TRANSPLANTATION FOR METABOLIC LIVER DISEASE – BACK TO THE FUTURE?

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Disclosure

• Relationships with commercial interests:
  • Grants/Research Support:
    • Cytonet, Abbvie, Ferring, Pentax, Alexion, Janssen
  • Honoraria:
    • Astellas pharma

I am the other Steve Martin
Objectives

- Provide an overview of transplant approaches to treating metabolic diseases (MLD).
- Briefly describe experience with liver cell transplant in metabolic liver disease.
- Introduce potential for using stem cell derived hepatocyte-like cells for transplant in MLD.
- Understand the current role for gene therapy in MLD.

Two major groups of metabolic disease:

<table>
<thead>
<tr>
<th>Progressive liver injury</th>
<th>Normal liver function</th>
</tr>
</thead>
<tbody>
<tr>
<td>• α-1-Antitrypsin deficiency</td>
<td>• Urea cycle disorders (most)</td>
</tr>
<tr>
<td>• Cystic Fibrosis</td>
<td>• Crigler-Najjar 1 (CN1)</td>
</tr>
<tr>
<td>• Wilson's</td>
<td>• 1o hyperoxaluria</td>
</tr>
<tr>
<td>• Tyrosinemia</td>
<td>• GSD 1</td>
</tr>
<tr>
<td>• GSD 3,4,6</td>
<td>• Hypercholesterolemia (HFH)</td>
</tr>
<tr>
<td>• PFIC</td>
<td>• Amino-acidopathies (MMA, PA)</td>
</tr>
<tr>
<td>• Hemochromatosis</td>
<td>• Coagulation disorders</td>
</tr>
<tr>
<td>• Nieman Pick type C</td>
<td>• Familial Amyloid Polyneuropathy</td>
</tr>
<tr>
<td>• Cholesterol ester storage disease</td>
<td>• MSUD</td>
</tr>
<tr>
<td>• Bile acid synthesis defects</td>
<td></td>
</tr>
<tr>
<td>• North American Indian Cirrhosis</td>
<td></td>
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<tr>
<td>• Mitochondrial disorders</td>
<td></td>
</tr>
</tbody>
</table>
Liver transplantation in metabolic disease with preserved liver function

<table>
<thead>
<tr>
<th>Prevent/Treat HCC</th>
<th>Prevent extra-hepatic injury</th>
<th>Quality of life</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Tyrosinemia</td>
<td>• Brain</td>
<td>• PFIC</td>
</tr>
<tr>
<td>• GSD 1, 3,6</td>
<td>• CN1, UCD, AA, tyrosinemia</td>
<td>• UCD</td>
</tr>
<tr>
<td>• NPC</td>
<td>• Heart</td>
<td>• Coagulopathy</td>
</tr>
<tr>
<td>• PFIC2</td>
<td>• HFH, AA</td>
<td>• Amino-acidopathy</td>
</tr>
<tr>
<td>• Mitochondrial disease</td>
<td>• Kidney</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Oxaluria, Amyloidosis</td>
<td></td>
</tr>
</tbody>
</table>

Caution:
Some problems may not be cured by a liver transplant

• Pre-existing neurological deficits
• Neurological deterioration
• TPN-dependent diarrhea
• Progressive renal disease
• Progressive cardiac dysfunction
LIVER TRANSPLANT

Metabolic disease - USA 1997-2017

Adult liver transplants
n=2887 (2.5%)

85% from only 3 diagnoses
AAT, Wilson’s, HHC

Pediatric liver transplants
n=1268 (10.3%)

UNOS data December 2017
Whole organ transplant - variations

Domino

- MSUD
- AAT

Normal donor

APOLT

- Parent
- CN1 child

Domino Auxiliary

- PA
- CN1

Challenges to liver transplant for metabolic disease in infants

- Ideal
  - Transplant early before extra-hepatic complications arise
  - Transplant in a stable state

- Reality
  - Recipient issues:
    - Technical challenges to transplanting very small infants
    - Metabolic disease makes the patient unstable
  - Donor issues:
    - Living/cadaveric donors may be too big
    - Living donors may be disease carriers
    - Pediatric cadaveric donors are rare

Rela M et al Pediatr Transpl 2016
Govil S et al Liver Transpl 2015
CELL TRANSPLANT

Animal & Human experience with LCT

<table>
<thead>
<tr>
<th>Animal models</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crigler-Najjar</td>
<td>✓</td>
</tr>
<tr>
<td>Urea Cycle defects</td>
<td>✓</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>✓</td>
</tr>
<tr>
<td>PKU</td>
<td>✓</td>
</tr>
<tr>
<td>GSD1, 1b</td>
<td>✓</td>
</tr>
<tr>
<td>Factor VII deficiency</td>
<td>✓</td>
</tr>
<tr>
<td>Refsum</td>
<td>✓</td>
</tr>
<tr>
<td>Wilson’s Disease</td>
<td></td>
</tr>
<tr>
<td>Tyrosinemia</td>
<td></td>
</tr>
<tr>
<td>MSUD</td>
<td></td>
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<tr>
<td>Hyperoxaluria</td>
<td></td>
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<tr>
<td>Porphyria</td>
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</tbody>
</table>

- Initial animal studies: 1970’s
- % liver mass infused:
  - 4-22%
- Initial human studies: 1990’s
- Duration of effect:
  - 1-36 months
Insights from animal models

• Hepatocyte engraftment is low in physiologic conditions
• Selective conditions promote high levels of re-population
• Only 30% of cells engraft
• Loss of engrafted cells is difficult to detect and mechanisms poorly understood.
• 5-15% replacement is often sufficient

Selective conditions enhance liver re-population

• Growth stimulus for engrafted cells
  • T3, other growth factors
  • Partial hepatectomy
  • Portal vein branch embolization

• Selective advantage for grafted cells
  • Host liver disease
  • Chemical
  • Liver irradiation
Selective advantage for infused cells

Tyrosinemia Model
Fah-/- mice transplanted with w.t. hepatocytes

Schaffritz DA Int J Biochem Cell Biol 2011;198

On NTBC

NTBC withdrawn

Human Heterologous Liver Cells for Infusion in Urea Cycle Disorders

• Pioneered by University of Heidelberg
• Cryopreserved human heterologous liver cells for infusion (HHLivC)
Catheter placement

- Broviac line allows repeated infusions
- Surgically placed
- Middle colic vein or SMV

Protocol

6 applications over 6 days.
- 0.5 - 3 x 10^8 viable cells/kg
- 5 % liver mass
- Infused by hand at 2ml/min
- Monitoring of PVP and PV flow
- Tacrolimus/Prednisone
# RESULTS

## Clinical outcomes: Calgary subjects

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<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Procedural</td>
<td>non-occlusive portal vein thrombosis -</td>
<td>none</td>
<td>none</td>
<td>non-occlusive portal vein thrombosis -</td>
<td>none</td>
</tr>
<tr>
<td>Complication</td>
<td>resolved</td>
<td></td>
<td></td>
<td>resolved</td>
<td></td>
</tr>
<tr>
<td>Time to Hyper-</td>
<td>14 mo</td>
<td>2 mo</td>
<td>10 mo</td>
<td>2 mo</td>
<td>3 mo</td>
</tr>
<tr>
<td>ammonemia</td>
<td>after apparent engraftment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>alive, transplanted</td>
<td>died, on wait list</td>
<td>exit from study at 24 m</td>
<td>alive, transplanted</td>
<td>alive, transplanted</td>
</tr>
</tbody>
</table>
Individual cases

13C-Ureagenesis Assay

Treatment Effect on Ureagenesis Capacity

Significantly increased ureagenesis capacity after treatment with HHLivC
(post treatment values are max. capacity measured)
Time to Hyperammonaemic Events

Bootstrap Analysis / Kaplan-Meier-Plots

- Subset of 20 patients and 63 historical controls with complete pre-post data; no protocol violations
- Matched for subtype diagnosis
- Adjusted for age at treatment

Perceived limitations of hepatocyte transplantation

Donor liver
- Scarcity of donors
- Quality of donor livers

Cell isolation
- Limited in-vitro culture
- Genetic manipulation not easily achieved
- Cryopreservation injury

Stem Cells?

Engraftment
- Loss of 70% of infused cells
- Low engraftment in non-selective conditions
- Immunogenicity
Sources of stem cells

- Non-invasive
- Immature phenotype
- Multiple sources
- Immunogenicity
- Ethical concerns

Somatic cells

- Mesenchymal stem cells

Somatic cells

- ESC
- cytokines
- media
- HLC

IPSC

- cytokines
- media
- HLC

IPc

- cytokines
- media
- HLC

TFs

- Re-programming
- HLC

Transforming growth factors (TFs)

Gene correction

Stem cells - potential concerns

- Immature phenotype of hepatocyte-like cells.
- Teratoma formation after transplant
- Epigenetic transcriptional memory of original somatic cell source
- Genetic instability in prolonged culture

Takebe T Nature 2013
Mature hepatocytes outperform stem cells in non-selective conditions


Promethera protocol

E. Sokal, Université catholique de Louvain, Brussels

Najimi M et al Cell Transplantation 2007
Promethera protocol (2)
Université catholique de Louvain, Brussels

Differentiation
EGF + βFGF
HGF + βFGF + ITS

Maturation
Oncostatin M
Dex
ITS

albumin

B

Najimi M et al Cell Transplantation 2007

Adult-derived human liver stem cells

• MSC phenotype
• Hepatocyte-like cells
  • Albumin and AFP mRNA, Glycogen storage
  • G-6-P, AAT, GGT, MRP2 HNF4, CYP3A4,CYP1B1
• Differ from mature hepatocytes
  • No CYP2B6, No biliary markers
• No Oct 4 expression
  • No reversion to osteocytes/adipocytes
  • No tumours in SCID mouse
  • No transformation in long-term culture

Phase 1/2 Clinical Trial 2012-2015

- 14 UCD (1.5m - 17 years);
- 6 Crigler-Najjar (3.5- 9 years)
- PV catheter by IR
- 0.6-6.1% of liver mass infused over 1 cycle 1-4 days
- Efficacy outcomes at 12 m

<table>
<thead>
<tr>
<th>UCD</th>
<th>CN</th>
</tr>
</thead>
<tbody>
<tr>
<td>13C Urea production</td>
<td>Bilirubin</td>
</tr>
<tr>
<td>Ammonia levels</td>
<td></td>
</tr>
<tr>
<td>Protein intake</td>
<td></td>
</tr>
<tr>
<td>Bilirubin</td>
<td></td>
</tr>
</tbody>
</table>

Sokal et al AASLD 2014

Results

- Ammonia levels
- Urea generation

CN patients

10-40% lower bilirubin in 3/6 pts

Sokal et al AASLD 2014
Summary

- LCT can successfully bridge to transplant in MLD.
- Currently, long-term, safe control of MLD can only rarely be achieved with LCT.
- Stem cells offer solutions to cell supply and ex-vivo gene manipulation but have not shown greater engraftment than hepatocytes.
- High re-population rates await reversible selective conditions.

Prevent cell loss
Control IBMIR
- Low MW dextran
- NAC
- KC, PMN depletion
- MSC supernatant
- TNF-alpha blockade
- Immunosuppression

Selective conditions
- Liver Irradiation
- PV occlusion

Pre-infusion
More cells
- Improve viability
- Pan-caspase inhibitors
- Vitrification
- Induce proliferation
- IL6, HGF

Access to lobule
- Splanchnic vasodilators
- Disrupt endothelium
- Cytosinephosphoamide
- Doxorubicin
- Rifampicin

Improving engraftment
Gene therapy - historical aspects

- **1970’s**
  - first direct human gene therapy trial for UCD using wild type-Shope papilloma virus - unsuccessful

- **1990’s**
  - First therapeutic gene therapy trial for ADA –SCID
    - Ex-vivo modification of ADA-deficient WBC’s to express normal gene
  - Explosion of interest
  - 1999 - death of OTC patient from multi-organ failure due to immune response to adenovirus vector

- **2012**
  - rAAV vector engineered to express LPL approved in Europe

Gene Delivery Tools

Adenoviral vectors
Retroviral vectors
Lentiviral vectors
Non-viral vectors
rAAV vectors

Gene Editing Tools

Zn finger
TALENS
CRISPR-Cas9

Schneller JM et al. BMC Medicine 2017

Promotorless gene targeting without nuclease

Liver Transplant
- Standard APOLT
- Proven track record
- Indicated for liver disease
- Complex
- Life-long immunosuppression
- Donor shortages
- Limited access for infants

Cell transplant
- Hepatocytes
- Stem Cells
- Less invasive
- Lower mortality
- Bridge to transplant

Gene therapy
- Ex-vivo
- In-vivo
- Simple administration
- Durable
- Monogenic disorders
- Immune responses
- Off-target effects
- Evolving field

Indications Addressed by Gene Therapy Clinical Trials
- Cancer diseases 65% (n=1688)
- Monogenic diseases 11.1% (n=287)
- Infectious diseases 7% (n=182)
- Cardiovascular diseases 6.9% (n=180)
- Neurological diseases 1.8% (n=47)
- Ocular diseases 1.3% (n=34)
- Inflammatory diseases 0.6% (n=15)
- Other diseases 2.2% (n=58)
- Gene marking 1.9% (n=50)
- Healthy volunteers 2.2% (n=56)