Delayed Biliary Complication after Warm Ischemic Insult in Rat and Effects of Immunosuppressants

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Introduction

• Shortage of deceased donor
  – Recent interest in marginal donors including donation from cardiac death (DCD)
• Liver graft from DCD
  – Possible prolonged warm ischemia
  – Biliary complication
  – Few studies about bile duct damage from DCD

Lee et al Liver Transpl 2007
Introduction

• **Rapamycin, Tacrolimus, MMF** have an **antiproliferative effect** on human intrahepatic biliary epithelial cells in vitro.  
  
  *Liu et al World J Gastroenterol 2005*

• **Rapamycin** has the strongest **growth-inhibitory effect**
  
  – Inhibit STAT3 activation in IL-6/STAT3 pathway  
  
  *Chen et al Liver Transpl 2010*

• **Controversy about effect of immunosuppressants** on the biliary complication and fibrosis

• The animal study about bile duct damage from warm ischemic insult such as DCD LT has not been well established
Purpose

• To evaluate the warm ischemic damage of bile duct in rat and to evaluate the effects of immunosuppressants.

• To suggest optimal immunosuppressant to protect bile duct after warm ischemic insult such as DCD LT
Methods I

- Male Strague-Dawley rats (280-300 g, 6~7 weeks old)
- Isoflurane, non-rebreathing circuit
- OP Procedure
  - Bile duct stenting and ligation of peribiliary plexus
  - Proper hepatic artery was ligated above gastroduodenal artery
  - Portal vein was clamped for 30 minutes.
Instrument
Operation
Rat Model of DCD

Normal Liver

- Bile duct stenting
- PHA ligation
- PV clamping for 25-30 minutes

Y. Abe et al. 2009
Pilot Study

• Sacrificed at POD#1, 3, 7

• No early biliary complication

H&E (x100)
Pilot Study

Sacrificed at Postop 6 weeks

H&E (x40)

H&E (x100)

Sirius red (x400)
Subgroup

Total (n=45)

Control (C, n=15)
- 1w (n=5)
- 3w (n=5)
- 6w (n=5)

Rapamune (S, n=15)
- 1w (n=5)
- 3w (n=5)
- 6w (n=5)

Tacrolimus (T, n=15)
- 1w (n=5)
- 3w (n=5)
- 6w (n=5)

C : no immunosuppression

S : Rapamycin 1 mg/kg from POD#1

T : Tacrolimus 1mg/kg from POD#1
Methods II

- Laboratory tests at postoperative 1 day, 1, 2, 3, 4, 5, 6 week in all rats - total bilirubin, ALP, ALT, GGT.
- H&E and sirius red staining was performed for evaluation of biliary hyperplasia and peribiliary fibrosis.
- The peribiliary fibrosis was evaluated by Sirius red staining and image analyzer and the ratio (fibrotic area: normal area) was evaluated in 8 areas in each slide.
C (sacrificed at 6 weeks)

Image Analyzer

Fibrotic area/Normal area
Results
Rat in Control (6w)
T-group (6w)

S-group (6w)
Laboratory Results

ALT

T.bil

no significant difference

ALP

GGT
% Fibrosis

- Image analyzer
- % fibrosis = (fibrotic area / normal area) * 100

<table>
<thead>
<tr>
<th></th>
<th>mean(range)</th>
<th>1 week</th>
<th>3 week</th>
<th>6 week</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C group (n=5)</strong></td>
<td>1.20(0.58-3.11)</td>
<td>3.37(1.67-7.04)</td>
<td>4.83(1.44-6.92)</td>
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<tr>
<td><strong>T group (n=5)</strong></td>
<td>1.57(1.04-2.61)</td>
<td>3.00(2.31-4.41)</td>
<td>2.43(0.75-6.57)</td>
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<tr>
<td><strong>S group (n=5)</strong></td>
<td>0.09(0.59-1.38)</td>
<td>1.49(0.78-1.91)</td>
<td>1.71(1.14-3.10)</td>
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</table>
**Trends of % Fibrosis**

- **C group (n=5)**
- **T group (n=5)**
- **S group (n=5)**

- **1 week**:
  - C group: 1.2
  - T group: 1.57
  - S group: 0.91

- **3 week**:
  - C group: 3.37
  - T group: 3.0
  - S group: 1.49

- **6 week**:
  - C group: 4.83
  - T group: 2.43
  - S group: 1.71

**Statistical Significance**

- P=0.145
- P=0.019
Effects of HA Ligation on BD Complication in Rat Model

<table>
<thead>
<tr>
<th></th>
<th>BD reconstruction</th>
<th>HA ligation</th>
<th>Numbe r</th>
<th>Complication</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Abscess</td>
</tr>
<tr>
<td>LT</td>
<td>Duct to duct</td>
<td>+</td>
<td>30</td>
<td>8/30</td>
</tr>
<tr>
<td></td>
<td>H-D</td>
<td>+</td>
<td>30</td>
<td>13/30</td>
</tr>
<tr>
<td>Without LT</td>
<td>Duct to duct</td>
<td>+</td>
<td>10</td>
<td>1 (10%)</td>
</tr>
<tr>
<td></td>
<td>Duct to duct</td>
<td>-</td>
<td>10</td>
<td>1 (10%)</td>
</tr>
<tr>
<td></td>
<td>H-D</td>
<td>+</td>
<td>10</td>
<td>3 (30%)</td>
</tr>
<tr>
<td></td>
<td>H-D</td>
<td>-</td>
<td>10</td>
<td>2 (20%)</td>
</tr>
</tbody>
</table>

Li et al. World J Gastroenterol 2011
Effects of HA Ligation

BD stenting

HA ligation(-), PV clamping(-) (n=2)

HA ligation(+), PV clamping(-) (n=3)
Effects of HA Ligation

BD stenting (n=2)  
HA ligation(-), PV clamping(-)  

BD stenting, HA ligation (n=3)  
HA ligation(+), PV clamping(-)
Summary

- No early bile duct proliferation in the H&E stained liver tissue.
- The laboratory results showed no significant difference between groups.
- Different trends of delayed bile duct hyperplasia and fibrosis in periductal area without septal fibrosis in H&E and Sirius red staining according to immunosuppressants.
- The mean ratio of fibrotic area of each groups at 6 weeks were 4.83 (C, range, 1.44-6.92), 2.43 (T, 0.75-6.57), 1.71 (S, 1.14-3.10).
- Rapamune medication group showed less peribiliary fibrosis compared with control group (p=0.033) in contrast to tacrolimus (p=0.145).
Conclusion

- **Warm ischemic insult** may cause **delayed biliary complication**
- **Rapamune** has a **protective effect on bile duct damage and peribiliary fibrosis** compared with tacrolimus after warm ischemic insult although it has antiproliferative effect on bile duct
- Further study with more number of cases for longer period
- Further study about biomolecular mechanism of bile duct ischemic damage, delayed complication and protective effects of immunosuppressant
Thank you for your attention