

The rat cell line R3/1 displays several phenotypical features of alveolar epithelial type I cells. In order to characterise the cell line as an *in vitro* model for drug disposition studies, R3/1 cells were cultured on Transwell filters and the transepithelial electrical resistance (TEER) was measured as a parameter for the integrity of cell layers. Presence of cell junctional proteins (i.e., E-cadherin, occludin, ZO-1 and ZO-2) was studied on mRNA (by reverse transcriptase-polymerase chain reaction, RT-PCR) and antigen level (by immunofluorescence microscopy, IFM). Moreover, the expression pattern of catabolic peptidases (carboxypeptidase M, aminopeptidases A, B, N and P, γ -glutamyltransferase, dipeptidylpeptidase IV, angiotensin-converting enzyme, and endopeptidases 24.11 and 24.15) was analysed in R3/1 cells and compared to rat alveolar epithelial I-like cells in primary culture.

TEER values were peaking at $\sim 99 \pm 17 \text{ } \Omega \cdot \text{cm}^2$ after 5 days in culture. Addition of 0.1 μM dexamethasone together with foetal bovine serum at 20% increased TEER by 65%. However, none of culture conditions used in our study yielded monolayers with TEER values comparable to those of primary cultures of rat pneumocytes. RT-PCR revealed the absence of transcripts encoding for E-cadherin and occludin. However, ZO-1 and -2 mRNA transcripts were found. IFM using a monoclonal antibody against occludin confirmed the absence of the antigen in R3/1 cells. From the range of investigated proteolytic enzymes, mRNA transcripts encoding aminopeptidases A and B as well as endopeptidases 24.11 and 24.15 were detected; a pattern similar to rat alveolar epithelial I-like cells in primary culture.

Although R3/1 cells express certain markers typical for type I pneumocytes (e.g., T1 α , ICAM-1, connexin-43, caveolins-1 and -2), their inability to form electrically tight monolayers prevents them from being used as an *in vitro* model for alveolar absorption. The R3/1 cell line might be useful for stability assays of inhaled proteins.