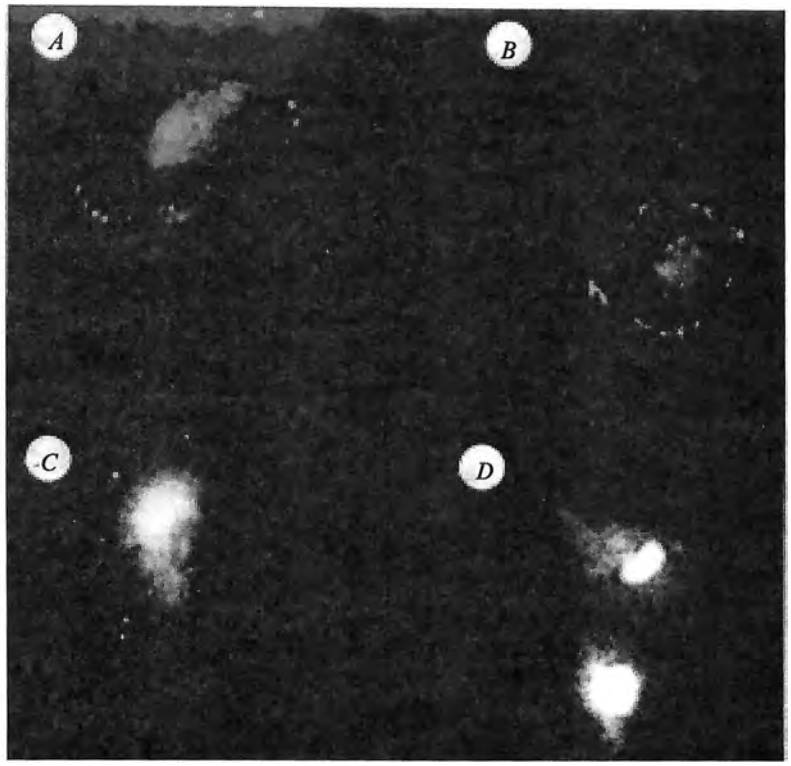

Fig. 2. Indirect immunofluorescence staining with mouse monoclonal anti-human paxillin antibody and CY2™ conjugated secondary anti-mouse antibody. The focal adhesions are visualized after 5 hours culturing on collagen IV covered glasses. *A* — non-treated cells; *B* — cell treated 2 hours with 0.9 mM halothane; *C* — cell treated 2 hours with 1.5 mM halothane; *B* — cell treated 2 hours with 2.1 mM halothane (all photos were taken at magnification 100×)

Acknowledgements: This work was supported by Ministry of Education and Sciences of Bulgaria (Grant MU-K 1301/03 — 2375) and by Medical University, Sofia.

References

1. Chang, L., R. D. Goldman. Intermediate filaments mediate cytoskeletal crosstalk. — Nature, **5**, 2004, 601-613.
2. Revenu, C. et al. The co-workers of actin filaments: from cell structures to signals. — Nature, **5**, 2004, 635-646.
3. Kinbara, K. et al. Ras GTPases: Integrins' friends or foes? — Nature, **10**, 2003, 767-776.
4. Hass, T.-A, E. Pox. Integrin-ligand interactions: a year in review. — Curr. Opin. Cell. Biol, **6**, 1994, 656-662.
5. Liu, S., D. A. Calderwood, M. H. Grinsberg. Integrin cytoplasmic domain-binding proteins. — J. Cell. Sci, **113**, 2000, 3563-3571.
6. Tran, H. et al. Integrin clustering induces kinectin accumulation. — J. Cell. Sci., **115**, 2002, 2031-2040.
7. Geiger, B., A. Bershadsky, R. Pankov, K.Yamada. Transmembrane cross-talk between the extracellular matrix and the cytoskeleton. — Nat. Rev. Mol. Cell. Biol, 2, 2001, 793-805.
8. Lieber, M. et al. A continuous tumor-cell line from a human lung carcionma with properties of type II alveolar epithelial cells. — Int. J. Cancer, **17**, 1976, 62-70.

9. T o n n e s e n, E. Immunological aspects of anaesthesia and surgery. — Dan. Med. Bull., 36, 1989, 263-281.
10. L a l c h e v, Z. et al. Alterations of biochemical and physicochemical quantities of pulmonary lavages attending halothane and penthrane treatment in rats. — Applied Cardiopulmonary Pathophysiology, 4, 1992, 315-322.
11. F r a n k s, N., W. L i e b. Molecular and cellular mechanisms of general anesthesia. — Nature, 333, 1994, 662-664.
12. D z o l j i c, M. et al. Ethanol and halothane differently modulate the HLA class I and class II oligomerization. A new look at the mode of action of anesthetic agents through fluorescence spectroscopy. — J. Photochem. Photobiol. B, 56, 2000, 48-52.
13. V a l t c h e v a, R. et al. Effect of Halothane on human lung carcinoma cells A 549. — Chem. Biol. Interact, 146, 2003, 191-200.
14. B a t a i, I., M. K e r e n y i. Halothane decreases bacterial adherence in vitro. — Acta Anaesthesiol. Scand., 43, 1999, 760-763.
15. B a z i l, C. W. et al. Equilibration of halothane with brain tissue in vitro: comparison to concentration during anaesthesia. — J. Neurochem, 49, 1987, 952-958.