THE SEQUENTIAL CHANGE OF A NAPHTHALENE-INDUCED BRONCHIOLAR DAMAGE AND OF PULMONARY CYTOCHROME P-450 IN MICE

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Abstract: Male ICR mice were exposed to 2 mmol/Kg body weight of naphthalene by single intraperitoneal injection. Treated mice were killed consecutively from 2 hours to 14 days after treatment. Tissues were examined for alterations of the bronchiolar epithelial cells by light and electron microscopes, and for amount of pulmonary cytochrome P-450 by ascorbate PES method.

Morphologically, naphthalene caused a remarkable increase in smooth-surfaced endoplasmic reticulum of the Clara cells within 4 hours after treatment and the subsequent lesions including the exfoliated ciliated cells. Foamy cell-like Clara cells were observed in the bronchiolar lumen from 6 to 24 hours followed by a pronounced increase in number of flat ciliated cells covering the denuded surface of the bronchiolar tree 48 hours after treatment. These lesions were almost completely recovered 7 days after treatment.

The amount of pulmonary cytochrome P-450 increased promptly and reached up to the maximum level 4 hours after treatment, paralleling with the remarkable increase in smooth surfaced endoplasmic reticulum of the Clara cells. Thereafter, cytochrome P-450 showed a decrease in amount simultaneously with degeneration and desquamation of the Clara cells and reduced by 40% of control amount 24 hours after treatment. These results seem to show that the activity of cytochrome P-450 is closely related with smooth surfaced endoplasmic reticulum of the Clara cells, and naphthalene-induced pulmonary damage is mediated by cytochrome P-450. (*J Toxicol Pathol* 1: 137~142, 1988)

Key words: Naphthalene, Bronchiolar damage, Cytochrome P-450

Introduction

Naphthalene (NP) is an important intermediate which is used as a dye and antioxidant. It is a major component of moth balls and has been used for insect and parasite elimination.

NP is known to damage selectively the non-ciliated bronchiolar epithelial cells: Clara cells of

the lung in the mouse^{1,2}. The recent papers^{3,4} on the sensitivity to several toxic chemicals of the Clara cells containing high levels of cytochrome P-450 enzymes⁵, have evoked attention on these cells as a target of xenobiotics⁶.

There have been several studies on NP-induced lung damage in mice⁷⁻¹⁰, but its early ultra-structural alterations have not been fully studied. The present study was carried out to disclose a correlation of NP-induced damage to the Clara cell with the activity of pulmonary cytochrome P-450 in mice.

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Materials and Methods

Male ICR mice weighing 26 to 35 g received a single intraperitoneal injection with 2 mmol/Kg naphthalene (NP) dissolved in corn oil.

Morphology

Mice were sacrificed under anesthesia using pentobarbital 2, 4, 6, 12, and 24 hours and 2, 7, and 14 days after treatment, respectively. The trachea was rapidly excised, and the fixative was injected through a silicone cannula inserted in the trachea. Lung expansion was easily achieved with 8 to 10 cm water pressure.

Light microscopy

Each three animals were examined at the time of sacrifice. Buffered formalin solution of 10% was used as a fixative. The lungs were cut and fixed overnight in the same fixative. The tissue specimen was prepared by the regular way.

Electron microscopy

Each two animals were examined at the time of sacrifice. 2.5% glutaraldehyde solution in 0.1 M phosphate buffer was used as a fixative. The lungs were cut into 1 mm³ strips and fixed in the same fixative. The strips were rinsed in 0.1 M phosphate buffer and post-fixed with 1% osmium tetroxide, dehydrated by passage through a graded series of concentrations of ethanol and acetone, and embedded in epon-araldite. Ultrathin sec-

tions were cut, stained with uranyl acetate and lead citrate and examined with JEM 100 CX-II electron microscope.

Measurement of cytochrome P-450

Mice were sacrificed 0 (control), 2, 4, 6, 24 hours after the treatment. Each 40 animals were used for each pool of lungs at the time of sacrifice and pulmonary microsomes were prepared by the method using calcium aggregation¹¹. Cytochromes P-450 were measured by the method of ascorbate-PES¹².

Results

Pulmonary cytochrome P-450 showed an increase in amount 2 hours after the treatment of naphthalene (NP) and reached to the maximum (1.6 times of control) 4 hours after the treatment. Thereafter, P-450 showed a gradual decrease and reduced by 37% of the normal amount 24 hours after the treatment (Fig. 1).

Morphology

Light microscopy; Terminal bronchioles are composed of two types of cells; ciliated cell and nonciliated: Clara cell. These two cells are easily distinguished in the control lungs.

In the NP-treated mice, Clara cells showed a swelling of cytoplasm from 2 to 4 hours after the treatment. Degeneration or necrosis of the Clara cells with subsequent change looking like a foamy

Fig. 1. Levels of cytochrome P-450 in lung microsomes obtained from control and naphthalene (NP)-treated mice.

Fig. 2. Light micrograph. 12 hours after treatment with NP. An accumulation of exfoliated Clara cells is seen as a debris in the bronchiolar lumen. (H.E., $\times 80$)

Fig. 3. 24 hours after treatment with NP. Bronchiolar wall is seen lined with flat ciliated cells. (H.E., $\times 80$)

cell and exfoliation of the ciliated cells in the bronchiolar lumen were seen 6 hours after the treatment. These alterations were also observed up to 24 hours after the treatment. Especially, an accumulation of the exfoliated Clara cells was seen as a debris in the bronchiolar lumen (Fig. 2). Gradual increase in number of flat ciliated cells on the surface of bronchiolar tree was seen 48 hours after the treatment (Fig. 3). Bronchiolar epithelium was almost completely recovered 7 days after the treatment.

Electron microscopy; Clara cells are columnar in shape. There are abundant smooth surfaced endoplasmic reticulum (SER) in their cytoplasm. The center of the Clara cell is occupied by the nucleus dividing cytoplasmic zones. The basal zone contains rough surfaced endoplasmic reticulum (RER) and mitochondria with scanty cristae. The upper zone contains many mitochondria similar to those in the basal zone and secretory granules with high density. Ciliated cells are columnar with poor organellae compared with Clara cell (Fig. 4).

In the naphthalene treated mice, the Clara cells showed a swelling of the cytoplasm with the increase in SER and circular vacuoles. However, RER was decreased 2 hours after the treatment (Fig. 5). The enlarged vacuoles increased with the other vacuoles formed by expansion of the antrum of SER 4 hours after the treatment. The

cytoplasm was occupied by SER and concentric lamellar bodies composed of SER also appeared therein (Fig. 6). Vacuolated Clara cells similar to foamy cells with decreased SER appeared 6 hours after the treatment (Fig. 8). The exfoliating cells, debris, and macrophage were seen scattered in the bronchiolar lumen because of damage of the Clara cells occurring 12 hours after the treatment. Flat ciliated cells of bronchiolar epithelium-origin were observed in this stage (Figs. 9 and 10). A part of the bronchi was denuded by the loss of epithelial cells 12 hours after the treatment (Fig. 9). However, mitosis (Fig. 7) and proliferation of the bronchiolar epithelium were seen 48 hours after the treatment. The bronchioli were indistinguishable from those of the control lungs by 7 days. No debris was seen in the lumen of the bronchioli.

Discussion

Naphthalene (NP) is known to damage selectively the Clara cells of lung in mice^{1,2}. And the enzyme of cytochrome P-450 has been suggested to be highly localized in these cells⁵. This study was carried out to disclose a correlation of NP-induced damage of the Clara cells with the activity of pulmonary cytochrome P-450 in mice.

Intraperitoneal injection of NP caused a remarkable proliferation and dilatation of the smooth surfaced endoplasmic reticulum (SER) in the Clara cells. Their alterations have been

Fig. 4. Electron micrograph. Ciliated cell and Clara cells from control mouse. * bar = $2\mu\text{m}$

Fig. 5. 2 hours after treatment with NP. Clara cell shows swelling of the cytoplasm with an increase in number of SER.

Fig. 6. 6 hours after treatment with NP. CLB and circular vacuoles are found in cytoplasm of Clara cells.

Fig. 7. 48 hours after treatment with NP. Mitosis in Clara cell.

Fig. 8. 6 hours after treatment with NP. Vacuolation of Clara cells like foamy cells and ciliated cells are seen.

Fig. 9. 12 hours after treatment with NP. Flat ciliated cell is replacing damaged Clara cells. A part of the basement membrane is denuded (arrow).

Fig. 10. 12 hours after treatment with NP. Exfoliation of Clara cells is seen as a debris in the lumen.

known to be induced by various lung toxins, such as butylated hydroxytoluene (BHT)^{13,14} and PCB¹⁵. Tong *et al.* examined the morphological change

and mixed function oxidase activity of lungs 24 hours after the treatment of NP in mice. They reported that cytochrome P-450 was most sensitive

to the effect of NP, being reduced by 65%⁹.

The amount of pulmonary cytochrome P-450 increased at an early period, and reached its maximum level 4 hours after the treatment, paralleled with the remarkable increase in SER of the Clara cells in the present study. Thereafter, P-450 showed a decrease in amount simultaneous with degeneration and desquamation of the Clara cells. These results indicate that cytochrome P-450 localized at SER membrane of the Clara cell plays an important role in drug metabolism of lung. Warren *et al.* reported that NP-induced pulmonary damage is mediated by the cytochrome P-450-dependent metabolism of NP¹⁰. Our study seems to support their views.

Concentric lamella bodies (CLB) are known to appear in tissues by various mechanisms inducing carcinogenesis^{16,17}. This figure is formed from SER and observed also in the Clara cell by BHT¹³ or adrenalin¹⁸. Foamy cell-like appearance of the Clara cells seems to reflect vacuolation produced by dilation of SER.

Whole area of the bronchi was denuded by the loss of Clara cells in the study of Tong *et al.*⁹. However, in our study, denudation was seen focally at the transitional area from bronchi to alveoli. Denudation at this area seems to be progressed on the basement membrane of the Clara cells replaced with the flat ciliated cells (Figs. 9 and 10). The flat ciliated cells seem to represent a reparatory change of the bronchiolar cells followed by the subsequent regeneration of the Clara cells. Contrary to these findings, Evans *et al.* suggested that the ciliated cells were derived from Clara cells based on their observation with treatment of NO₂¹⁹. Ebe suggested the existence of the intermediate cell transferring from Clara cell to type II alveolar cell²⁰. These studies suggest that bronchiolar epithelial cells have a high potency in differentiation.

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