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Transforming monocytes into hepatocyte surrogates

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CHALLENGES OF IDIOSYNCRATIC DILI (iDILI)

- Rare occurrence (e.g. 1 in 10,000)
- Numerous individual factors that influence susceptibility
- May be difficult to diagnose and especially to identify the causative agent in polymedication
- Lack of experimental models

NEED: Models for iDILI
Positive test supporting the diagnosis and causality assessment
MONOCYTE DERIVED HEPATOCYTE-LIKE CELLS (MH CELLS)

BACKGROUND

- Monocytes seem to be important for hepatic repair in models of acute liver injury (1)
- Monocytes may be capable to transform into hepatocytes (2)
- Previous data suggest that cells with hepatocyte-like functions can be generated from peripheral monocytes (3, 4)

MONOCYTE DERIVED HEPATOCYTE-LIKE CELLS (MH CELLS) GENERATION

ISOLATION:
- 20ml EDTA-plasma
- Gradient centrifugation
- Adherence separation of monocytes from EDTA-plasma sample

CULTURE:
- Cultivation in presence of adenosine, IL-3, M-CSF, deoxycholate and caffeine followed by medium containing EGF, FGF4, heparin, glucagon, insulin, deoxycholate and caffeine (1)

Transforming monocytes into hepatocyte surrogates

MONOCYTE DERIVED HEPATOCYTE-LIKE CELLS (MH CELLS) GENE EXPRESSION COMPARED TO HEPATOCYTES

Gene expression profiles of monocytes (left) and MH cells (right) compared to primary human hepatocytes (PHH) from the same donor
MONOCYTE DERIVED HEPATOCYTE-LIKE CELLS (MH CELLS)
DONOR CHARACTERISTICS – EXAMPLE: CYP2C9

CYP450 activities and induction levels of primary hepatocytes are reflected by MH cells from the same donor.
MH CELLS FROM PATIENTS WITH iDILI

CASE: iDILI BY OMEPRAZOLE

Abbreviations:

- con: vehicle control
- positive con: lysis with TWEEN20
- APAP: acetaminophen (µM)
- Diclo: diclofenac (µM)
- Omep: omeprazole (µM)
- Panto: pantoprazole (µM)

- MH cells of the patient exhibit omeprazole toxicity
- No omeprazole toxicity in MH cells of healthy donors nor patients with iDILI by another drug
RESULTS

SPECIFICITY OF MH CELL TESTING

<table>
<thead>
<tr>
<th>MH</th>
<th>healthy</th>
<th>iDILI diclo</th>
<th>iDILI other than diclo</th>
<th>non DILI</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥2</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&lt;2</td>
<td>81</td>
<td>0</td>
<td>27</td>
<td>23</td>
</tr>
</tbody>
</table>

MH testing is positive for diclofenac in 4 iDILI cases with diclofenac as the drug with the highest causality likelihood.
No diclofenac toxicity is observed in MH cells derived from healthy donors, iDILI patients with another drug as cause and non DILI patients, respectively.
MH CELLS
PILOT STUDY IN PATIENTS WITH ACUTE LIVER INJURY

Study aim:
- Investigation of potential individual toxicity response of MH cells from iDILI patients in comparison to MH cells from patients with acute liver injury of other origin

Methods:
- Patients treated with at least one drug and acute liver injury according to (1):
  \[ \text{ALT} \geq 5\times \text{ULN} \text{ or } \text{AP} \geq 2\times \text{ULN} \text{ or } \text{ALT} \geq 3\times \text{ULN} \text{ and } \text{Bili} \geq 2\times \text{ULN} \]
- Diagnostic Workup: laboratory testing, imaging, histology (where available), RUCAM-score, drug signature (e.g. LiverTox website (2))
- MH cell generation, toxicity testing, data analysis

ClinicalTrials.gov Identifier: NCT02353455

### Classification for every drug

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>iDILI</th>
<th>non DILI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>classification:</strong></td>
<td>„definite“</td>
<td>„highly likely“</td>
</tr>
<tr>
<td>other causes of liver injury</td>
<td>definitely excluded</td>
<td>definitely excluded</td>
</tr>
<tr>
<td>drug signature</td>
<td>typical</td>
<td>atypical OR comedication with compatible signature</td>
</tr>
</tbody>
</table>

**Transforming monocytes into hepatocyte surrogates**

**Exclusion of other causes**

**Drug signature**
RESULTS: PATIENT CHARACTERISTICS

31 patients with iDILI and 23 with other causes for acute liver injury

<table>
<thead>
<tr>
<th></th>
<th>iDILI (n=31)</th>
<th>non DILI (n=23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>gender</td>
<td>15 female (48%)</td>
<td>9 female (39. %)</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>16 male (52%)</td>
<td>14 male (61%)</td>
<td></td>
</tr>
<tr>
<td>ethnicity</td>
<td>30 caucasian (97%)</td>
<td>23 caucasian (100%)</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>1 hispanic (3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pattern</td>
<td>22 hepatocellular (71%)</td>
<td>13 hepatocellular (57%)</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>2 mixed (7%)</td>
<td>0 mixed (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 cholestatic (23%)</td>
<td>10 cholestatic (43 %)</td>
<td></td>
</tr>
</tbody>
</table>
## RESULTS: DRUGS WITH HIGHEST CAUSALITY LIKELIHOOD IN iDILI

<table>
<thead>
<tr>
<th>Class</th>
<th>n</th>
<th>drug</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NSAID</strong></td>
<td>8</td>
<td>diclofenac</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>metamizol</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>indomethacin</td>
<td>1</td>
</tr>
<tr>
<td><strong>ORAL ANTICOAGULANT</strong></td>
<td>4</td>
<td>phenprocoumon</td>
<td>4</td>
</tr>
<tr>
<td><strong>ANTITHYROID</strong></td>
<td>2</td>
<td>carbimazole</td>
<td>2</td>
</tr>
<tr>
<td><strong>ANTI-INFECTIVE</strong></td>
<td>2</td>
<td>ciprofloxacin</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>piperacillin + tazobactam</td>
<td>1*</td>
</tr>
<tr>
<td><strong>IMMUNEMODULATOR</strong></td>
<td>2</td>
<td>glatirameracetat</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pembrolizumab</td>
<td>1*</td>
</tr>
<tr>
<td><strong>ANTIPSYCHOTIC</strong></td>
<td>2</td>
<td>olanzapine</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fluspirilene</td>
<td>1</td>
</tr>
<tr>
<td><strong>PROTON PUMP INHIBITOR</strong></td>
<td>2</td>
<td>omeprazole</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pantoprazole</td>
<td>1</td>
</tr>
<tr>
<td>antihistamine</td>
<td>1</td>
<td>cetirizin</td>
<td>1</td>
</tr>
<tr>
<td>antifibrotic</td>
<td>1</td>
<td>4-potassium aminobenzoate</td>
<td>1</td>
</tr>
<tr>
<td>low-molecular weight heparin</td>
<td>1</td>
<td>enoxaparin</td>
<td>1</td>
</tr>
<tr>
<td>phosphodiesterase 5-inhibitor</td>
<td>1</td>
<td>tadalafil</td>
<td>1</td>
</tr>
<tr>
<td>serotonin-receptor antagonist</td>
<td>1</td>
<td>sumatriptan</td>
<td>1</td>
</tr>
<tr>
<td>oral contraceptive</td>
<td>1</td>
<td>ethinylestradiol + levonorgestrel</td>
<td>1</td>
</tr>
<tr>
<td>tyrosin-kinase inhibitor</td>
<td>1</td>
<td>sunitinib</td>
<td>1</td>
</tr>
<tr>
<td>vitamin A analogue</td>
<td>1</td>
<td>acitretin</td>
<td>1</td>
</tr>
<tr>
<td>muscle relaxant</td>
<td>1</td>
<td>flupirtin</td>
<td>1</td>
</tr>
</tbody>
</table>

*positive rechallenge
RESULTS: DRUG WITH HIGHEST CAUSALITY LIKELIHOOD RUCAM AND MH TOXICITY IN UNEQUIVOCAL CASES

- RUCAM $\geq 6$ is found in 2 non DILI cases
- MH testing is negative in 1 case with unequivocal iDILI
RESULTS: DRUG WITH HIGHEST CAUSALITY LIKELIHOOD
RUCAM AND MH TOXICITY IN TOTAL STUDY POPULATION

- RUCAM $\geq 6$ is found in 29/31 iDILI cases and 6/23 non DILI cases
- MH testing is negative in 2 iDILI cases, no positive MH testing in non DILI cases

*different cases
RESULTS: COMEDICATIONS
RUCAM AND MH TOXICITY IN TOTAL STUDY POPULATION

Test results for comedications in all iDILI (n=31) and non DILI (n=23) cases (up to 4 drugs per patient):
- RUCAM is ≥6 for 27/53 comedications in iDILI cases and 2/39 non DILI cases
- MH testing is positive for 4 comedications in iDILI cases, no positive results in non DILI cases
RESULTS: ALL DRUGS IN TOTAL POPULATION TEST RESULTS COMPARED TO CAUSALITY LIKELIHOOD

- RUCAM yields a relevant proportion of false results for drugs with intermediate causality likelihood
- MH testing shows stable results independent on causality likelihood
SUMMARY

- Our data suggest that monocytes can acquire some hepatocyte properties in vitro and seem to reflect donor specific characteristics.
- In this pilot study toxicity was higher in MH cells derived from iDILI patients compared to patients with non DILI acute liver injury or healthy donors.
- MH cells offer the possibility to assist with diagnosis of iDILI and causality assessment.

OUTLOOK

- Ongoing research further characterises the model using Omics-technologies.
- Further data are needed from patients who tolerate potential iDILI drugs.