

Isolation and Cultivation of Lung Epithelial Type 2 Cells. The Effect of Cadmium

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Abstract. The effects of cadmium exposure on rat lung type 2 cells were evaluated by morphological and biochemical examinations. The results showed dose dependent reduction in the marker enzyme (alkaline phosphatase), changes in cell membranes and in the antioxidant status.

Key words: Type 2 Pneumocytes — Cadmium — Alkaline phosphatase — *In vitro* Antioxidant status

Introduction

Lung is a very complex organ, consisting of more than 40 different cell types (Sorokin 1970). Each cell type has a unique morphologic characteristic associated with its function. One of the most important from the toxicological point of view are the alveolar epithelial type 2 cells. The alveolar epithelial surface is built up of two distinct epithelial cell types, type 1 and type 2 cells. Type 2 cells cover approximately 5% of the alveolar surface area, they are of cuboidal shape and contain lamellar body structures, where the surfactant is stored. The pulmonary surfactant is a complex of phospholipids and tissue specific proteins that lowers surface tension and prevents atelectasis. In the bronchoalveolar region of the lung only the apical portion of alveolar type 2 cells is associated with alkaline phosphatase (Miller et al. 1987). The lamellar bodies and the alkaline phosphatase are used as markers for this type of cells.

Type 2 cells represent specific targets for a variety of pulmonary toxicants. Cadmium belongs to the most frequent environmental contaminant. It is generated by car emissions, cigarette smoking and metal processing factories. Its inhalation may lead to chronic lung diseases. Type 2 cells are progenitors of certain types of tumours, and cadmium has been classified as a group 1 carcinogen in humans (IARC 1993). The possible involvement of type II cells in cadmium pulmonary toxicity was the reason for studying its toxicity in these cells by morphological and biochemical methods.

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Materials and Methods

Animals

Male Albino Wistar rats (Velaz, Prague, Czech Republic) weighing 180–220 g were used in these experiments. The animals were maintained under standard laboratory conditions and were given conventional laboratory diet (MOK, Velaz, Prague, Czech Republic) and tap water ad libitum.

Reagents and media

DMEM was purchased from Pansystem GmbH Aidenbach (Germany), fetal calf serum (FCS) from SEBAK GmbH Aidenbach (Germany), Durcupan ACM (Fluka, Germany), total antioxidant status kit from Randox Laboratories Ltd (United Kingdom), Bio-Rad protein assay from Bio-Rad (USA). All other chemicals were supplied by Sigma (USA).

Cell isolation and culturing

Rat alveolar type 2 cells were isolated according to methods described previously (Richards et al. 1987, Hoet et al. 1995). The cells were cultured on 96 or 24 well plates (Falcon) in DMEM supplemented with 10% FCS (37°C, 95% air/5% CO₂). The plating density was 100,000 or 250,000 cell/well, respectively. The medium was changed 20 h after plating and replaced with a medium containing various concentration of CdCl₂. The cells were cultured for additional 24h.

Cell identification

The cultured cells were stained for AP by the method of Bingle et al. (1990). Lamellar inclusion bodies were counted using phase contrast microscopy. Only cells with at least 4 lamellar bodies were considered as type 2. Biotin labeled Maclura pomifera agglutinin (MPA) was used for detection of the type 2 cell membranes.

Transmission electron microscopy (TEM)

In the isolated cells the purity was checked by TEM. After fixation in 2.5% glutaraldehyde (pH 7.4) cells were dehydrated, embedded in Durcupan, cut and stained with uranyl acetate and lead citrate. The preparations were examined in a JEOL JEM 100 transmission electron microscope.

Biochemical estimations

Protein was measured using the method of Bradford (1976) and expressed in mg ml⁻¹, total glutathione (GSH) was determined using the GSH reductase method of Anderson (1985) and calculated in nmol mg protein⁻¹, the activity of alkaline phosphatase was estimated according to Kováčiková (1990) and calculated in nmol mg protein⁻¹. The total antioxidant status was evaluated with the Randox kit and expressed in mmol mg⁻¹ protein.

Results

The purity of freshly isolated cells was checked by TEM, their average purity was 76 % (Fig. 1). The cultured pneumocytes type 2 showed very intense alkaline phosphatase positivity in the cytoplasm (Fig. 2) and their cell membranes reacted with MPA (Fig. 3). After exposure to cadmium chloride the activity of alkaline phosphatase decreased at the concentration 10^{-6} mol.l⁻¹, and completely disappeared at the concentration 10^{-5} mol.l⁻¹. Reaction with MPA did not change at LC₅₀ (5×10^{-6} mol.l⁻¹, data not shown) but at 5×10^{-5} mol.l⁻¹ it turned into irregular in the cell membranes (Fig. 4).

The alkaline phosphatase activity was simultaneously estimated also biochemically. The activity decreased gradually: 54 % of the control activity was detectable at 5×10^{-5} mol.l⁻¹. The decrease of total glutathione was clearly dependent on the concentration of cadmium. The total antioxidant status of the cells was decreasing parallelly and reached 50 % at 10^{-6} mol l⁻¹. The results are summarized in Fig. 5.



Figure 1. Electronmicrograph of type 2 cells from the rat lung. The structure of type 2 pneumocytes is well preserved containing osmiophilic lamellar bodies. Original magnification $\times 5000$

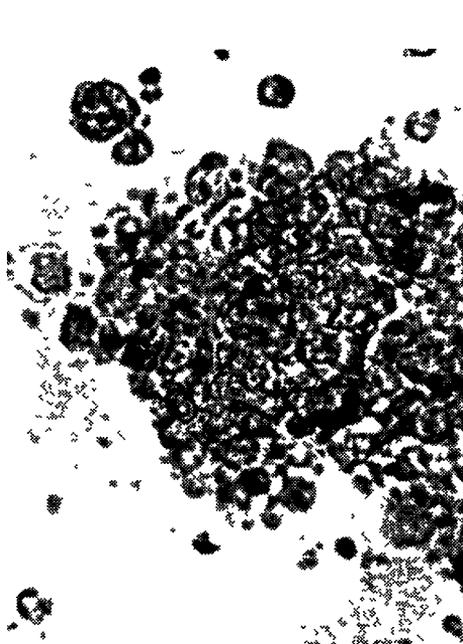


Figure 2. Type 2 cells from the rat lung after isolation, under phase contrast microscopy. Alkaline phosphatase reaction. The cells show a very intense reaction. Magn $\times 320$

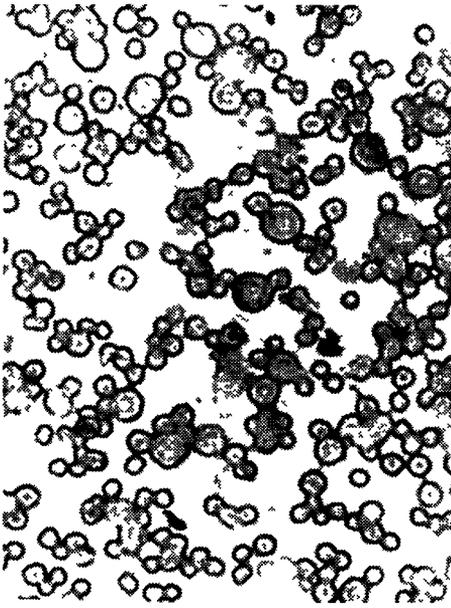


Figure 3. Type 2 cells from the rat lung after isolation. Reaction with MPA. The surface membrane of pneumocytes reacts very strongly with MPA. Magn. $\times 320$

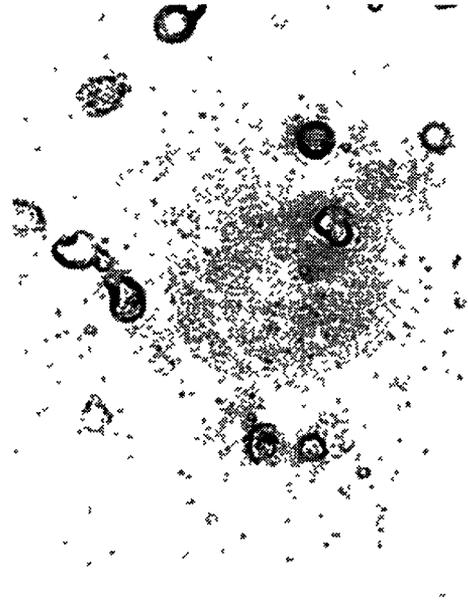


Figure 4. Type 2 pneumocytes from the rat lung after exposure to cadmium chloride, concentration $5 \times 10^{-5} \text{ mol l}^{-1}$, reaction with MPA. The reaction with MPA in cell membranes becomes irregular and disappears. Magn. $\times 320$

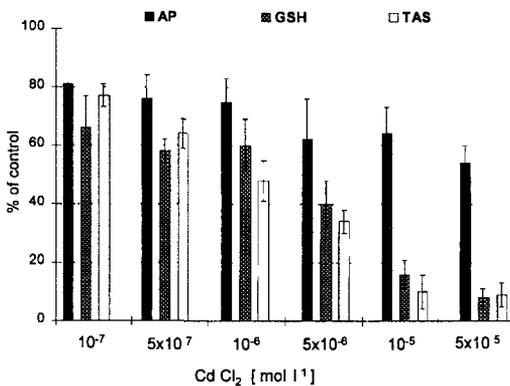


Figure 5. Cadmium induced changes in the activity of alkaline phosphatase, total glutathione and total antioxidant status expressed in percentages of the corresponding values in control cells. Data are means \pm SD ($n = 3-4$). AP - activity of alkaline phosphatase, GSH - total glutathione, TAS - total antioxidant status

Discussion

The mechanism of toxicity of cadmium has not been fully understood, but depletion of antioxidants and enhancement of reactive oxygen species have been proposed to be involved in its deleterious effect (Stoys and Bagchi 1995). As the lung is one of

the main target organ for cadmium and type 2 cells are highly sensitive to toxic agents we studied these cells after cadmium exposure in vitro. The adverse effect of cadmium was evaluated in inhalation studies, but in those cases changes of the whole cell population were estimated (Grose et al. 1987), but no particular information about type 2 cells was reported. Morphological examinations showed that cadmium induced cytotoxicity, which was evidenced by decrease of alkaline phosphatase activity and membrane damage, the staining of membranes with MPA became uneven and irregular. Biochemical estimation of alkaline phosphatase activity seemed to be more sensitive, the gradual decrease of activity showed the toxic effect of cadmium, but still at $5 \times 10^{-5} \text{ mol.l}^{-1}$ 54 % of the control cells activity was detected. The results clearly indicate that cadmium was able to cause oxidative cellular damage in type 2 cells manifested by reducing the amount of glutathione and the total antioxidant status. A close correlation was observed between the concentration of cadmium and the two above mentioned alternations. Our results showed a good agreement with the results of Yang et al. (1997) who studied cadmium toxicity on human fetal lung fibroblasts in vitro.

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