

Original Article

The Clara cells activated by acetaminophen

Birendra Man Amatya¹, Yuji Kimula² and Morio Koike¹

1) Department of Human Pathology, Digestive and Metabolic Diseases, Tokyo Medical and Dental University, Graduate School

2) Ome Municipal General Hospital, Tokyo, Japan

Many high mountaineers die of acute mountain sickness in the high mountains of Nepal during their adventure. Most of them administer acetaminophen (AP) as an analgesic. Various cells with higher expression of cytochrome P-450 (CYP) would metabolize AP and lead to cellular impairment. CYP is a major enzyme system in the metabolism of xenobiotics in different organs of the human being including in Clara cells of the lungs. Clara cells contain the highest concentration of CYP. We injected AP to C57BL/6J mice to examine the toxicity of AP in Clara cells. In 8 hours Clara cells show more prominent apical protrusion with edematous smooth endoplasmic reticulum (sER), reduced secretory granules, and edema. We speculate that using AP in a high mountain area might be harmful for Clara cells being a trigger of high altitude sickness.

Key words: cytochrome P-450, Clara cell, acetaminophen, high altitude sickness.

Introduction

Many high mountain trekkers to the Himalayas of

Nepal have been reported to develop acute mountain sickness (AMS) frequently. Approximately 20% of all trekkers who visited the higher Pheriche (4,243m from sea level) were diagnosed with AMS¹. Most of them were characterized by pulmonary edema². High altitude pilgrims, especially women, are more prone to develop AMS³. Some trekkers experience a headache at night and are found to be dead the next morning. Trekkers are used to taking acetaminophen (AP) with ease when they feel chills or febrile. Various cells with higher expression of cytochrome P-450 (CYP) would metabolize AP. CYP 2E1 has been identified as a dominant isoform in the production of N-acetyl-p-benzoquinone imine (NAPQI) from AP in the human being⁴.

CYP dependent mono-oxygenase system functions as a vital pathway in a number of organs for the metabolism of numerous environmental xenobiotics^{5,6}. The Clara cells, being metabolically active cells, are located at the critical junction between conducting airways and alveoli⁷. Secretory granules of the Clara cells are being considered to be Clara cell 10-kDa (CC10), surfactant protein-A, B, D, leukocyte protease inhibitor, beta-galactoside binding lectin, and trypsin like protease⁸. Clara cells predominate throughout the tracheobronchial airways of a rabbit and have the pulmonary CYP monooxygenase system, CYP isozymes, I, II and V, and NADPH-CYP reductase^{9,10,11}. Ultra-structurally Clara cells possess numerous round mitochondria and sER¹². They maintain normal proliferating epithelium of the distal airway that account up to 44% in the respiratory bronchioles¹³.

The high level of CYP activity in Clara cells renders the cells highly susceptible to injury by a wide range of

Corresponding Author: Birendra Man Amatya
Department of Human Pathology, Digestive and Metabolic Diseases, Tokyo Medical and Dental University, Graduate School, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
Phone: (+81) -3-5803-5172, FAX: (+81) -3-5803-0123
E-mail: amatyabirendra@hotmail.com
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xenobiotics. The metabolism of AP, being an agent commonly used by trekkers, has not been considered in the Clara cells yet. This study has been designed to determine an influence of AP metabolism in Clara cells morphologically.

Materials and methods

Animals

Thirty-five male C57BL/6J mice (Nihon Clea, Tokyo, Japan) at five weeks old, weighing from 18 to 22 grams, were selected to undergo experimental study. The mice had been divided into 7 experimental groups, 5 mice in each. One fourth of the lethal dose of AP (Sigma, Tokyo, Japan), 225mg/kg body weight in saline, was administered to each experimental group intraperitoneally and each group was killed by cervical dislocation at various time intervals after AP administration; 0 hour, 1 hour, 2, 4, 8, 16, and 32 hours. Before the removal of the viscera, the lungs were perfused via the trachea with 1% glutaraldehyde phosphate buffer solution. All mouse work was performed in accordance with the guideline established by the Animal Experiment Committee of the Tokyo Medical and Dental University.

Histopathology

The right upper, middle, and cardiac lung lobes, heart and liver of the mice were fixed in neutral formalin solution, processed routinely, and stained with hematoxylin and eosin and Azan stains. The right lower lung lobe was submitted for transmission electron microscopic examination and the left lung was examined by the scanning electron microscopy.

Scanning Electron microscopy

After fixation with 1% of glutaraldehyde in 0.1M phosphate buffer, the specimens underwent dehydration with routine ethanol and n-pentyl-acetate series followed by critical point drying system, JCPD-5 (JEOL, Japan), and then finally were coated with gold by an ion sputtering device, JFC-1100 (JEOL, Japan). Lastly prepared specimens were observed under a scanning electron microscope, JSM-T20 (JEOL, Japan).

Calculation of Clara cells measurement

The scanning micrographs taken by 1000 times in magnification from the terminal bronchioles were analyzed by Scion Image system (Scion Cooperation, MD, USA). The area of the apical protrusions of the

Clara cells and the number of the Clara cells were calculated. The calculation was analyzed statistically at the level of the significance by means of Student's t-test.

Transmission Electron Microscopy

The lung samples were fixed by 1% glutaraldehyde in 0.1M phosphate buffer, and again fixed by 2% osmium tetroxide in 0.1M phosphate buffer. The tissues were embedded in epoxy resin. The ultra-thin sections were made by ultramicrotome, Ultracut E (Leica, Germany) and stained with uranyl acetate and lead citrate, and observed with a transmission electron microscopy, JEM-100SX (JEOL, Japan).

Results

Morphology of Clara cells by the scanning electron microscope

The terminal bronchioles of the control mice reveal smooth apical protrusions of Clara cells sized approximately 7.8 μm in diameter. There are an equal proportion of the Clara cells and ciliated cells in the terminal bronchioles (Fig. 1a). Morphological changes of the Clara cells are observed in all experimental groups after exposure to AP. The apical protrusions of the Clara cells become prominent after 1 hour, 8.1 μm in diameter (Fig. 1b) and increase in size up to 8 hours after AP administration, 8.4 μm . So the average area of the apical protrusions of the Clara cells of the experimental mice is significantly greater than that of the control group ($p < 0.0001$). The highest value has been achieved after 8 hours of AP injection (Fig. 1c). The surface areas of apical protrusions after 16 and 32 hours also show enlargement when compared to control group statistically ($p < 0.0001$). However, there is no significant difference among 1 h, 2 h, 4 h, 16 h, and 32 h groups ($p > 0.05$). After 8 hours of AP exposure, the Clara cells start to develop the regenerative changes showing homogenously enlarged apical protrusion by 32 hours (Fig. 1d). Neither Clara cell necrosis nor any significant change in ciliated cell has been revealed with this sublethal dose of AP.

The increase in size of the apical cytoplasm of the Clara cells has been noticed as early as 1 hour after exposure and achieves the biggest size after 8 hours (Fig. 2). The apical caps of Clara cells after 16 and 32 hours also show larger surface areas than the control mice ($P < 0.0001$).

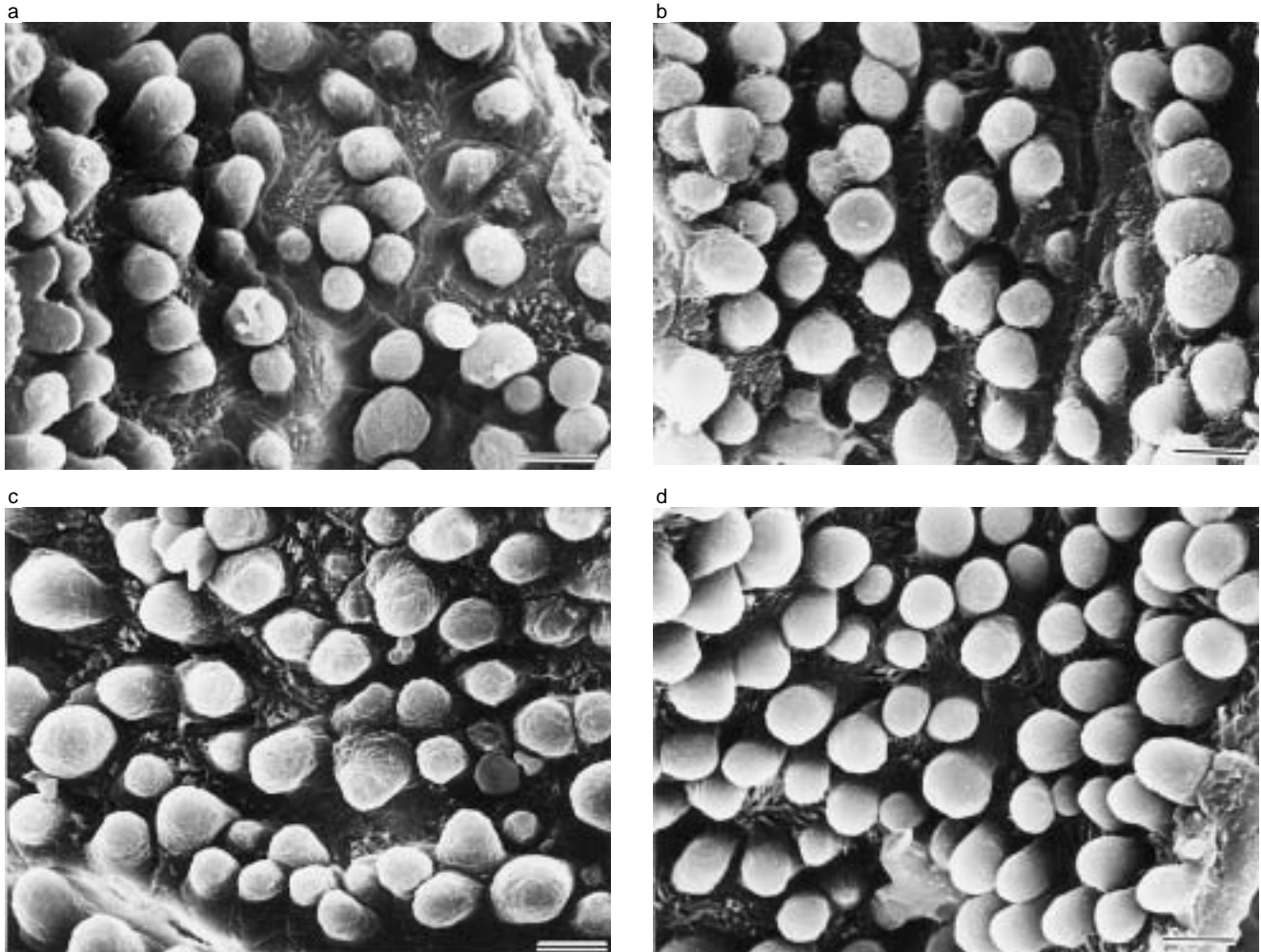


Fig. 1. Scanning electron micrographs of terminal bronchioles. (a) Apical protrusions of Clara cells from control mouse intermingled with ciliated cells. (b) Prominent apical projections of Clara cells after one hour of AP injection. (c) Large apical protrusions of Clara cell after 8 hours of AP treatment. (d) Terminal bronchiole of the mouse after 32 hours. Each Bar = 10 μm .

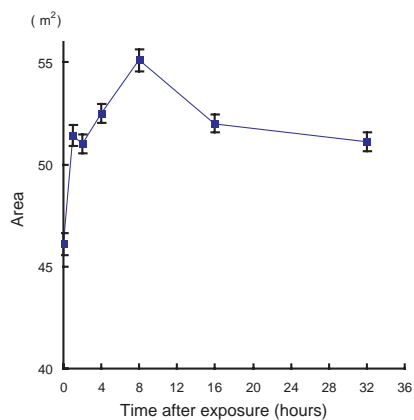


Fig. 2. Time-lapse change in surface area of apical protrusion of the Clara cells after AP treatment. The maximum measurement was obtained after 8 hours of AP exposure. Apical caps of the Clara cell in all AP treated mice are larger than control mice ($p < 0.0001$). Error bars indicate SEM.

Transmission electron microscopic findings

The normal Clara cells demonstrate secretory granules of various sizes with prominent sER. The mitochondria scatter throughout the cytoplasm (Fig. 3a and 3b). However secretory granules of the Clara cells disappear at 8 hours after AP administration. The population of the mitochondria seems to be reduced and are mostly distributed in the perinuclear zone with remarkable edema (Fig. 3c). The swollen and irregularly arranged sER are more prominent in apical protrusions of the Clara cells (Fig. 3d). The cellular height of the Clara cell is approximately double the height of the ciliated epithelial cells in the 8-hour group. The Clara cells show a feature of regeneration

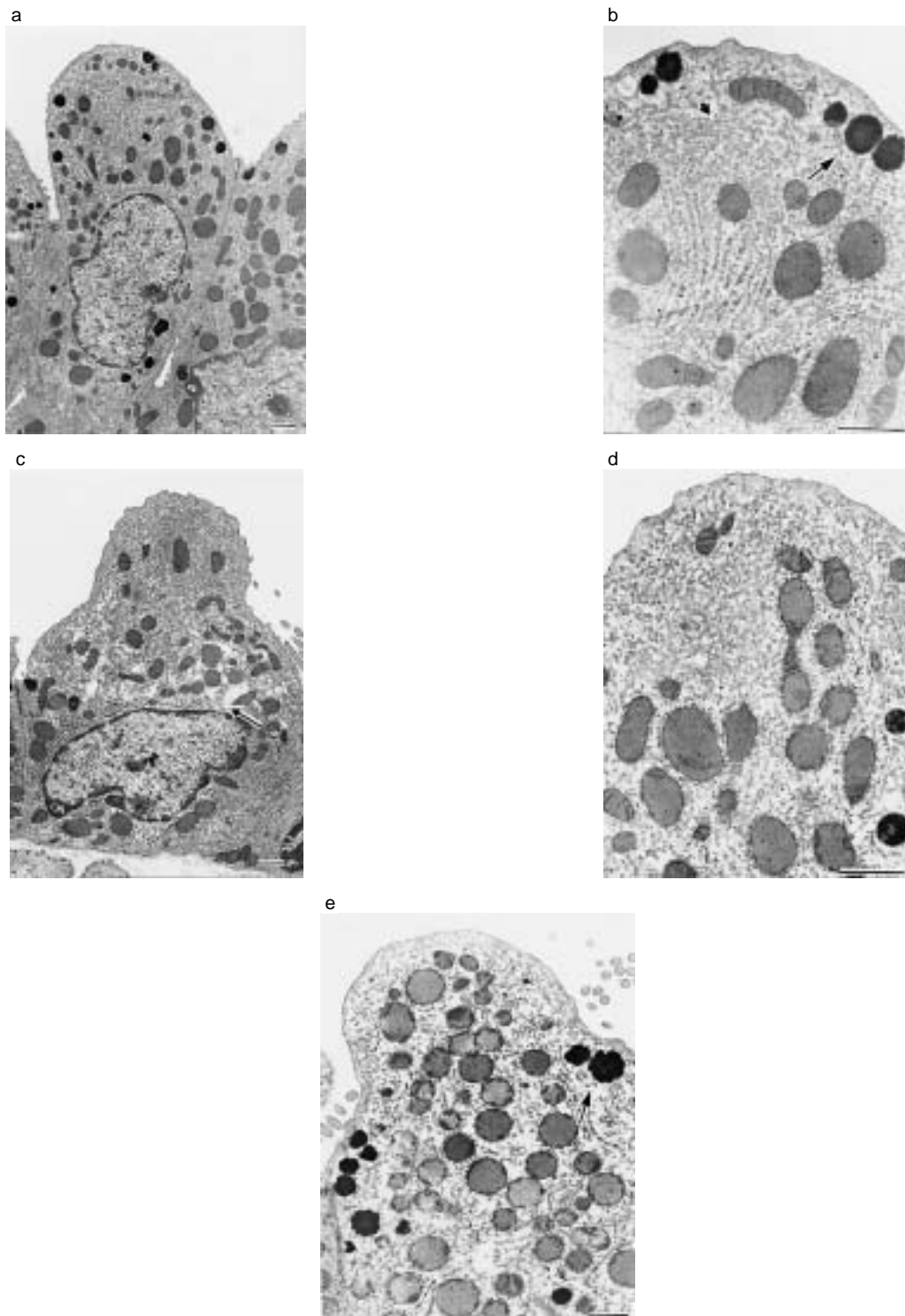


Fig. 3. Transmission electron micrographs of Clara cells. Uranyl acetate and lead citrate stain. (a) Clara cell from control mouse. (b) The higher magnification demonstrates several secretory granules (open arrow) and laminar arrangement of sER (arrowhead) around mitochondria. (c) Clara cell after 8 hours showing reduced number of secretory granules and perinuclear edema (long arrow). (d) Higher magnification of the Clara cell after 8 hours shows irregular arrangement of sER in the apical cap with a few secretory granules. (e) Clara cell after 32 hours showing features of regeneration after acute injury. An arrow demonstrates secretory granule. Each Bar = 1 μ m.

by 32 hours, which is characterized by many secretory granules and heavily laden mitochondria, and a lesser degree of edema in the cytoplasm (Fig. 3e).

Histopathology

The sections from the lungs and heart are unrevealing in hematoxylin and eosin and Azan stains. The liver starts to show the feature of degeneration after one hour of AP exposure and massive centrilobular necrosis by 8 hours.

Discussion

The present study has revealed a striking morphological alteration in Clara cells after the administration of AP. The size of Clara cells increases first, peaks at 8 hours after exposure, and then gradually reduces. However, the apical caps of Clara cells after 16 and 32 hours still reveal larger surface areas than the control mice. Transmission electron micrographs clarify that this cellular enlargement is attributable to intracellular edema and swelling of sER collected in apical caps. The most of AP might have been metabolized in the first 8 hours of injection, so this group of mice have been severely affected than others. AP might have been started to undergo catabolism since first hour of injection. The generation of highly reactive metabolite of AP, probably NAPQI would be a possible pathogenesis of an acute toxicity in the Clara cells. It can therefore react with nucleophilic sites in the cell macromolecules resulting in injury to the cells. This mechanism of toxicity in Clara cells could be a phenomenon similar to that found in liver cells. However an effect of AP in the human Clara cells has not been studied so far. We presume that frequent administration of AP or high dose of AP might bring similar effects in human Clara cells. Clara cells would reduce surfactant protein secretion under an influence of AP toxicity that might predispose an individual to develop high altitude sickness in trekkers. However we need further studies to show an exact relationship between AP metabolism in human Clara cells and high mountain sickness.

One of the phenotypic characteristics of the Clara cells is an abundance of the CYP dependent monooxygenases system that renders the cells highly susceptible to injury by a wide range of pulmonary toxicants^{8,14}. Many studies have shown an active participation of Clara cells in bio-activation of chemicals through the CYP mediated metabolism, which is characterized by various ways of toxicity leading to cell

death. Naphthalene causes severe injury to Clara cells characterized by apical blebs containing abundant swollen sER followed by exfoliation¹⁵. 4-ipomeanol metabolism in Clara cells demonstrates the potentially toxic product covalently binding to cellular macromolecules leading to pulmonary edema and hemorrhage¹⁶. Similarly oral or inhalation of carbon tetrachloride shows severe dilation of sER and occasional cellular necrosis of the Clara cells¹⁷.

Prevention of AP induced olfactory toxicity in mice can be achieved with dially sulfide (DAS), which will completely inhibit CYP 2A5 and 2G1¹⁸. Acute cataract and other ocular tissue damage in mice treated with a high dose of AP (350mg/kg b w) can be prevented completely with the combination of dially disulfide (DADS) and N-acetyl L-cysteine (NAC)¹⁹.

The present paper concludes that being endowed with higher expression of CYP, the Clara cells manifest a feature of acute metabolic insult caused by environmental xenobiotics including AP that might have a detrimental effect on the physiology of the Clara cells. The consequence of acute toxicity of AP observed in the Clara cells might trigger the development of high altitude sickness in trekkers. Use of CYP inhibitor and/or NAC in an attempt to prevent the toxicity of AP would be the most promising intervention in trekkers, who administer AP very frequently. However we need further studies to elucidate the toxicity of AP in the Clara cells of the human being.

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References

1. Basnyat B, Savard GK, Zafren K. Trend in the workload of the two high altitude aid posts in the Nepal Himalayas. *J Travel Med* 1999; 6: 217-22.
2. Goodman T, Basnyat B. A tragic report of probable high altitude pulmonary edema in the Himalayas: preventive implications. *Wilderness Environ Med* 2000 Summer; 11:99-101.
3. Basnyat B, Subedi D, Sleggs J. et al. Disoriented and ataxic pilgrims: an epidemiological study of acute mountain sickness and high altitude cerebral edema at a sacred lake at 4300 m in

- the Nepal Himalays. *Wilderness Environ Med* 2000 Summer; 11: 89-93.
4. Sarich T, Kalthorn T, Magee S. et al. The effect of omeprazole pretreatment on acetaminophen metabolism in rapid and slow metabolizers of S-mephenytoin. *Clin Pharmacol Ther* 1997; 62: 21-28.
 5. Yokose T, Doy M, Kakiki M. et al. Expression of cytochrome P450 3A4 in foveolar epithelium with intestinal metaplasia of the human stomach. *Jpn J Cancer Res* 1998; 89: 1028-32.
 6. Yokose T, Doy M, Taniguchi T. et al. Immunohistochemical study of cytochrome P-450 2C and 3A in human non-neoplastic and neoplastic tissues. *Virchows Arch* 1999; 434: 401-11.
 7. Clara Max. Zur Histobiologie des Bronchalepithels, *Z, Mikrosk. Anat Forsch* 1937; 3: 321-47.
 8. Massaro GD, Singh G, Mason R. et al. Biology of the Clara cell. *Am J Physiol* 1994; 266: 101-06.
 9. Plopper CG, Cranz DL, Kemp L. et al. Immunohistochemical demonstration of cytochrome P-450 monooxygenase in Clara cells throughout the tracheobronchial airways of the rabbit. *Exp Lung Res* 1987; 13: 59-68.
 10. Devereux TR, Serabjit Singh CJ, Slaughter SR. et al. Identification of cytochrome P-450 isozymes in non-ciliated bronchial epithelial (Clara cells) and alveolar type II cells isolated from rabbit lung. *Exp Lung Res* 1981; 2: 221-30.
 11. Serabjit-Singh CJ, Nishio SJ, Philpot RM. et al. Distribution of Cytochrome P-450 Monooxygenase in cells of rabbit lung: An ultrastructural immunocytochemical characterization. *Mol Pharmacol* 1988; 33: 279-89.
 12. Kimula Y. The Clara cell and bronchial diseases. *The Journal of the Japan Society for Bronchology* 1988; 10: 467-78.
 13. Boers JE, Ambergen AW, Thunnissen FB. Number and proliferation of Clara cells in normal human airway epithelium. *Am J Respir Crit Care Med* 1999; 159: 1585-91.
 14. Philpot RM, Smith BR. Role of cytochrome P-450 and related enzymes in the pulmonary metabolism of xenobiotics. *Environ Health Perspect* 1984; 55: 359-67.
 15. Van Winkle LS, Johnson ZA, Nishio SJ. et al. Early events in naphthalene-induced acute Clara cell toxicity: comparison of membrane permeability and ultrastructure. *Am J Respir Cell Mol Biol* 1999; 21: 44-53.
 16. Hukkanen J, Lassila A, Paivarinta K. et al. Induction and regulation of xenobiotic-metabolizing Cytochrome P-450s in the Human A549 Lung adenocarcinoma cell line. *Am J Respir Cell Mol Biol* 2000; 22: 360-66.
 17. Boyd MR, Statham CN, Longo NS. The pulmonary Clara cell as a target for toxic chemicals requiring metabolic activation; studies with carbon tetrachloride. *J Pharmacol Exp Ther* 1980; 212: 109-14.
 18. Genter MB, Liang HC, Gu J. et al. Role of CYP 2A5 and 2G1 in acetaminophen metabolism and toxicity in the olfactory mucosa of the CYP-1a2 (-/-) mouse. *Biochem Pharmacol* 1998; 55: 1819-26.
 19. Zhao C, Shichi H. Prevention of acetaminophen-induced cataract by a combination of diallyl disulfide and N-acetylcysteine. *J Ocul Pharmacol Ther* 1998; 14: 345-55.