Dissecting the idiosyncratic drug-induced liver injury (iDILI)-initiating mechanism using the individual-centric model: the role of the innate immune response

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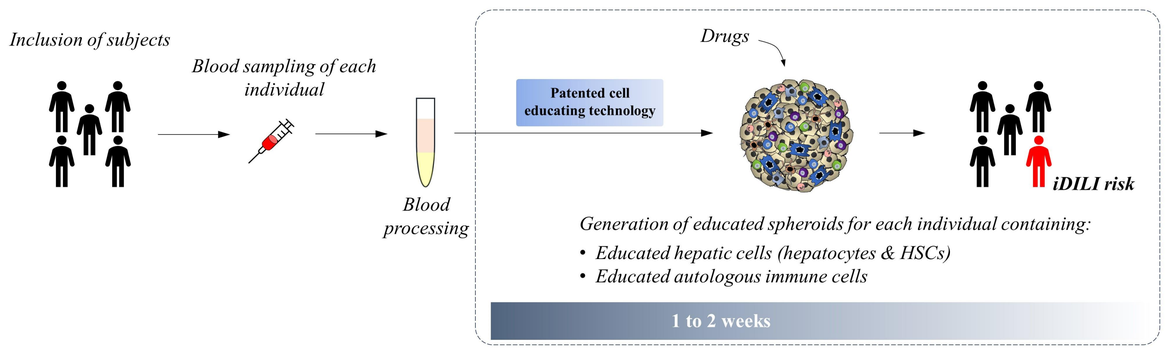
Introduction

Idiosyncratic drug-induced liver injury (iDILI) is a rare event that is difficult to detect and to dissect its mechanism at preclinical stage. Although there is evidence that most idiosyncratic drug reactions are mediated by an adaptive immune response against drug-modified proteins that are formed by a covalent binding of reactive metabolites and liver proteins [1], little is known about the early steps of iDILI-initiating mechanisms, i.e., the role of the innate immune response. Macrophages and dendritic cells play an important role in initiating innate immune responses [2]. We have developed a cell line-based individual-centric spheroid model that contains autologous monocyte-derived macrophages and dendritic cells that can detect troglitazone (TGZ)-mediated idiosyncratic liver injury at therapeutic dose [3, 4]. We used this model to analyze the response of immune cells to TGZ for a better understanding of iDILI mechanism and to explore new strategies to mitigate iDILI occurrence.

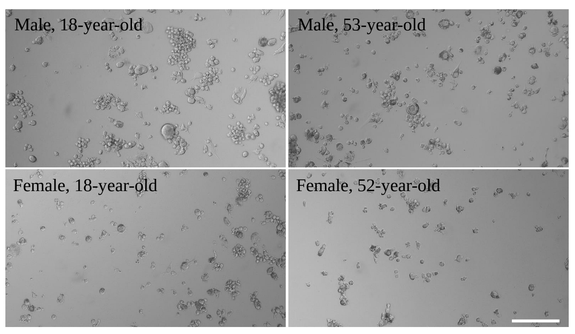
Materials & Methods

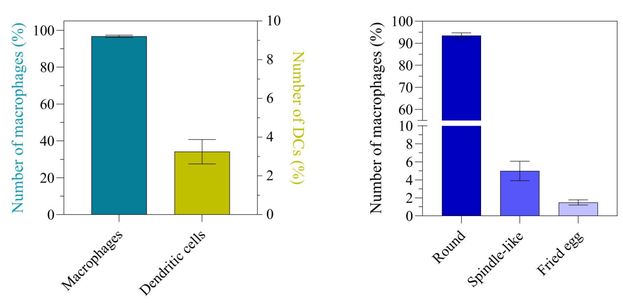


Individual-centric spheroids were generated with the cell educating technology (PCT/EP2024/052109) using processed blood from healthy donors. The spheroids that contain educated hepatocytes, and stellate cells, were supplemented with educated autologous monocyte-derived macrophages, and dendritic cells (DCs). Individual-centric spheroids and monocyte-derived macrophages and DCs were treated with troglitazone with concentrations up to 100x C_{max}. The cell viability was measured using CellTiterGlo (Promega). The expression of pro- and anti-inflammatory cytokines were measured by qPCR using QuantStudio 5 DX IVDR-compliant real-time PCR system (ThermoFisher Scientific).

Results

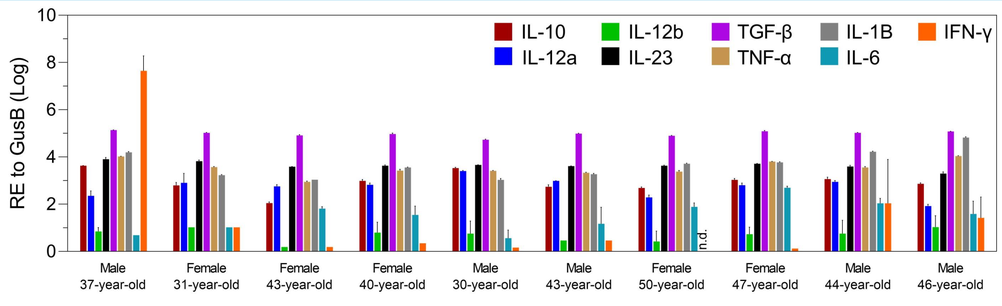
The differentiation of monocytes into macrophages and dendritic cells was individual dependent





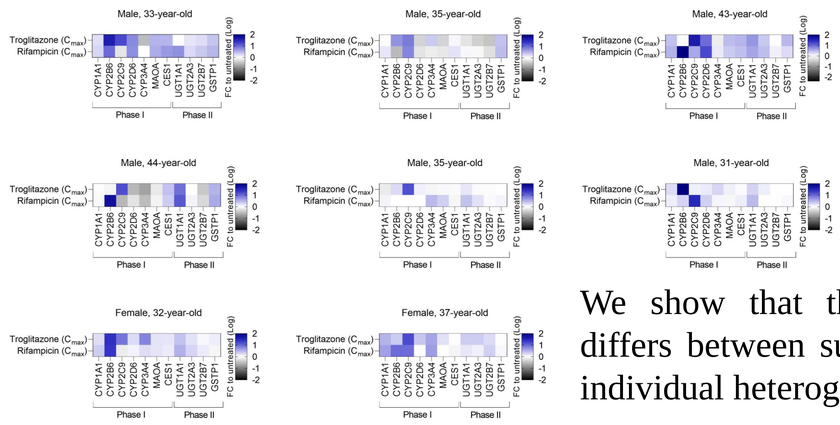
The differentiation of monocytes into macrophages and DCs was person dependent and 96.7 ± 0.9% of differentiated monocytes were macrophages while only 3.7 ± 0.9% were DCs. Among the macrophages, 93.5 ± 1.6% have a round shape, 4.9 ± 1.5% have a spindle-like shape, and 1.6 ± 0.5% have a fried egg shape.

Pro- and anti-inflammatory cytokines are differentially expressed in educated macrophages and dendritic cells



We analyzed the basal expression level of a panel of pro- and anti-inflammatory cytokines in monocyte-derived macrophages and DCs and found an heterogeneity of cytokine landscape. Moreover, we did not observe any specific expression patterns that could be associated to the age nor the gender of the donor.

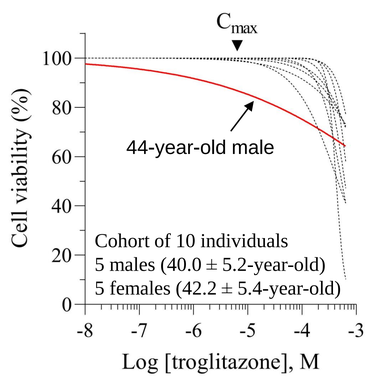
Interindividual variability of induction of drug metabolizing enzymes in educated spheroids

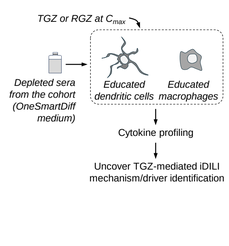


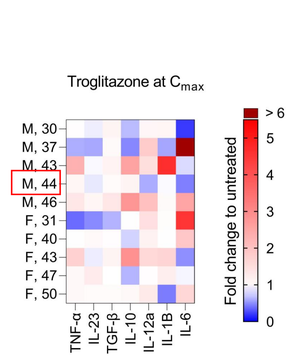
We analyzed the magnitude of induction of DMEs in response to troglitazone and rifampicin in educated spheroids from 12 individuals.

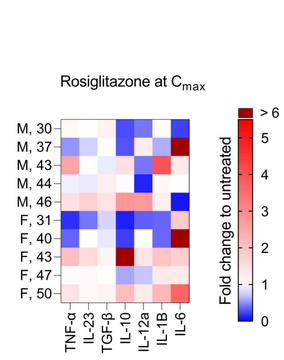
We show that the magnitude of induction differs between subjects confirming the inter-individual heterogeneity to iDILI occurrence

TGZ-mediated suppression of IL-12a expression by immune cells is correlated with iDILI occurrence



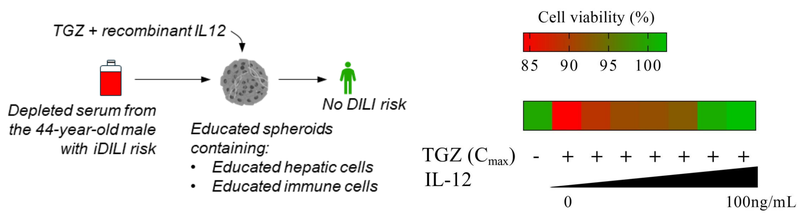






We found that only one subject, a 44-year-old male, showed toxicity already at concentrations below C_{max} confirming TGZ-iDILI [4]. No toxicity was detected with rosiglitazone at C_{max}. Cytokinome analysis on educated macrophages and DCs revealed that TGZ suppressed IL-12a expression correlated with iDILI occurrence.

Recombinant human IL-12 rescued TGZ-induced iDILI in a 44-year-old male



We performed a cell viability recovery experiment on fully reconstructed spheroids with increasing doses of IL-12.

We demonstrated that combinatory treatment of educated spheroids with troglitazone and IL-12, dose dependently rescued TGZ-mediated cell death confirming that IL-12 could prevent troglitazone-mediated iDILI in the 44-year-old male.

Conclusion

We provide evidence that the preclinical individual-centric model is a valuable model to de-risk immune-mediated iDILI occurrence and to uncover iDILI-initiating mechanism at early stage of drug development opening a perspective for new strategies to mitigate idiosyncratic drug reactions.

Acknowledgements

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Litterature cited

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