Use of Immortalized Proximal Tubular Epithelial Cells (ciPTEC) in Nephrotoxicity Testing

For the efficient development of new drugs, it is important to identify safety risks at the earliest stage. Due to the lack of adequate in vitro models, the organ specific toxic effect to kidney cells (nephrotoxicity) is very often detected too late. To overcome this limitation, scientists from Radboud University immortalized primary proximal tubular epithelial cells isolated from human urine. In the kidney, the proximal tubular epithelium is responsible for the reabsorption of filtered solutes and the excretion of waste products, which is facilitated by multiple organic ion transporters. These transporters mediate the specific uptake of substances from the blood, which thereby can exhibit a kidney cell-specific toxicity that would not be prominent in other cell types.

Introduction

In the kidney, the proximal tubular epithelium is responsible for reabsorption of filtered solutes and excretion of waste products. The excretion of metabolic waste products and drugs is facilitated by multiple organic ion transporters. These transporters mediate the uptake from the blood and the efflux to the urine. The conditionally immortalized ciPTEC cells proliferate at 33°C in a humidified atmosphere and 5% CO2, where T-antigen is active. After cultivation at 37°C for seven days, cells stop proliferating and differentiate into cells with properties of proximal tubular epithelial cells. The OAT1 and OAT3 expression is recovered in the cells by recombinant expression.

Both the slow growth of ciPTEC at the low permissive temperature and the time-consuming differentiation at the non-permissive temperature can be an obstacle to a routine use of the cell in early, high-throughout nephrotoxicity testing. The preparation of assay ready cell banks from differentiated cells could overcome this bottleneck of cell cultivation. In addition, when an optimized cryopreservation process is applied, the full physiological function of the cells can be maintained during the cryo-passage. These assay ready cells can be used instantly after thawing without prior cultivation or passaging. ciPTEC-OAT1, ciPTEC -OAT3, and parental ciPTEC were expanded to 1 billion cells each and differentiated at the non-permissive temperature. The differentiated cells were detached gently and cryopreserved at specifically optimized conditions. After thawing, assay ready ciPTEC cells recovered at a high viability of about 90%, re-adhered quickly within 24 hours, and displayed the typical morphology of differentiated ciPTEC cells (Fig 1).

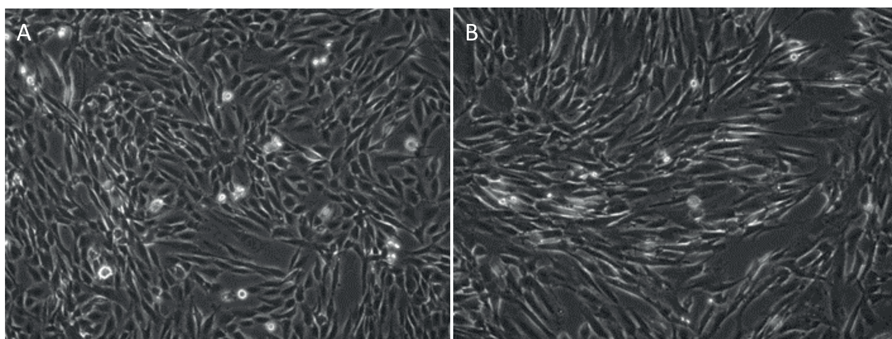

Fig. 1: Morphology of undifferentiated (A) and differentiated (B) ciPTEC-OAT-1 Cells

thawing of assay ready cells

One vial of assay ready ciPTEC cells was thawed for 2 minutes in a water bath at 37°C. The content was transferred into 8ml prewarmed assay medium and centrifuged at 100xg for 3 minutes. The cells were resuspended in 10ml of assay medium. An aliquot of the suspension was used to determine cell count and viability parameters in a CASY TT. Instantly after thawing, viability was at 98,4%, amount of debris was below 20% and aggregation as low as during normal cell cultivation.

characterization of ciPTEC

To further characterize the assay ready ciPTEC, the cells were plated instantly after thawing and fixed with 4% paraformaldehyde after 24 hours of cultivation. To access intracellular proteins the cells were permeabilized with HBBS-Triton X (0.1%). Transmembrane aminopeptidase N and intracellular Cystatin C, two proteins specifically expressed in proximal tubular epithelial cells, were detected with primary mouse monoclonal antibodies against CD13 (Santa Cruz Biotechnology) and Cystatin C (Santa Cruz Biotechnology), followed by an incubation with PE-labelled antibody against mouse IgG (Santa Cruz Biotechnology). The expression of the marker proteins was analyzed by fluorescence microscopy (Fig 2). The assay ready

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ciPTEC cells showed a strong staining for aminopeptidase N (Fig 2A) located in the cell membrane and expression Cystatin C (Fig 2 B) accumulated predominantly around the nucleus.

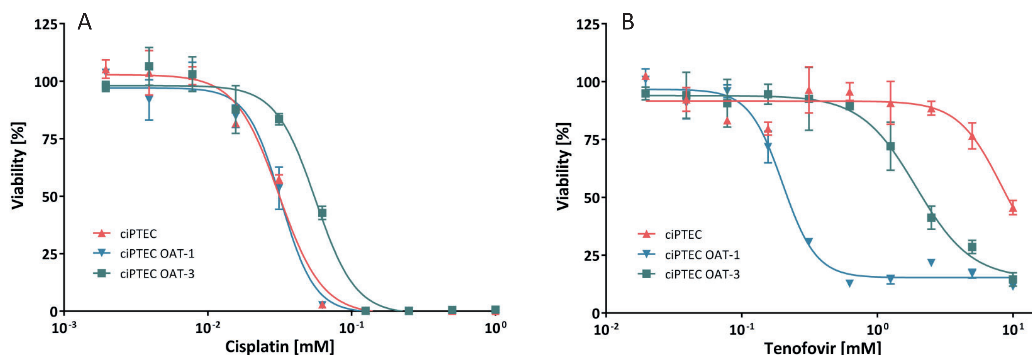

Fig. 3: Nephrotoxicity of Cisplatin (A) and Tenofovir

nephrotoxicity assay

Assay ready cells from parental ciPTEC, ciPTEC-OAT1 and ciPTEC-OAT3 were thawed and directly seeded into a 96-well plate at 20.000 cells/well. The cells were incubated at 37°C for 24h to attach before cisplatin and tenofovir were added in serial dilution. Cisplatin is a cytostatic drug which is know to exhibit nephrotoxic site effect mediated by organic cation transporter 2 (OCT2), which is expressed in ciPTEC cells. Tenofovir is an acyclic nucleotide analogue reverse-transcriptase inhibitor structurally similar to the nephrotoxic drugs adefovir and cidofovir, and is known to be a substrate for OAT1 and OAT3. The cells were incubated with the dilutions for another 48h at 37°C, before 50µl of XTT/PMS staining solution was added to each well. After 4h, the absorption of the metabolized formazan was quantified at 480nm against 690nm for reference in a Safire2 plate reader.

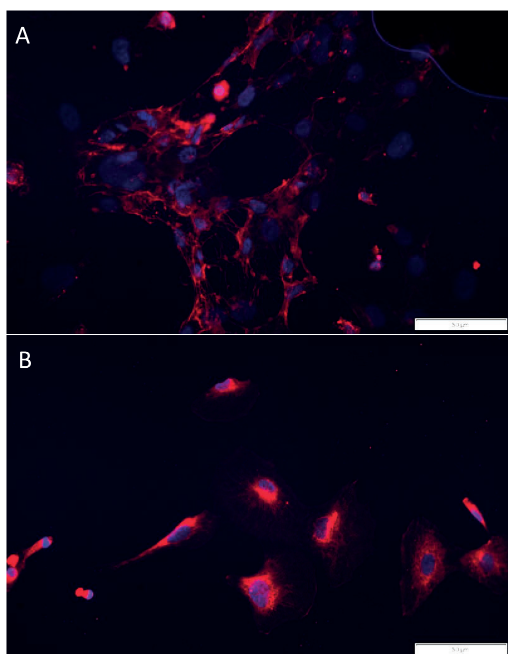

Fig 2: Immunofluorescence of CD13 (A) and Cystatin C (B)

Cisplatin had a toxic effect on all three cell lines, but slightly reduced in ciPTEC-OAT3 cells (IC50 of 56µM compared to 32µM In ciPTEC). Tenofovir is highly toxic to ciPTEC cells, which expressed OAT1 (IC50 of 0,2mM) and to a lesser extant to ciPTEC-OAT3 (IC50 of 6,1mM). Parental ciPTEC, which do not express OATs, exhibited a reduced viability only at high concentration of tenofovir (> 2 mM).

discussion

The function and regulation of the renal transport system requires a suitable in vitro cell model for physiological and pharmaceutical research. Currently available proximal epithelial cells express only a few transporters, whereas primary PTEC can only yield a limited amount of material, as proliferation stops after a few passages. ciPTEC, ciPTEC-OAT1 and ciPTEC-OAT3, are promising models for in vitro nephrotoxicity. Here, we demonstrated that ciPTEC cell lines can be cryopreserved after differentiation while maintaining their functionality. These assay ready frozen cells can be used instantly in nephrotoxicity testing without prior cultivation or further differentiation. Furthermore, we showed that assay ready ciPTEC and ciPTEC-OAT1 cells can discriminate between chemicals of general toxicity and those of particular nephrotoxicity. Assay Ready ciPTEC cells are a valuable tool for nephrotoxicity testing that upon systematic validation could improve translation of in vitro findings to clinical research. By using assay ready cells, which have been prepared in large homogenous batches, the cell-dependent variability in nephrotoxicity testing can be reduced while increasing the reliability of the assay. Since assay ready cells are instantly available from a frozen stock, screenings can be performed at a higher flexibility without significant lead time for the pre-cultivation and differentiation.

related products

Assay Ready ciPTEC
Proximal Tubular Epithelial Cells

RE421	ciPTEC 14.4 (parental)
RE422	ciPTEC-OAT1
RE423	ciPTEC-OAT1