In vitro assessment of respiratory toxicology: Selection of a suitable cell line

With the human lung having more than 60 different cell types and a variety of different cell functions, the in vitro assessment of respiratory toxicity is a challenging endeavor. Each cell type and the function it performs represents a potential target for a toxic mechanism of action. The first question when selecting a suitable cell line for in vitro studies of respiratory toxicity is therefore which factors are responsible for the susceptibility of the lung and which of these can be adequately reproduced in vitro by selecting an appropriate cell line. A further point to consider when making the selection is the endpoint of interest and whether this can be assessed with the cell line under selected culture conditions. When investigating airborne particles, the expected deposition region in the lung can also play a role in the selection of the cell line.

According to Cohen et al., the chemically induced, organ-specific toxicity of a substance can be based on the following, not clearly separable factors (Cohen, 1986): the specific distribution of a substance in the tissue, the local tissue-specific metabolism of a substance and/or the specific biochemistry or physiology of an organ. For example, the lung-specific toxicity of the herbicide paraquat is due to selective accumulation in Clara cells and type I (ATI) and type II alveolar epithelial cells (ATII). It is actively transported into the cells due to structural similarity to endogenous polyamines (Brooke-Taylor et al., 1983; Smith et al., 1978) and the polyamine transport system is considered to be important for its lung-specific toxicity (Smith et al., 1990). The tissue-specific metabolization of a substance is primarily due to the organ's characteristic expression pattern of xenobiotic-metabolizing enzymes. Different cell types in the lung express such enzymes, which results in the potential of the lung to metabolize numerous xenobiotics. Xenobiotic metabolism is of particular importance for the toxicity of chemical compounds, as the toxicity of many substances is terminated by metabolic inactivation. On the other hand, the metabolic modification of a substance can also lead to a more reactive or toxic product. A process known as bioactivation. The investigation of potential lung toxicity of inhalable substances is still largely based on animal experiments. However, the known species differences in xenobiotic-metabolizing enzymes, especially in the lungs, cast doubt on the predictive capacity of animal test data for lung toxicity in humans. The development of alternative in vitro methods using continuous human cell lines of pulmonary origin is therefore of great importance, and the suitability of a particular cell line depends, among other things, on how representative its expression pattern of xenobiotic-metabolizing enzymes is for the human lung.

Due to the physiological function of gas exchange, the lung is also the primary target organ for the toxicity of gaseous substances and aerosols. In vivo, lung epithelial cells are only separated from the inhaled test atmosphere by a thin viscous liquid layer. In order to capture these conditions in vitro and to enable an appropriate investigation of inhalable test atmospheres, an appropriate cell culture technique and the selection of a suitable cell line are necessary. A limited number of continuous human lung epithelial cell lines are available. These include the bronchial epithelial cell lines 16HBE14o-, Calu-3 and BEAS-2B and the alveolar epithelial cell line A549 (Forbes, 2000). From these cell lines A549 and 16HBE14o- are widely used cell lines of particular relevance. These are described in more detail in the following sections.

Humane Alveolarepithelzelllinie A549

A549 cells are a continuous human alveolar epithelial cell line isolated from an adenocarcinoma of the lung (Lieber et al., 1976). They exhibit numerous properties characteristic of type II alveolar epithelial cells (ATII). They possess the typical lamellar bodies, show a phosopholipid synthesis pattern expected for cells secreting pulmonary surfactant, and display the same phospholipid composition as ATII primary cells (Lieber et al., 1976; Nardone & Andrews, 1979; Shapiro et al., 1978). In addition, they exhibit an expression pattern for the cytochrome P450 isoenzymes essential for xenobiotic metabolism that qualitatively matches in vivo values and show transport properties comparable to type II alveolar epithelial cells (Foster et al., 1998). In culture, they have extensive cytoplasmic extensions and exhibit a pronounced polarity (Stearns et al., 2001).

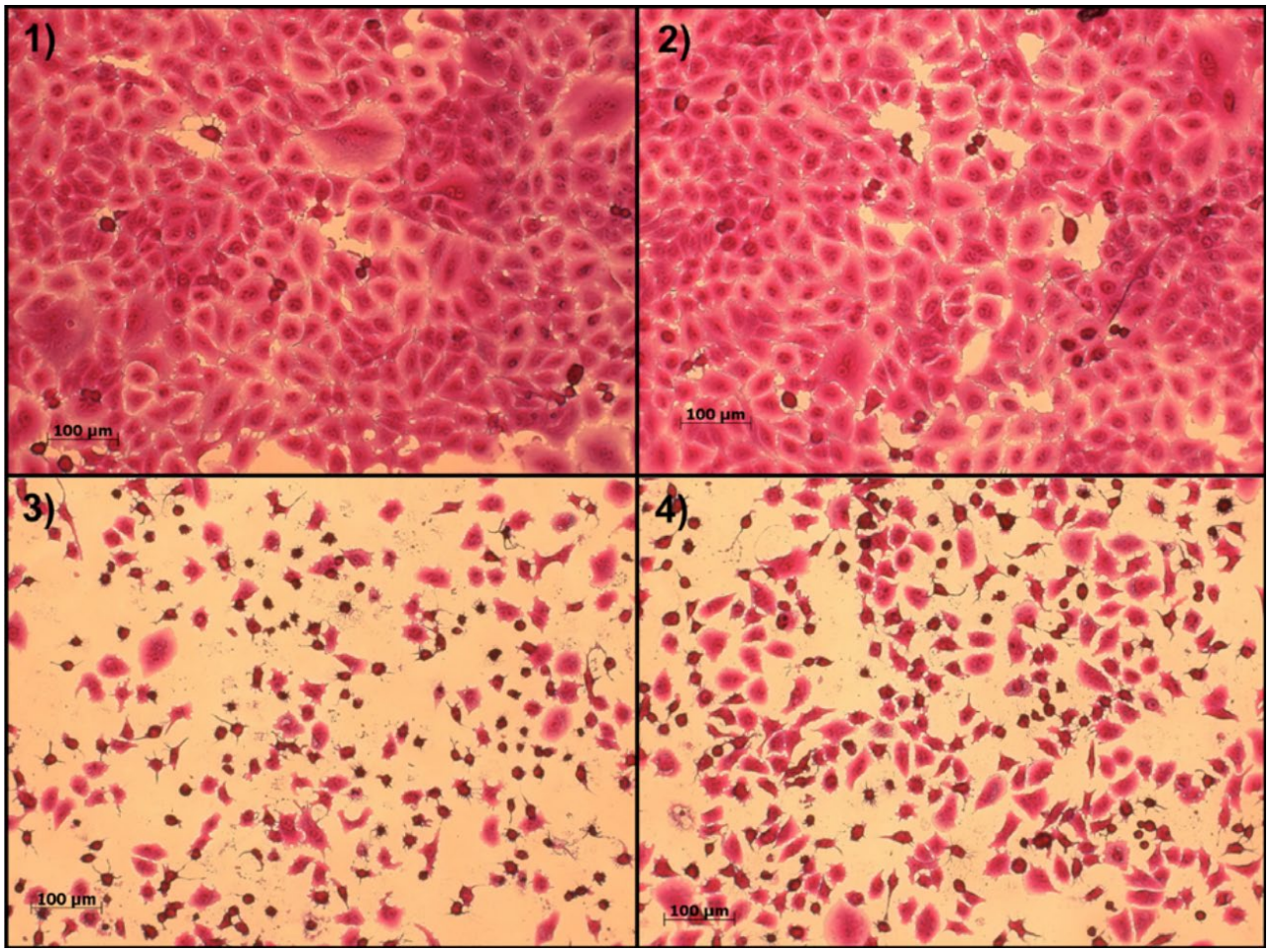

Figure 1: Fixed A549 cells after a 24h substance-exposure period and SRB-staining. (1) control; (2) 100 µM terbutaline; (3) 100 µM BIIF 1149 (NK1 receptor antagonist); (4) 100 µM BIIM 1310 (NK1 receptor antagonist)[Source: (Matt, 2008)*]*

The expression and typical localization of E-cadherin, a protein that is expressed at cell-cell contacts and is important for the formation of a polarized epithelium, confirms the polar character of A549 monolayers (Rothen-Rutishauser et al., 2005). Various research groups have also shown that the cells express the tight junction proteins occludin, claudin-2 and the intracellular tight junction protein zonula occludens 3 (ZO-3) (Blank et al., 2006; Rothen-Rutishauser et al., 2005; Stearns et al., 2001). Despite various attempts (Elbert et al., 1999; Rothen-Rutishauser et al., 2005), the detection of ZO-1, another intracellular protein of the tight

junction complex, has so far only been achieved by Winton et al. However, only sporadic and incoherent expression at the cell-cell junctions was found (Winton et al., 1998).

Despite their strong differentiation and the numerous functional markers detected, A549 cells are not fully suitable for studying lung toxicity in vitro. The cells do not capture all cytotoxicity mechanisms that can occur in vivo. The differentiation between paraquat and diquat, a herbicide from the same structural class as paraquat but which does not show lung-specific toxicity, was not successful in in vitro toxicity studies with A549 cells (Brooke-Taylor et al., 1983; O'Brien et al., 1987). As already mentioned, the organ-specific toxicity of paraquat is due to selective accumulation via an active polyamine transporter in different lung-specific cell types such as type I and type II alveolar epithelial cells. Lack of expression of a functional polyamine transporter in A549 cells may be one of the reasons for why the differentiation between paraquat and diquat toxicity failed.

Further limitations concern the metabolism of foreign substances. Although the activity profile of the A549 cells for the most important CYP isozymes qualitatively largely corresponds to in vivo values, the activity of the enzymes is quantitatively significantly below the activities achieved in the human lung. In addition, the various CYP isozymes react to different inducers of xenobiotic metabolism, but not to the same extent as the in vivo system. In contrast to the activity levels of phase I enzymes, the activity levels of phase II enzymes in A549 cells are in a range comparable to lung tissue (Castell et al., 2005; Hukkanen et al., 2000). According to Castell et al., A549 cells are the most suitable cell line for the investigation of chemically induced lung-specific toxicity, despite the limitations with regard to xenobiotic metabolism. However, for substances whose toxicity requires prior bioactivation, the predictive capacity is limited (Castell et al., 2005).

Humane Bronchialepithelzelllinie 16HBE14o-

The human bronchial epithelial cell line, 16HBE14o- (16HBE), is widely used as a model for respiratory epithelial diseases and barrier function (Callaghan et al., 2020). It was established by transforming primary bronchial epithelial cells from a one-year-old heart-lung patient with the large SV40 T-antigen. The cell line was originally developed to investigate the chloride channel activity of the ABC transporter CFTR, which when lacking or impaired is the cause of cystic fibrosis (Cozens et al., 1994). The transformed cells have retained numerous characteristics of differentiated bronchial epithelial cells and exhibit typical epithelial barrier function properties when cultured appropriately (Callaghan et al., 2020). The cells form a polarized cell layer with cilia and microvilli and have retained the stimulation of active chloride ion transport by β-adrenergic stimuli, bradykinin and calcium ionophores. Submerged cultured monolayers of cells form functional tight junctions and other cell-cell junctions and associated measurable transepithelial resistance (Ehrhardt et al., 2002). The measurement of the transepithelial resistance (TEER), which increases with the "tightness" of the tight junctions, thus enables the investigation of paracellular integrity and the impact of compounds on paracellular integrity.

Contact

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