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Blood-brain barrier permeation in the autologous stem-cell derived four-organ-chip

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INTRODUCTION

Over the last years microphysiological systems have been increasingly accepted by academia and industry as a valuable tool in drug development to test substances for their safety and efficacy. Current systems still face the problems of heterogeneous tissue sources, hindering the development of patient specific chip systems. To overcome these limitations we established the four-organ-chip (Chip4) combining miniaturized autologous human intestine, liver, brain and kidney equivalents to study absorption, distribution, metabolism and excretion (ADME) derived from one single human induced pluripotent stem cell (hiPSC) line.[1] Understanding the ability to pass the blood-brain barrier (BBB) is crucial for assessing safety and efficacy in the development of neurological-active compounds. Therefore, we have enhanced our neuronal model[2] by introducing brain microvascular endothelial cell (BMEC)-like cells.[3]

Here, we present results from the adaption of the new neurovascular model to the Chip4. Furthermore, we show results of carbamazepine, propranolol and atenolol and their metabolization and permeation across the the BBB.

BASIC EXPERIMENTAL SETUP

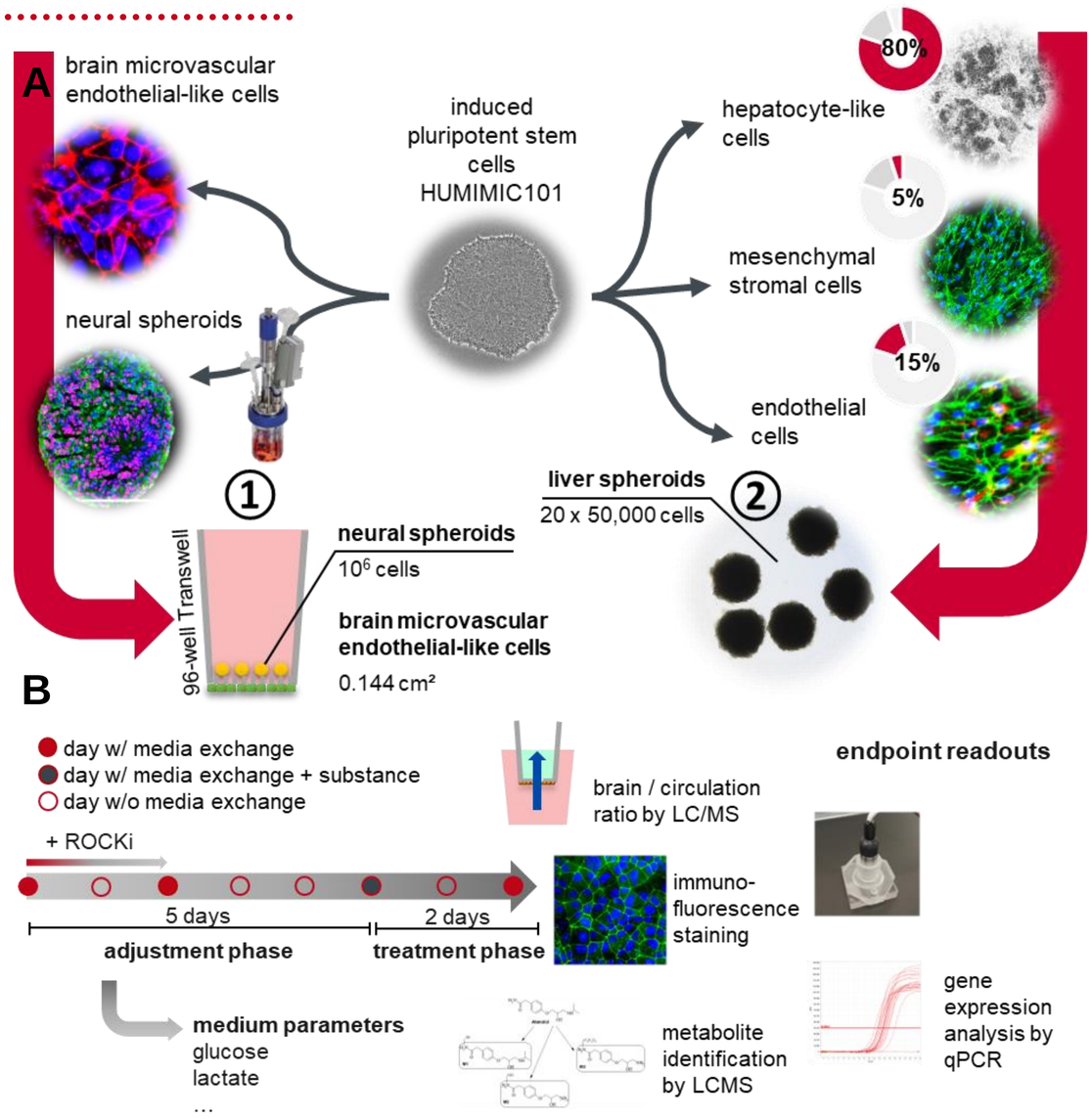


Fig. 1 A: Schematic iPSC differentiation into brain microvascular endothelial-like cells, neural spheroids, endothelial cells, mesenchymal stromal cells and hepatocyte-like cells for formation of the Transwell-based BBB/brain model and the liver spheroid model. B: Schematic of the dynamic coculture in the HUMIMIC Chip4, with in-process measurement of medium parameters and end-point analysis.

BLOOD-BRAIN-BARRIER LIKE ENDOTHELIAL CELLS

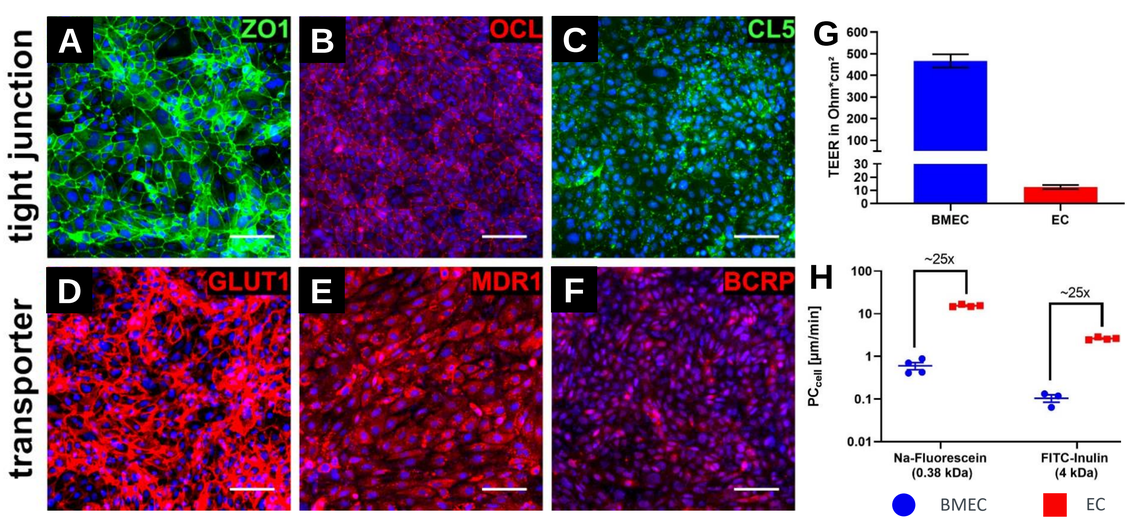


Fig. 2 Immunofluorescence staining of hiPSC-derived brain microvascular endothelial cells showed expression of the tight junction proteins ZO-1 (A), occludin (B), claudin-5 (C) and the transporters GLUT1 (D), MDR1 (E) and BCRP (F). Nuclei were counterstained with DAPI. Scale is 100 µm. Comparison of barrier tightness of BMEC-like cells and endothelial cells, G: average TEER values of hiPSC-derived BMEC-like cells and hiPSC-derived endothelial cells (EC) after two days of culture on PET 96-well Transwell membranes (n=8). H: average blank corrected sodium fluorescein and 4kD FITC-Inulin permeation coefficients of BMEC-like cells and ECs (n=4).

BBB/BRAIN - LIVER COCULTURE

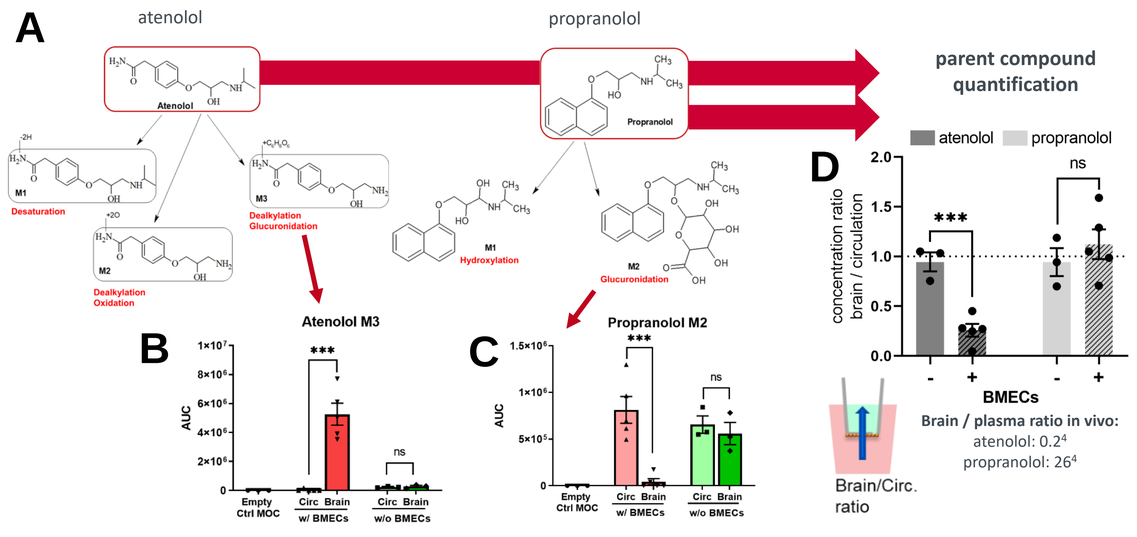
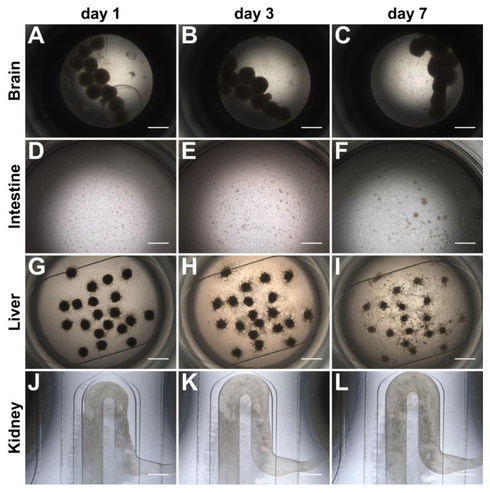


Fig. 3 A: Proposed metabolic pathway for propranolol and atenolol in the Chip4. An identical instrument response is assumed for parent and metabolites. Identification of atenolol metabolites is based on accurate mass only. B/C: Distribution of glucuronidated metabolites of propranolol and atenolol between medium circulation (Circ) and the brain compartment. D: Measured concentration ratio between the brain compartment and the medium circulation of propranolol and atenolol without BMEC-like cells and with BMECs from two experiments (N=2, n=3-5). Differences between circulation and brain compartment were compared by an unpaired t-test, (*** p<0.001).

BBB/BRAIN - LIVER – KIDNEY – INTESTINE COCULTURE



A	atenolol		carbamazepine		verapamil	
	mean	SD	mean	SD	mean	SD
recovery 24h [%]	70.69	*13.16*	60.52	*13.15*	6.43	*1.07*
Intestinal absorption 24h [%]	80.09	*5.20*	84.36	*7.01*	98.66	*0.30*
Apparent permeability circulation to brain (0h to 1/6h)	4.36	*3.47*	60.10	*81.03*	10.58	*8.97*
(1/6h to 24h)	6.38	*2.78*	43.62	*29.66*	18.75	*5.68*
Permeability coefficient [10⁻⁶ cm/s] (24h to 48h)	3.33	*1.52*	22.63	*4.42*	23.78	*2.92*

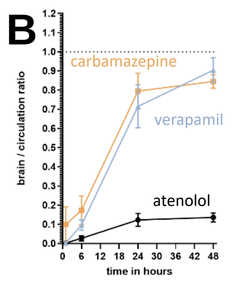


Fig. 4 Exemplary brightfield images of the brain (A-C), intestine (D-F), liver (G-I) and kidney (J-L) compartment over one week of continuous culture in one Multi-Organ-Chip. Images are taken at day 1, day 3 and day 7 of Chip4 cultivation, scale is 1000 µM.

Fig. 5: A: Mean percentage of recovered compound after application to the Chip4. Intestinal absorption is the calculated missing percentage that was not found in the intestinal compartment . Apparent permeability values for atenolol, carbamazepine and verapamil for different permeation periods. B: Mean brain / circulation ratios + s.e.m. (n=4).

SUMMARY & CONCLUSION

- hiPSC-derived liver spheroids maintained their differentiation in the Chip4
- BMEC-like cells demonstrated presence of tight junction and strong barrier formation compared to classical iPSC-derived endothelial cells
- Permeation behaviour of atenolol and propranolol and their metabolites in the Chip4 system matches their BBB permeation properties in vivo
- hiPSC-derived co-culture of autologous kidney, liver, intestine and brain models was stable for 7 days and enabled to assess distribution of atenolol, carbamazepine and verapamil across the intestinal, brain and kidney barrier

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References 1- Ramme, Anja Patricia, et al. Future science OA 5.8 (2019): FSO413. **2-** Koenig, Leopold, et al. Appl. Note 364 (2018): 1-12. **3-** Koenig, Leopold, et al. Cells 11.20 (2022): 3295. **4-**Neil-Dwyer, G. J. J. J., et al. British journal of clinical pharmacology 11.6 (1981): 549-553.

The presented work is supported by public funding from the German Ministry for Education and Research BMBF (HiPSTAR, funding code: 01EK1608) and European Union's Horizon 2020 research and innovation programme under grant agreement No 964537.