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Modeling of tacrolimus nephrotoxicity using kidney organoids derived from human iPS cells

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Objectives: Tacrolimus, a calcineurin inhibitor, was clinically used as an immunosuppressive agent in organ transplantation or glomerulonephritis. Despite the therapeutic benefits, tacrolimus's use is limited due to its nephrotoxicity. To reduce or avoid nephrotoxicity, the effective experimental models to recapitulate nephrotoxicity are essential. Animal models or in vitro study using cell lines are limited by species difference from human, inter-donor variability or loss of their function during passaging. Recently, we and others have established protocols for the generation of kidney organoids from human pluripotent stem (hPS) cells, containing nephron-like structures with podocytes, proximal tubules, and distal tubules. Here, we recapitulated tacrolimus nephrotoxicity using kidney organoids derived from human iPS cells.

Methods: Kidney organoid differentiated from the CMC11 iPSC cell line (male donor). Kidney organoids were re-seeded in 96-well plates and tacrolimus was treated at doses of 0 μ M, 30 μ M, 60 μ M, or 120 μ M for 24 h and compare to biopsy samples of kidney transplant recipients with tacrolimus-induced acute kidney injury.

Results: Cell viability assessed by CCK-8 assay and live/dead cell staining decreased at dose-dependent manner. Proximal tubular cells (LTL- positive area) as well as distal tubular cells (E-cadherin-positive area) were decreased according to the concentration of tacrolimus. Ultrastructural analyses showed the vacuoles throughout the cytoplasm of tubule-like structures. Podocyte loss and injured podocytes with unipolarized and diffuse pattern of ZO-1 tracks were observed after treatment of tacrolimus. These findings were similar to the pathologic findings of human tacrolimus nephrotoxicity. Tacrolimus treatment in kidney organoids resulted in accumulation of damaged mitochondria and the increased immunoreactivity of 8-OHdG, a marker of oxidative DNA damage. Persistent autophagy activation induced by tacrolimus enhanced the cellular apoptosis of human iPSC-derived kidney organoids.

Conclusions: Our data suggest that iPSC-derived kidney organoids can recapitulate human tacrolimus nephrotoxicity and serve the effective in vitro model to investigate its pathogenic pathway.