Cell Transplantation: from hepatocytes to stem cells

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Conflict of Interest:

Consultant: Cambrex (Lonza)

Stockholder in Stemnion, LLC

1. Although the stock is worthless, I still must show this slide.
Recognition

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Outline

- Historical Background
- Describe the techniques involved
- Spotlight specific transplant cases
- Future directions.
Need for Liver Support

- >14,000 patients with liver insufficiency are listed for Tpx.
- Estimated 1,300 patients die each year
- Estimated 3.9 Million people with HCV in US. 1 in 4 will develop cirrhosis or end stage liver failure.
- Estimated 12,000 will die of the disease each year.
Options for the Treatment of Liver Disease

- Oltx is major surgery
- High incidence of surgical, medical complications
- Expensive Surgery
- Expensive maintenance therapy.
- Limited donor numbers.
- Timing is critical

- Hepatocyte transplant
- Less invasive
- Less costly
- Fewer complications
- Cryopreserved cells available “on call”
- New cell sources available
Hepatocyte Transplantation: current clinical uses

- “Bridge” patients to whole organ transplantation.
- Repopulation of liver in FHF without whole organ transplantation.
- Correction of metabolic defects without whole organ transplantation.
- Support for end-stage cirrhosis
Acute Liver Diseases Corrected by Hepatocyte Transplantation

(Animal models)

- Fulminant Hepatic Failure
  - 90% hepatectomy, Galactosamine,
  - CCL₄, Thioacetamide, Fas-Ligand
- Ischemia/Reperfusion Injury
- Anhepatic phase
- Acute decompensation of chronic liver failure
Metabolic Liver Diseases Corrected by Hepatocyte Transplantation

- Tyrosinemia Type 1 (fah)
- Analbuminemia
- Wilson’s Disease (Cinnamon Rat, Toxic milk mouse)
- Crigler-Najjar, Gunn Rat, CN-Type 1
- Ornithine Transcarbamylase Deficiency, (urea cycle defect)
- Ascorbic Acid Deficiency (ODS rat)
- Familial Hypercholesterolemia.
- Uric Acid metabolism (Dalmation Dog)
- Hemophilia, Factor 7, 9
- Glycogen storage disease
- Dubín-Johnson Syndrome, (MRP2 deficiency)
- Progressive Familial Intrahepatic Cholestasis
  - PFIC-3 (mdr2).
Hepatocyte Transplantation

- First clinical transplants
- 1992
- Mito, Kasai, Kusano in Japan, and
- Strom and Fisher in the US
- Followed soon by Habibullah, Bilir, Soriano, Fox and Strom, Sokal, Muraca, Dhawan, Ott, Allen, and now more.
Hepatocyte Transplantation

- How does it work?
- How is it done?
Transplantation Techniques

- In acute liver failure or if architecture is normal, transplant cells into the portal vein.

- Chronic liver disease with cirrhosis, transplant cells into spleen.
How do cells engraft?
Blood Flow Through Liver

- Cells block the terminal portal veins.
- Transient increase in portal pressure facilitates entry of cells into sinusoidal areas.
- Un-engrafted cells are cleared within 48 hrs.
Hepatocyte Integration Following Tpx

1 hr post transplant. Hepatocytes (red) fill portal vein (PV) radicle, and spill into sinus region.

24 hrs post transplant. Arrows show individual hepatocytes integrated into parenchyma.

Koenig et al., 2005
Detection of h-Albumin in Transplanted Human Hepatocytes (+4 days)
Cell Source?

- Remaining lobes or segments from partial grafts or split liver transplants. (quite rare).

- Organs rejected for whole organ transplant.
  - Frequently with high levels of steatosis.
  - Frequently with extended cold ischemic times.
Hepatocyte Transplantation
Clinical Experience

- “Bridge” patients to whole organ transplantation.
- Repopulation of liver in Acute Liver Failure without whole organ transplantation.
- Correction of metabolic defects without whole organ transplantation.
Hepatocyte Transplants
To:
Bridge Patients
to OLTx
The Hepatocyte Bridge

- Patient listed for whole organ transplantation
- Patient deteriorates, progresses to end stage liver failure.
- Then:
- Hepatocyte transplantation provides temporary liver function to keep patient alive long enough to receive OLTx
Cell Transplants as Bridge to OLTx
(First 20 patients, Strom, Fisher, Fox)

- 7 Deaths, 3.5 ± 2.3 days
- 11 Survivors, OLT 5 ± 4 days
- Controls Received No Cell Transplant (4 cases)
  No Survivors, Death at 2.8 ± 2.5 days
- 2 Patients recovered without OLT
# Clinical Values

(N=18)

<table>
<thead>
<tr>
<th></th>
<th>Pre-HTx</th>
<th>Post-HTx</th>
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<tbody>
<tr>
<td>Intracranial Pressure</td>
<td>21 ± 8</td>
<td>12 ± 5*</td>
</tr>
<tr>
<td>Cerebral Perfusion Pressure</td>
<td>60 ± 8</td>
<td>75 ± 11*</td>
</tr>
<tr>
<td>Ammonia (μ Mol / L)</td>
<td>198 ± 58</td>
<td>65 ± 18*</td>
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Hepatocyte Transplants
For:
Acute Liver Failure
Acute Liver Failure

- **Idea:** Partially repopulate the liver with donor hepatocytes.

- **To:** Supplement liver function with transplanted cells to keep a patient alive long enough to allow the native liver to regenerate.

- **However:** Long-term survival requires regeneration of native liver.
Hepatocyte Transplantation for HBV-Induced Fulminant Hepatic Failure

- 37, F, 58kg,
- Febrile 2 wks
- Illicit drug use, HBsAG Positive
- Grade 1 encephalopathy
- prothrombin time pt>100 sec
- Factor 7 <1%

- NH₃ = 150 μmol/L
- ALT= 4079 U/L
- Bilirubin 11.4 mg/dl
- Glucose = 70 mg/dl, ON I.V. D₁₀
- 26 U Fresh Frozen Plasma,
- Transhepatic Portal Vein Catheter
HBV, Acute Liver Failure
37, F, Day 7 Post Cell Transplant
Post Cell Transplant

- Ammonia 34 - 40 < 1 week
- Factor 7= 25% (day 7)
  64% (day 14)
- No FFP or factor support after cell transplantation.
- Discharged, 14 days

- 6-weeks Post TPX
- bilirubin 1.2 mg/dl
- ALT 38 U/L
- Factor 7, 70-80%
- pt = 9.7
- HBsAG Negative
Day 107  Post Cell Transplant.
HBV, FHF
Htx. for Acute Liver Failure

- 5 Patients recovered without Oltx
  - Acetaminophen (2), Viral (1) Mushroom Poisoning (1), Idiopathic (1 pediatric).

- Promising results:
  - Problem, requires large numbers of cells
  - Usually cryopreserved
Cell Therapy of Metabolic Liver Disease

- Idea:

  Correct enzyme or protein deficiency in a metabolic disease patient by transplantation of hepatocytes.
Basic Concepts

- Able to transplant of up to 5% of liver mass/transplant event in an undamaged liver.
- Attains 1-5% repopulation of native liver.
- Repeated transplants are safe.
- Most effective on metabolic diseases which are corrected with <10 activity.
- Is MOST effective when there is growth stimulation or a selective growth advantage for transplanted cells.
Metabolic Liver Diseases Corrected by Hepatocyte Transplantation

- Tyrosinemia Type 1 (fah)
- Analbuminemia
- Wilson’s Disease (Cinnamon Rat, Toxic milk mouse)
- Crigler-Najjar, Gunn Rat, CN-Type 1
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- Uric Acid metabolism (Dalmation Dog)
- Hemophilia, Factor 7
- Glycogen storage disease
- Dubín-Johnson Syndrome, (MRP2 deficiency)
- Progressive Familial Intrahepatic Cholestasis
  - PFIC-3 (mdr2).
HTx for Crigler-Najjar

- 10 y.o. Female CN-Type 1
- Received approximately 4% of liver mass in single transplant event.
- Approximately 7.5 Billion Cells via portal vein
UDP-Glucuronosyltransferase Activity

% of Normal

5
3
1

Pre-Tpx

Post Tpx

HTx for Crigler-Najjar

UDP-Glucuronosyltransferase Activity

Total Bilirubin (mg/dl)

Days

-30 0 30 60 90 120 150 180 210 240 270
HTx for Crigler-Najjar

- **1.5 Years Post Transplantation**
- Bilirubin reduced >65% stable at 12-13 mg/dl
- Phototherapy reduced by 50% to 6 hrs/day
- Bilirubin conjugates in bile:
  - >80% diglucuronide
- **Concluded:** More than 1 tx will be needed to completely normalize bilirubin levels.
Clinical Transplants for Metabolic Liver Diseases (numbers of patients)

- Familial Hypercholesterolemia. (5)
- Crigler-Najjar, CN-Type 1 (5)
- Ornithine Transcarbamylase Deficiency (4)
- Factor 7 Deficiency (2)
- Glycogen Storage Disease (2)
- Infantile Refsum Disease (1)
- Progressive Familial Intrahepatic Cholestasis (PFIC-2, BSEP). (2)
- A1AT Deficiency (2)
- Arginosuccinate Lyase (ASL) (1)
Summary, Metabolic Liver Disease

- Cell transplant alone can correct metabolic liver disease.
- Longest correction > 500 days.
- In most transplants to date, insufficient numbers of viable cells were transplanted to attain stable corrections.
- Complete corrections were attained when sufficient numbers of cells and multiple transplants were conducted.
Arginosuccinate Lyase (ASL) deficiency

- Urea Cycle defect, hyperammonemia.

- 3.5 y.o. F, 13.5 kg

- Complete correction required 3 transplants.
  - Cells from 3 different donors (4.7 Billion)
    - 7 infusions over 1 month, 1.7 billion cells
    - 2 infusions /2 days (+2.5 months), 1 billion cells
    - 2 infusions/2 days (+4.5 months), 2 billion cells
Current Problems:

- **Cell Source?**
  - Organs rejected for transplant
  - Immortalized Cells?
  - Xenotransplants?
  - Fetal liver?
  - Stem/Progenitor Cells?
Stem Cells from Placenta

- Amnion membrane an fetus, 9 Weeks.

- For our studies, stem cells are isolated from amnion at or near full term, following the live birth of baby.
Histology of Placenta Tissue (H&E) and Before/After Trypsinization

Before

Amniotic Epithelial cell
Amniotic Mesenchymal cell

After

x400
Expression of molecular markers for stem cells

Cytospin cell immunohistochemistry / Fluorescent microscopy

Oct-4
SOX-2
FGF-4
Rex-1
TERT
β-actin

Relative Expression

Oct-4

Nanog

Days

Days

Cytospin cell immunohistochemistry / Fluorescent microscopy

Oct-4

Nuclear

Merged
Differentiation potential into all three germ layer cell lineages

Endoderm (Pancreatic cells)
- Pdx-1
- Pax-6
- Nkx 2.2
- Insulin
- Glucagon
- \(\beta\)-actin

Day 0  Day 7

Mesoderm (Cardiac cells)
- MLC-2A
- MLC-2V
- hANP
- cTnT
- GATA-4
- Nkx 2.5
- \(\beta\)-actin

Day 0  Day 14

Ectoderm (Neural cells)
- NSE
- NF-M
- MBP
- Nestin
- GFAP
- GAD
- \(\beta\)-actin

Day 0  Day 7

Differentiation of hAE Cells to Hepatocyte-like Cells, \textit{In Vitro}

- Cytokeratins: 8, 18, 19
- Albumin, Alpha 1-Antitrypsin, c-met
- **CYP 7A1**, marker of definitive endoderm
  (not expressed in visceral endoderm)
- HNF1, HNF 4 alpha, C/EBP (alpha and beta), Oatp, PXR, CAR, RAR, RXRa, PPAR, and more...
- **CYP450** gene expression (Mature Liver)
  - 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, **3A4**, 3A7, 7A1
  - Inducible CYP 1A1, 1A2, 2B6, 2C9, 3A4, 3A7
Methods

60% Partial Hepatectomy

Liver tissue
- Histology
- DNA analysis
- RNA analysis

SCID/beige mice (8-9 wks)

70mg/kg Retrorsine

1 week → 2-4 weeks → 60% Partial Hepatectomy

1 day → CTx

6 months

AE cells (0.5 x 10^6 cell/mouse) transplanted via spleen

Liver tissue
- Histology
- DNA analysis
- RNA analysis
DNA quantification:
Quantitative RT-PCR using SYBR green Human Alu primers

mRNA expression:
Quantitative RT-PCR using Taqman® gene expression assays

Data analysis:
Gene expression arbitrary values normalized to human Cyclophilin expression
CYP2B6

mRNA levels relative to CyclinHin

AE Cells | AE Tx | Fetal Liver | Adult Liver
MDR-1 (P-gp)

The graph shows mRNA levels relative to Cyclophilin across different samples:

- AE Cells
- AE Tx
- Fetal Liver
- Adult Liver

The y-axis represents the mRNA levels, ranging from 0.000 to 0.020. The adult liver sample has the highest mRNA levels, followed by the AE Tx and fetal liver samples. The AE Cells have the lowest levels.
MRP2

mRNA levels relative to Cyclophilin

AE Cells  AE Tx  Fetal Liver  Adult Liver
Bile Salt Export Pump (BSEP)

mRNA levels relative to Cyclophilin

AE Cells  AE Tx  Fetal Liver  Adult Liver
Summary

• In animals receiving human AE transplants a number of genes are expressed at or near normal adult levels
  
  – CYPs : CYP3A4, 3A7, 1A2,
  
  – 2B6, 2C8, 2C9, 2D6, 7A1
  
  – Hepatic Transporters :
    P-glycoprotein, MRP2, BSEP
Transplantation of Amnion-derived Stem Cells

Results in Full Adult Expression of:

• Alpha-1 antitrypsin (A1AT)
• Ornithine Transcarbamoylase (OTC)
• Glucose-6-phosphatase (G6P)
• While UDP glucuronosyltranferase (UGT)1A1 Approaches normal values
Amnion Stem Cells

- May be a significant source of Cardiac, Neural or Beta cells and Hepatocytes for cell transplantation and regenerative medicine.

- NO Social, Ethical or Religious objection to the use of this stem cell source.
Hepatocyte Transplants

Summary

- Useful for “bridging” patients to whole organ transplantation.
- Can sometimes obviate whole organ transplantation (Acute Liver Failure)
- Provides long-term correction of metabolic diseases.
Hepatocyte Transplant Centers

- **Worldwide Clinical Effort**

- 14 Transplant Centers in 12 Countries
  - Italy, Belgium, UK, Germany, Australia, Korea, Japan, China, India, Spain, Sweden, USA.

- Additional efforts China, Italy, Egypt
Thank You

どうもありがとうございます

謝 謝