Abstract No: 648

The ADME-Chip: Studying different application routes on a PB/PK compliant preclinical tool

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ABSTRACT

Complex human in vitro absorption, distribution, metabolism and excretion (ADME) models involving co-culture of key organs to mimic certain exposure routes present a challenge to establish physiologically relevant organ models as well as physiologically based pharmacokinetic (PBPK) distribution behaviour in the culture environment. In our recent study, we developed a PBPK compliant ADME 4-Organ-Chip (Chip4) with a downscale factor of 1:100.000 of human body. The integration of an intestinal barrier model for absorption, liver micro-tissues for the main metabolism, a kidney model with proximal tubular-like cells and podocytes for excretion, and neuronal spheroids as a potential target organ were optimized in the chip and co-cultured for 14 days. We exposed the Chip4 to Haloperidol, an antipsychotic medication in butyrophenone family systemically and to Carbamazepine, a tricyclic compound with anticonvulsant properties orally with a repeated dose regime. We demonstrate uptake, distribution, metabolic capacity and toxicity assessment at an organ-specific level. We aim to develop a testing system as a potential new approach methodology for toxicological testing and to increase predictability in the preclinical stage with the multi-organ-chip platform.

CHIP4

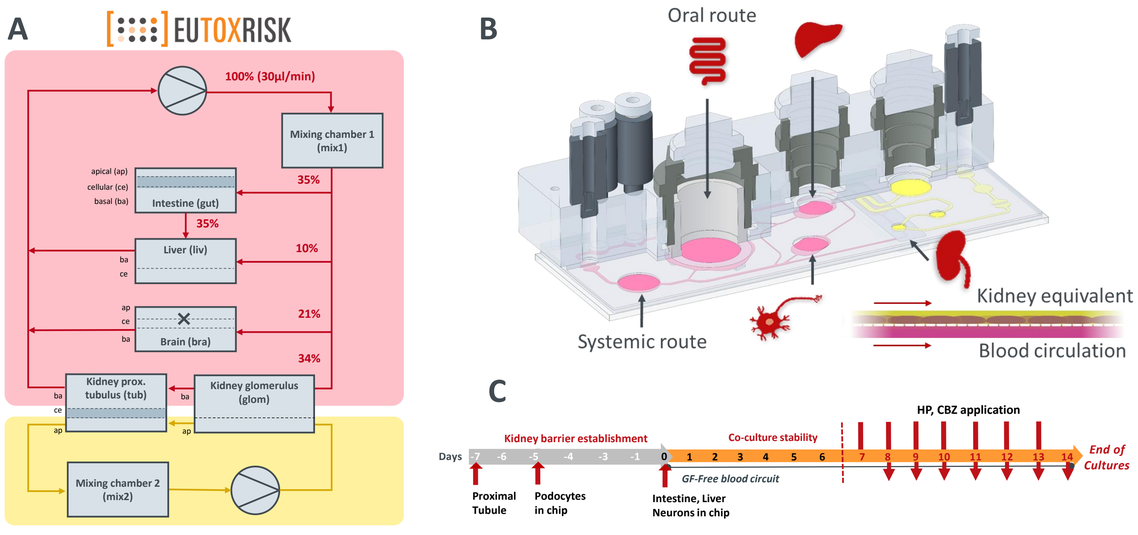


Fig. 1: Chip4 is modelled to be PBPK compliant and mimics the human ADME organ axis combining the intestine for absorption, the circulation with relevant flow rates for distribution, the liver for metabolism and the two functional kidney compartments for excretion and the neuronal model as the target organ. Simulated organ perfusion rates in Chip4 correlate to human in vivo data (marked in red). An organ downscale factor of 100,000 was applied **(A)**. Chip4 architecture and culture compartments show blood and kidney circuits with parallel perfusion at the kidney equivalent **(B)**. Experimental set-up of the ADME-N Chip4 co-culture with 7-day repeated Haloperidol (HP) and Carbamazepine (CBZ) application in 3 experiments **(C)**.

BARRIER INTEGRITY

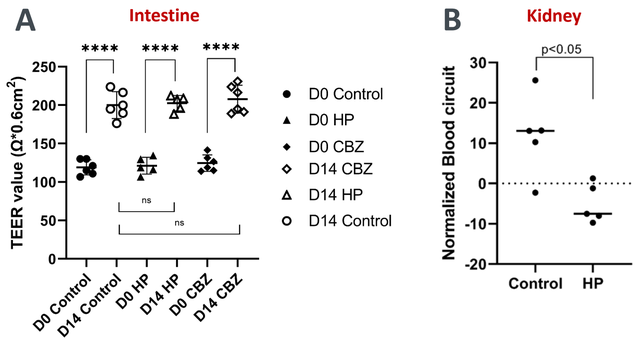
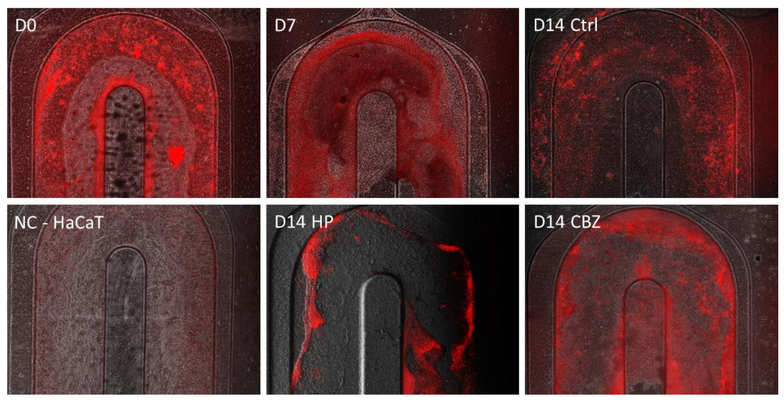


Fig. 2: TEER is measured in order to evaluate intestinal barrier integrity at D0 and D14 of the co-culture. All conditions showed elevated TEER at D14 in comparison to D0 ($p<0.0001$) **(A)**. Kidney barrier integrity is measured via FITC-Inulin application to the kidney circuit. HP exposure reduced the barrier integrity of proximal tubular cells ($p<0.05$) **(B)**.

FUNCTIONAL PROXIMAL TUBULE

Fig. 3: Proximal tubule-like (PTL) cells were challenged for Albumin-Alexa594 uptake at D0, D7 (before treatment) and at D14. Vehicle control, HP and CBZ-treated PTL cells were able to uptake Albumin. Cell type negative control was HaCaT cells in the PTL compartment which did not show any Albumin uptake.



CalceinAM / CellTox

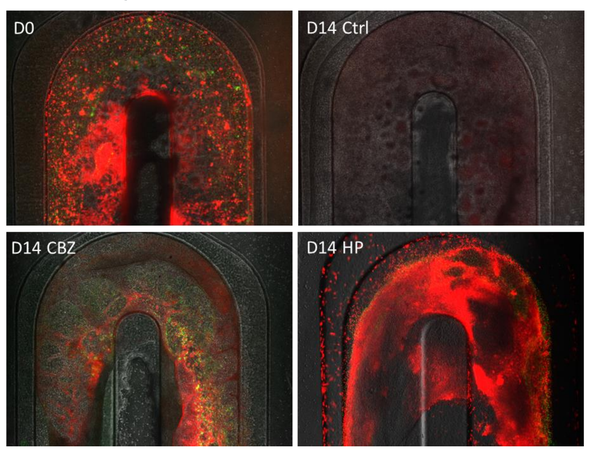


Fig. 4: CalceinAM is used for efflux testing while CellTox stained the dead cells. The D14 control group showed matured tubular efflux in comparison to the D0 control where the efflux of CalceinAM was rapid (20 min assay duration). HP is known to inhibit CalceinAM efflux protein PgP in the proximal tubule [1] where we see the retained dye in the HP-treated group while the CBZ-treated group showed a delay in maturation. The CellTox staining indicates cytotoxicity for CBZ and at a lower amount for HP-treated PTL cells.

1- Iwaki, Koichi et al. *The Journal of pharmacy and pharmacology* vol. 58,12 (2006): 1617-22.

METABOLIC COMPETENCE

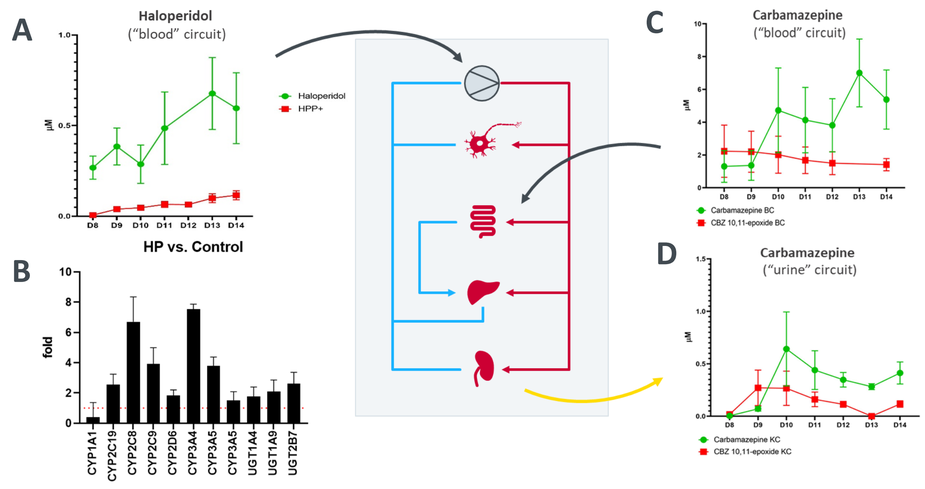


Fig. 5: Systemically applied 1,5 mM HP was found in the blood circuit with the metabolite of interest HPP+ in the blood circuit **(A)**. Differentially expressed genes related to phase I and II liver metabolism are listed in comparison to D0. Among up-regulated enzymes are CYP 2C8, 2C9, 2C19, and CYP3A4/3A5 and transporters UGT1A4, UGT1A9 and UGT2B7, all involved in either first and secondary toxic metabolite formation or in the generation of glucuronide conjugates for direct excretion **(B)**. Orally applied CBZ and its toxic 10,11-epoxide metabolite have been detected in the blood **(C)** and the kidney circuit **(D)** indicating oral uptake and excretion.

IMMUNOFLUORESCENCE

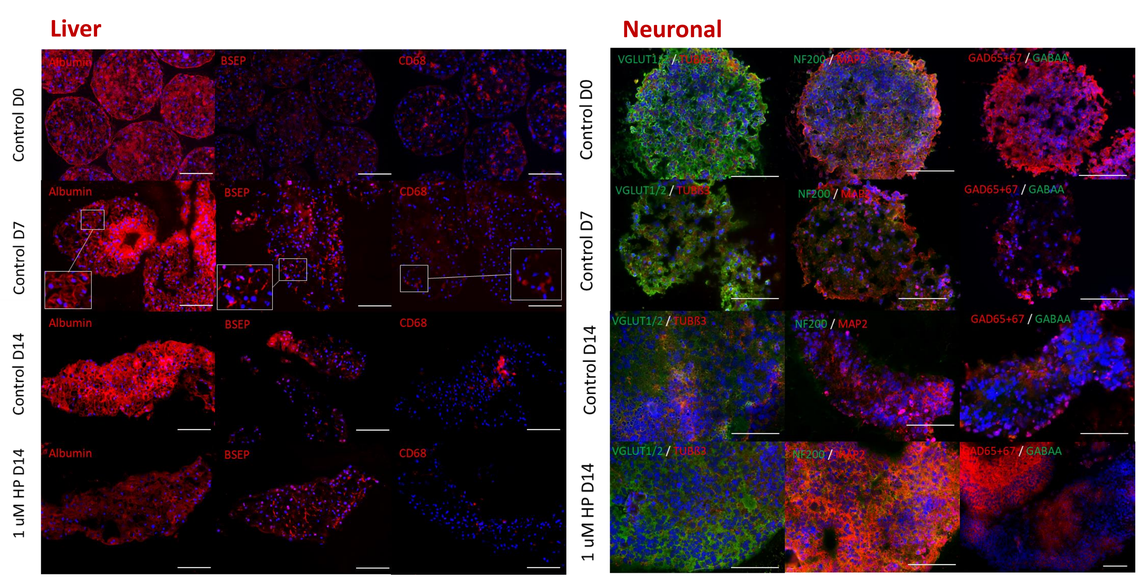


Fig. 6: Immunofluorescence marker staining of the cryosectioned liver (left) and neuronal (right) models at day 0, day 7 and day 14 control and 1uM HP treatment are shown. Intercellular Albumin, BSEP and CD68 are stained on liver sections while VGLUT1/2, TUBβ3, NF200, MAP2, GAD65 and 67, GABAA are stained on neuronal sections. Scale bar: 100um.

SUMMARY & CONCLUSION

- We designed and prototyped the ADME-N Chip4 and applied two study compounds to challenge the system orally and systemically.
- Intestinal barrier integrity increased in all the chip conditions including apically applied CBZ.
- We observed slight toxicity of HP on proximal tubule with reduced barrier integrity with CellTox positive cells.
- We were able to show ADME of CBZ on Chip4.
- The liver showed a clear metabolic adaptation on HP by transcriptional upregulation of involved phase I and II enzymes.

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