Using individual-centric model to detect immune-mediated idiosyncratic drug-induced liver injury (iDILI) at preclinical stage

Sara CHERRADI[1], Salomé ROUX[1], and Hong Tuan DUONG[1]

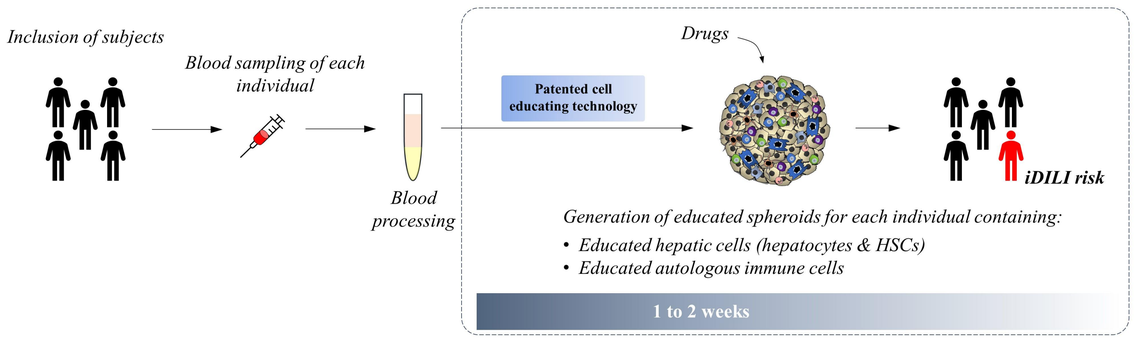
[1]PredictCan Biotechnologies, Biopôle Euromédecine, Cap Sigma, 34790 Grabels, FRANCE

Introduction

Drug-induced liver injury (DILI) is a main cause of drug development failures and market withdrawals. Despite the availability of a large panel of *in vitro* and animal models to screen for DILI risk, its detection remains problematic as it does not occur in each person. Indeed, idiosyncratic DILI (iDILI) is usually dose-independent and occurs in only a small fraction of subjects. Furthermore, there are evidence that most iDILI is immune mediated suggesting that its occurrence may also depend on the age and the sex of the subject as these non-genetic factors influence the immune cell landscape [1] [2].

We have recently developed a subject-dependent educated spheroid system that can detect DILI risk of difficult-to-detect molecules [3]. Unlike the cell reprogramming technique, our cell educating technology does not modify the cell fate but induces an individual specific behavior to the cells, thanks to the composition of the serum supplemented in the cell culture medium. In the present work, we use this technology to add educated autologous monocyte-derived macrophages and dendritic cells into our hepatic spheroids and demonstrated that our system can detect immune-mediated iDILI at therapeutic doses.

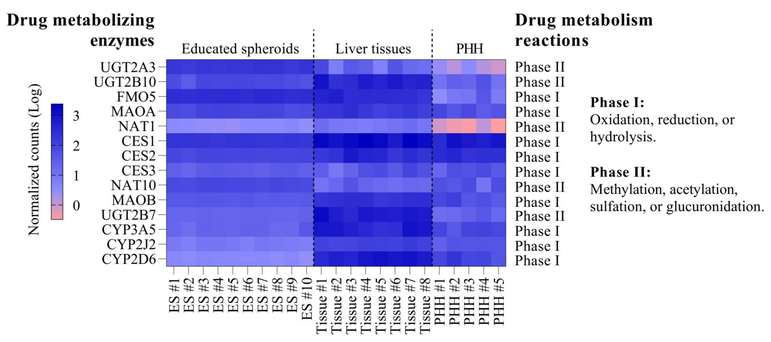
Materials & Methods



Educated spheroids were generated (PCT/EP2024/052109) from a co-culture of HepG2 and TWNT-1 in ultra-low attachment plates, using processed blood from healthy donors. The system is cultured with educated autologous macrophages and dendritic cells for 3 days prior being treated for 72h with diclofenac or with acetylsalicylic acid (ASA). Transcriptomic profiling and gene expression were performed by RNAseq and quantitative PCR, respectively. Cell viability was measured using CellTiterGlo™ (Promega). CYP3A4 and 2C9 activity were measured using P450-Glo™ CYP3A4 Assay and P450-Glo™ CYP2C9 Assay from Promega.

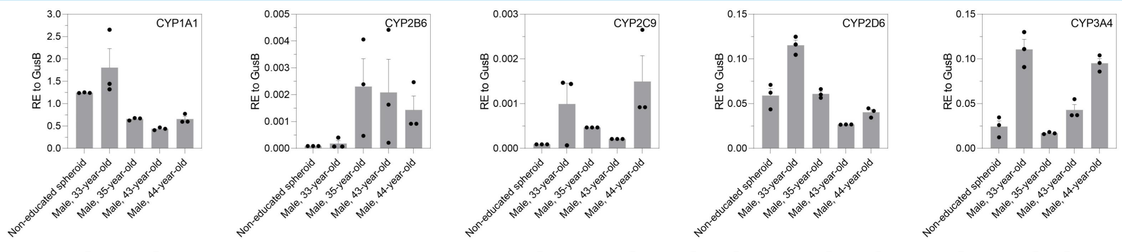
Results

The expression of phases I and II drug-metabolizing enzymes in educated spheroids (ES) is individual-depedent

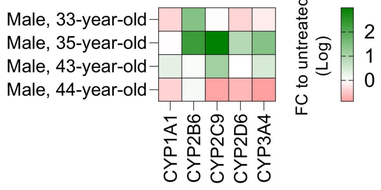


Transcriptomic profiling of educated spheroids revealed that the expression of some phases I and II drug-metabolizing enzymes is comparable to the healthy liver tissue

Basal CYP expression after the cell educating step and expression level after diclofenac treatment

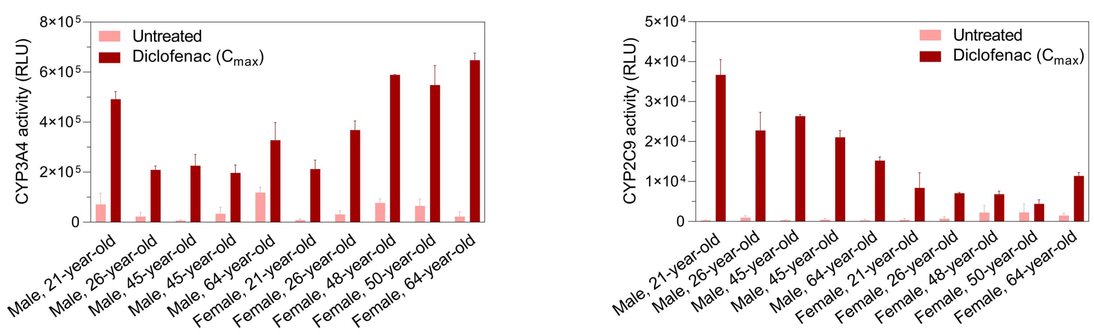


We analyzed CYP expression in non-educated and educated spheroids and showed an upregulation in an individual manner after the cell educating step.



Educated spheroids from 4 individuals were treated with diclofenac at C_{max} and the expression of CYP was quantified by qPCR. We observed an individual dependent alteration of CYP expression upon diclofenac treatment.

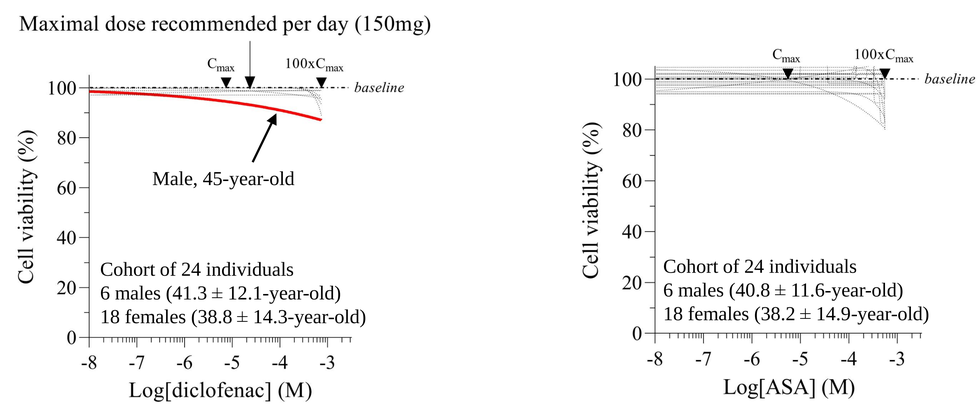
Increased CYP3A4 and CYP2C9 activity upon diclofenac treatment at C_{max} in an individual-dependent manner



Among the cytochrome P450 family, CYP3A4 and CYP2C9 are responsible for the metabolism of more than 40% of clinical drugs and are sequentially involved in diclofenac biotransformation [4] [5].

We found that diclofenac at C_{max} mediated an increase of CYP3A4 and CYP2C9 activity in a subject-dependent manner. Furthermore, the increase of CYP3A4 activity seems to be more pronounced in females than males while the activity of CYP2C9 was more induced by diclofenac in males than females.

A 45-year-old male showed iDILI at therapeutic dose out of a cohort of 24 individuals



As expected, we found that 20% of subjects (5 out of 24) displayed cell death with concentrations below 100x C_{max} suggesting that diclofenac is a DILI risk compound [3]. More importantly, only one subject, a 45-year-old male, showed toxicity already at concentrations below C_{max} confirming diclofenac-iDILI. No toxicity was detected with ASA at C_{max}.

Conclusion

We present here a powerful educated spheroid system containing autologous educated macrophages and DCs, that can detect immune-mediated iDILI risk of diclofenac at therapeutic concentration. As the system does not require primary cells and it is easy and quick to build up, it could be used for HTS of new molecules supporting lead compounds selection. Our model will strengthen drug development pipelines of pharmaceutical companies de-risking clinical trial failures.

Acknowledgements

This study was funded by the Region Occitanie and the French Government as part of the France 2030 Plan “Projets d’Innovation” DOS0222542/00 & DOS0222543/00, and by the French Government as part of the “Programme d’investissements d’avenir” (BFTE DOS0178148/00).

This work is supported by the Etablissement Français du Sang Hauts de France - Normandie, Montpellier Méditerranée Métropôle, and French Tech Méditerranée.

Litterature cited

- [1] Huang, Z., Chen, B., Liu, X., Li, H., Xie, L., Gao, Y., Duan, R., Li, Z., Zhang, J., Zheng, Y., and Su, W. (2021). Effects of sex and aging on the immune cell landscape as assessed by single-cell transcriptomic analysis. Proc Natl Acad Sci U S A 118.
- [2] Allison, R., Guraka, A., Shawa, I.T., Tripathi, G., Moritz, W., and Kermanizadeh, A. (2023). Drug induced liver injury - a 2023 update. J Toxicol Environ Health B Crit Rev 26, 442-467.
- [3] Cherradi, S., Taulet, N., and Duong, H.T. (2023). An original donor-dependent spheroid system for the prediction of idiosyncratic drug-induced liver injury risk. In vitro models https://doi.org/10.1007/s44164-023-00057-w.
- [4] Den Braver, M.W., Den Braver-Sewradj, S.P., Vermeulen, N.P., and Commandeur, J.N. (2016). Characterization of cytochrome P450 isoforms involved in sequential two-step bioactivation of diclofenac to reactive p-benzoquinone imines. Toxicol Lett 253, 46-54.
- [5] Zhao, M., Ma, J., Li, M., Zhang, Y., Jiang, B., Zhao, X., Huai, C., Shen, L., Zhang, N., He, L., and Qin, S. (2021). Cytochrome P450 Enzymes and Drug Metabolism in Humans. Int J Mol Sci 22.