P2-174

In vitro evaluation of drug-induced kidney injury using three-dimensional culture of human proximal tubular epithelial cells

ヒト近位尿細管上皮細胞三次元培養モデルを用いた薬物誘発性腎毒性評価

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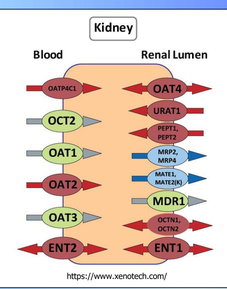
Introduction

Kidney-derived cell lines[1)] or Primary human kidney cells[2)] do not maintain the gene expression related to kidney function such as drug transporters[3)]. Conventional kidney cells have not yet been used in drug discovery. Therefore, many of the drug-induced kidney injury (DIKI) has been evaluated by animal studies. However, in vitro evaluation of DIKI using human cells is desired from the viewpoint of low predictivity to the clinical trial, species difference and animal welfare.

We investigated the usefulness of evaluating DIKI using three-dimensional cultured human proximal tubular epithelial cells (3D-RPTEC), whose expression levels of major drug transporters are comparable to those of human kidney cortex. To detect for DIKI more sensitively, we investigated the usefulness of High Content Analysis (HCA) using a confocal image cytometer.



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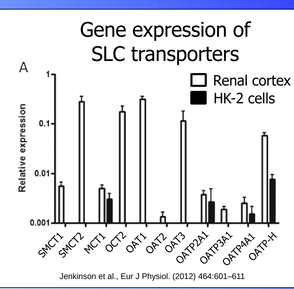
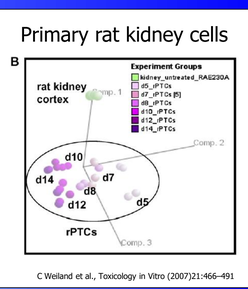


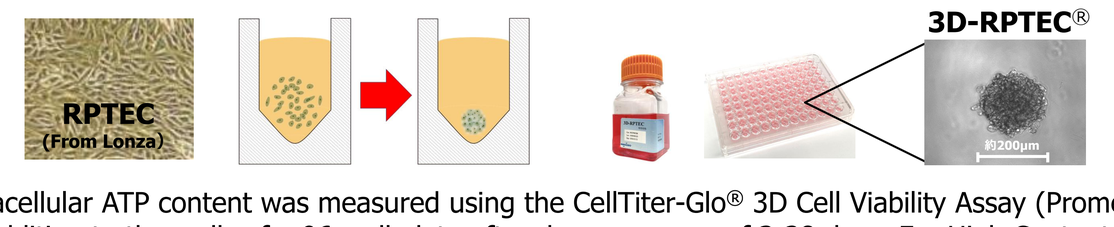
Jenkinson et al., Eur J Physiol. (2012) 464:601–611



C Weiland et al., Toxicology in Vitro (2007)21:466–491

Methods

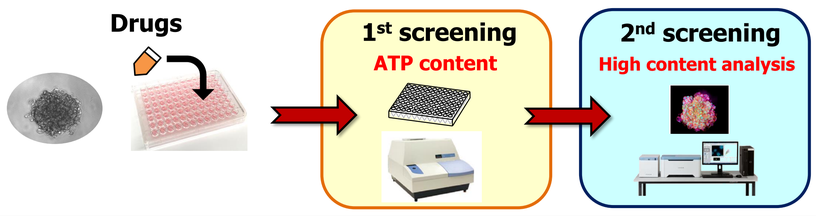
2D-culture : RPTEC (LONZA, CC-2553, Passage 3) was thawed and cultured in REGM (LONZA).
3D-culture : RPTECs were cultured as spheroids in ultra-low attachment 96-well culture plate (PrimeSurface, Sumitomo Bakelite). The medium was changed once every 2-3 days. This spheroid of RPTEC (3D-RPTEC®) will be available from Nikkiso from July 2023.



Intracellular ATP content was measured using the CellTiter-Glo® 3D Cell Viability Assay (Promega) by addition to the wells of a 96-well plate after drug exposure of 3-28 days. For High Content Analysis (HCA), the images of cells were taken using a confocal image cytometer CQ1 (Yokogawa).

Conclusion

- **新規なヒト腎細胞3D-RPTEC®を用いた*in vitro*腎毒性モデル**
  - 長期培養できる腎細胞（薬物トランスポーターやメガリンを発現）
  - トランスポーターを介した腎毒性を検出
- **ATP測定によりヒト腎毒性リスクを評価**
  - ATP測定とHCAを組み合わせて高い確度で腎毒性を検出



問い合わせ先

3D-RPTEC®について興味またはご質問のある方は以下までお問い合わせ下さい。

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https://www.nikkiso.co.jp/products/industrial/3drptec/

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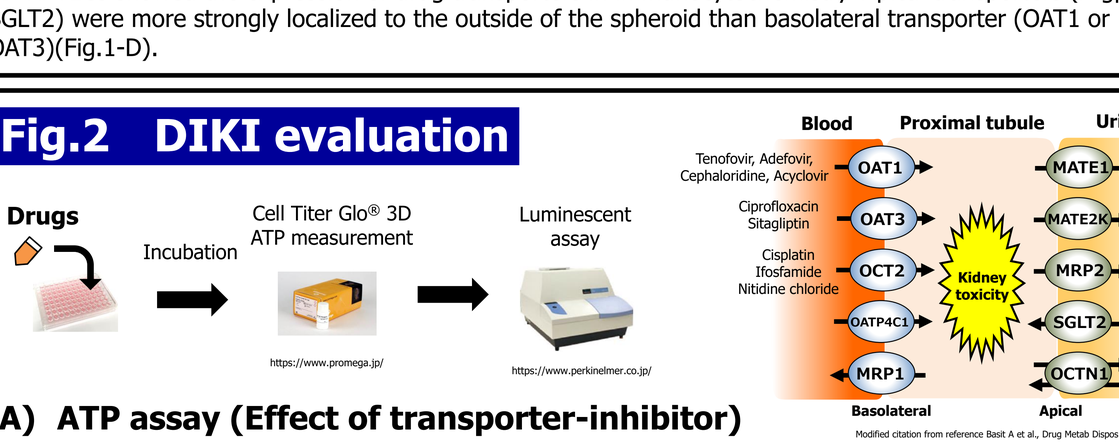
Results

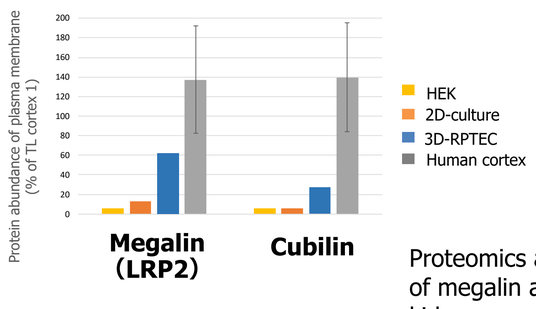
Fig.1 Features of 3D-RPTEC

(A) Drug transporter (Microarray)

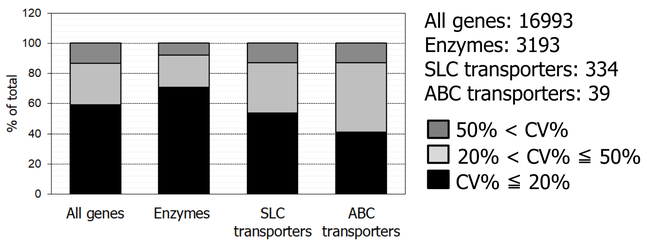


3D-RPTEC showed improved expression of drug transporters and was maintained in long-term culture.(Fig.1-A).

(B) Endocytosis (Proteomics)



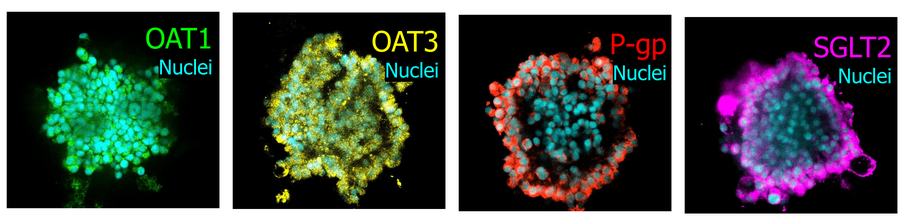
(C) Donor-to-donor variability



All genes: 16993
Enzymes: 3193
SLC transporters: 334
ABC transporters: 39

Proteomics analysis of plasma membranes showed higher expression of megalin and cubilin, which are responsible for endocytosis in kidney, compared to 2D-culture (Fig.1-B).

(D) Immunocytochemistry



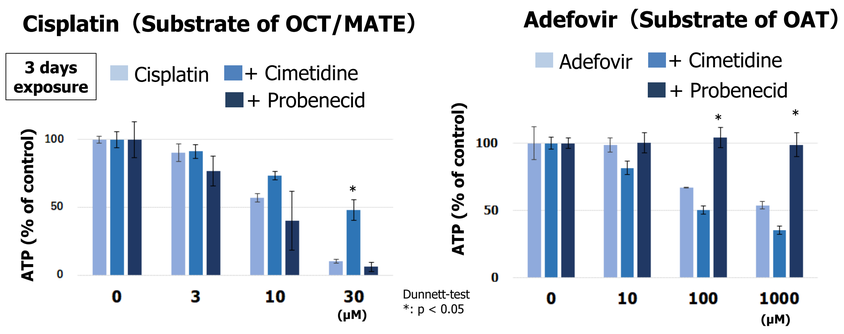
Antibody
anti-OAT1(Transgenic, KE038)
anti-OAT3(Transgenic, KE032)
anti-Pgp (abcam, ab129450)
anti-SGLT2(Proteintech, 24654-1-AP)

3D-RPTECs showed the expression of drug transporters in immunocytochemistry. Apical transporters (P-gp or SGLT2) were more strongly localized to the outside of the spheroid than basolateral transporter (OAT1 or OAT3)(Fig.1-D).

Fig.2 DIKI evaluation

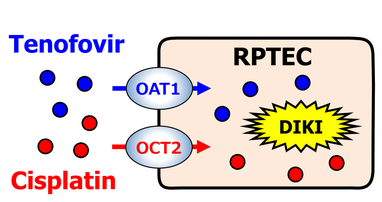
Modified citation from reference Basit A et al., Drug Metab Dispos. (2019) 8:802-808

(A) ATP assay (Effect of transporter-inhibitor)



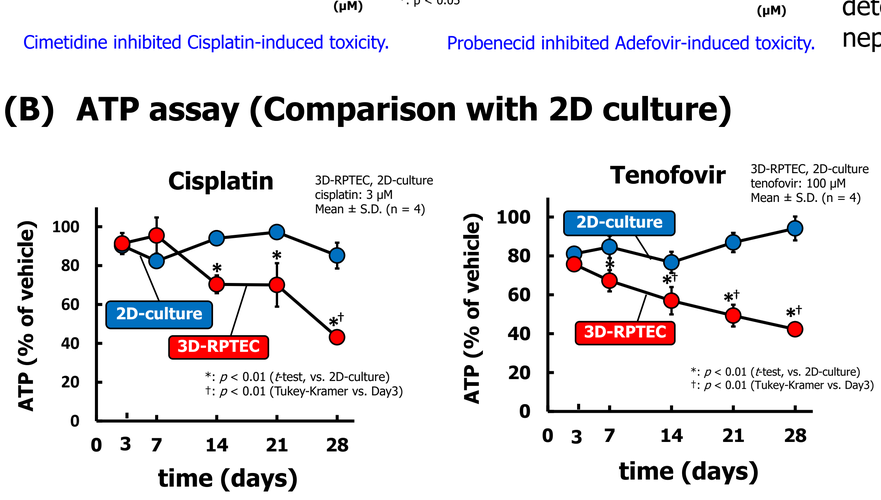
Cimetidine inhibited Cisplatin-induced toxicity.

Probenecid inhibited Adefovir-induced toxicity.



ATP assay of 3D-RPTEC was detected transporter-dependent nephrotoxicity (Fig.2-A).

(B) ATP assay (Comparison with 2D culture)

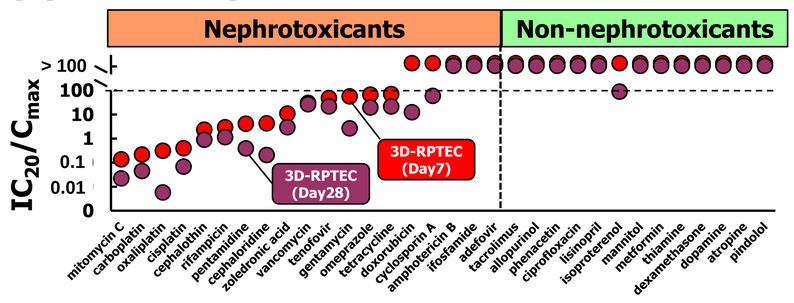


Long-term exposure of tenofovir, an OAT substrate, and cisplatin, an OCT2 substrate, significantly decreased the amount of intracellular ATP in 3D-RPTEC compared to 2D culture (Fig.2-B).

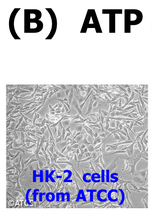
DIKI by long term exposure of drugs could be evaluated in 3D-RPTEC.

Fig.3 Safety margin for DIKI

(A) ATP assay in 3D-RPTEC



(B) ATP assay in HK-2



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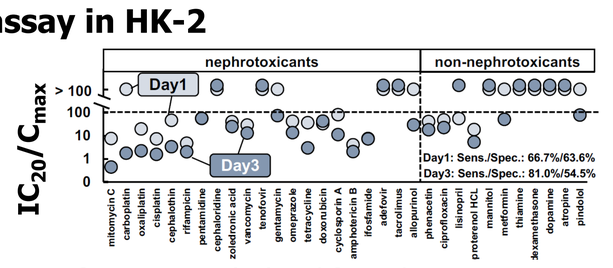


The safety margin of DIKI was calculated from the concentration that inhibits ATP by 20% (IC_{20} value) and the effective blood concentration (C_{max}).

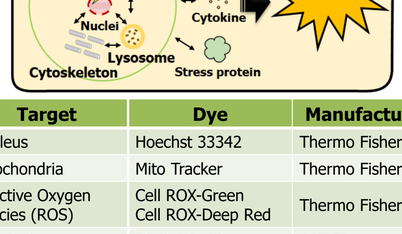
Sensitivity: 66.7% (day7), 76.2% (day28) Specificity: 100% (day7), 100% (day28)

Compound	C_{max} total* (μM)	7 days exposure	
		IC_{20} (μM)	IC_{20} / C_{max}
Mitomycin C	1.77	0.24	0.14
Carboplatin	67.3	14.5	0.22
Oxaliplatin	4.37	1.35	0.31
Cisplatin	7.47	2.93	0.39
Cephalothin	54.7	127	2.32
Rifampicin	12.2	35.4	2.91
Pentamidine	0.9	3.7	4.11
Cephaloridine	192	812	4.22
Zoledronic acid	0.97	10.5	10.8
Vancomycin	38	1140	30
Tenofovir	0.93	44.2	47.68
Gentamycin	15.7	878	55.9
Omeprazole	3.18	212	66.5
Tetracycline	3.67	256	69.9
Doxorubicin	0.04	4.8	>100
Cyclosporin A	0.21	> 100	> 100
Amphotericin B	1.52	> 100	> 100
Ifosfamide	199	> 300	> 100
Adefovir	0.07	77.6	> 100
Tacrolimus	0.03	> 10	>100
Allopurinol	73.5	> 3000	> 100
Phenacetin	38.4	> 3000	> 100
Ciprofloxacin	12.1	> 1000	> 100
Lisinopril	0.09	> 10	> 100
Isoproterenol HCL	0.95	> 100	> 100
Mannitol	43.1	> 10000	> 100
Metformin	12.4	> 1000	> 100
Thiamine	6.8	> 1000	> 100
Dexamethasone	0.68	> 100	> 100
Dopamine	0.08	> 10	> 100
Atropine	0.09	> 10	> 100
Pindolol	0.2	> 30	> 100

* : Schulz. M. et al., Crit Care.(2012)16: R136

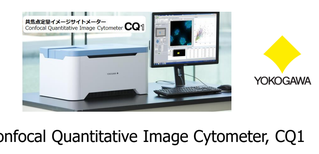
Fig.4 High Content Analysis

(A) Target molecule in DIKI

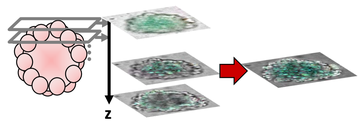


Target	Dye	Manufacturer
Nucleus	Hoechst 33342	Thermo Fisher
Mitochondria	Mito Tracker	Thermo Fisher
Reactive Oxygen Species (ROS)	Cell ROX-Green Cell ROX-Deep Red	Thermo Fisher
Cytoskeleton	Phalloidin-iFluor	AAT Bioquest
Endoplasmic Reticulum	ER Tracker	Thermo Fisher
Lysosome	Lyso Tracker	Thermo Fisher

(B) Image analysis



Confocal Quantitative Image Cytometer, CQ1



consolidates 21 sliced images into one (MaxIP)

3D-RPTEC could analyze the images inside the spheroid (Fig.4B).

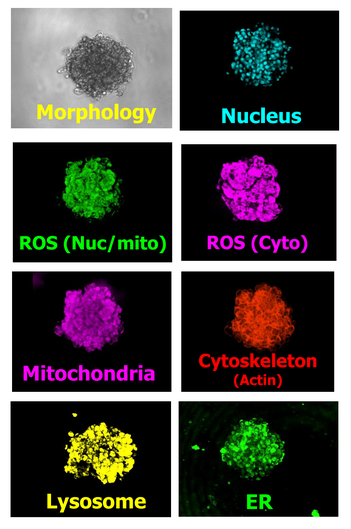
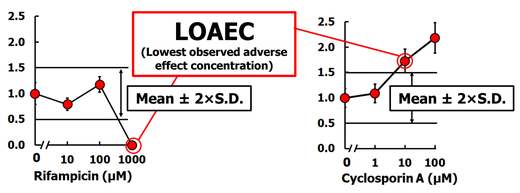


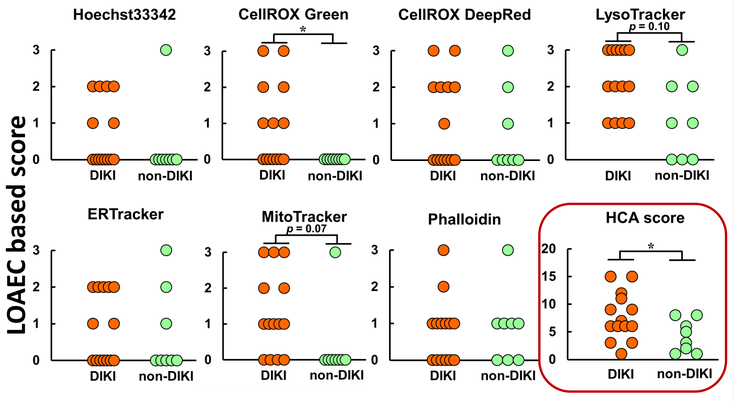
Fig.5 DIKI evaluation by combination of ATP and HCA

(A) Evaluation method in HCA



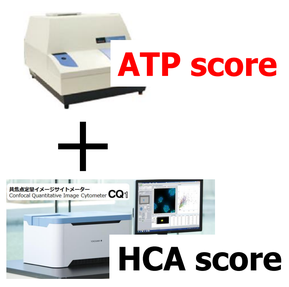
LOAEC based score	
3	within 1 × C_{max}
2	within 10 × C_{max}
1	within 100 × C_{max}

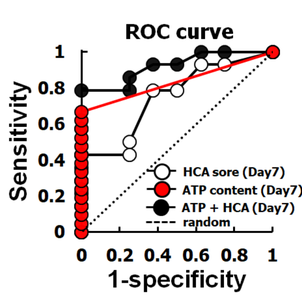
(ATP score)
LOAEC based score were calculated from IC_{20} value.



* $p < 0.05$ (Wilcoxon rank-sum test vs. non-DIKI)

(B) Combination of HCA and ATP





3D-RPTEC	ROC-AUC (95% CI)	sensitivity/ specificity
HCA score (Day7)	0.750 (0.538, 0.962)	78.6 / 62.5%
ATP content (Day7)	0.833 (0.730, 0.936)	66.7 / 100%
ATP + HCA (Day7)	0.916 (0.810, 1.03)	78.6 / 100%

* : $p < 0.05$ (chi-square test vs. ROC curve AUC=0.500)

LOAEC based score were calculated from the change of each fluorescent intensity. The HCA score by adding each score was higher in DIKI compounds than in non-DIKI compounds (Fig.5-A). ATP score from Luminescent assay (AUC = 0.833) and HCA score (AUC = 0.750) calculated from confocal image analysis in 3D-RPTEC can classify DIKI. The combination of HCA score and ATP (AUC = 0.916) classified DIKI more accurately (Fig.5-B).

COI Disclosure Information

I have no COI regarding this presentation.
Presenting author: Etsushi Takahashi
Organization: Nikkiso Co. Ltd

Acknowledgement

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References

(1) Jenkinson et al., Eur J Physiol. (2012) 464:601–611
(2) C Weiland et al., Toxicology in Vitro (2007)21:466–491