Chronic Calcineurin Inhibitor Nephrotoxicity: Myth or Reality?

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Division Chief Nephrology
University of Wisconsin School of Medicine and Public Health
CNIs, Mechanism of Action

Samaniego, Becker, Djamali, Nature Clin Pract Nephrol, 12, 2006 VOL 2 NO 12
Calcineurin Inhibitors are the backbone of maintenance immunosuppression

2008 OPTN / SRTR Annual Report
The Natural History of Chronic Allograft Nephropathy


![Graph showing the natural history of chronic allograft nephropathy](image)

N Engl J Med 2003;349:2326-33
CsA associated with a 25% greater risk of ESRD than TAC
CNI nephrotoxicity: pathogenesis

Normal glomerulus

Glomerulus + CsA

Adapted from English et al. Transplantation 1987;44:135
Reducing Risk Exposure to Immunosuppressive drugs

- Avoidance
- Withdrawal
- Minimization
Minimizing Strategies

Ekberg, Halloran et al, Elite-Symphony Trial, NEJM 2007

KTR
N=1645

- CsA, MMF, P
- Daclimuzab
- CsA, MMF, P
- Daclimuzab
- TAC, MMF, P
- Daclimuzab
- SRL, MMF, P

Primary endpoint: 12M eGFR

- CsA: 57
- L-CsA: 59
- L-TAC: 65
- L-SRL: 56

Secondary Endpoint: 12M Rejection

- CsA: 30%
- L-CsA: 27%
- L-TAC: 15%
- L-SRL: 40%

P<0.05
Avoidance:
Belatacept eGFR higher than CsA in BENEFIT and BENEFIT-EXT trials

Withdrawal:
Everolimus-based, CNI-free regimen in recipients of de-novo kidney transplants: an open-label, randomized, controlled trial

Budde K et al, Lancet 2011; 377: 837–47
Identifying specific causes of kidney allograft loss

- Specific cause identified in 80%
- CNI only in 1%

Specific Causes of Kidney Allograft Loss

- ABMR (50%)
- Mixed Rejection (18%)
- Glomerulonephritis (11%)
- Medical/Surgical Conditions (7%)
- Polyoma Virus Nephropathy (9%)
- Probably ABMR (5%)

CNI nephrotoxicity was associated with improved Graft Survival

Long-term Deterioration in Kidney Allograft Function (DeKAF) Study

Gaston et al, Transplantation 2010;90: 68–74
The Histology of Solitary Renal Allografts at 1 and 5 Years After Transplantation

Stegall et al, AJT 2011; 11: 698–707

<table>
<thead>
<tr>
<th>Banff summary</th>
<th>All 1 yr</th>
<th>All 5 yr</th>
<th>p-Value2</th>
<th>Paired Bx (n = 296)</th>
<th>p-Value3</th>
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<td></td>
<td></td>
<td></td>
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<td>1 yr</td>
<td>5 yr</td>
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<tr>
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<tr>
<td>None</td>
<td>47% (n = 209)</td>
<td>38% (n = 130)</td>
<td>0.0347</td>
<td>46% (n = 136)</td>
<td>38% (n = 112)</td>
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<tr>
<td>Mild</td>
<td>40% (n = 178)</td>
<td>45% (n = 153)</td>
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<td>39% (n = 115)</td>
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<td>Mod-sev</td>
<td>13% (n = 60)</td>
<td>17% (n = 60)</td>
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<td>15% (n = 45)</td>
<td>19% (n = 55)</td>
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<tr>
<td>None</td>
<td>80% (n = 337)</td>
<td>40% (n = 136)</td>
<td>&lt;0.0001</td>
<td>81% (n = 227)</td>
<td>41% (n = 119)</td>
</tr>
<tr>
<td>Mild</td>
<td>17% (n = 71)</td>
<td>41% (n = 137)</td>
<td></td>
<td>17% (n = 47)</td>
<td>40% (n = 117)</td>
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<tr>
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<td>3% (n = 14)</td>
<td>19% (n = 65)</td>
<td></td>
<td>2% (n = 7)</td>
<td>19% (n = 56)</td>
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<td>None</td>
<td>98% (n = 437)</td>
<td>92% (n = 313)</td>
<td>&lt;0.0001</td>
<td>98% (n = 290)</td>
<td>92% (n = 273)</td>
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<tr>
<td>Mild</td>
<td>2% (n = 9)</td>
<td>3% (n = 9)</td>
<td></td>
<td>2% (n = 5)</td>
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<tr>
<td>Mod-sev</td>
<td>0% (n = 1)</td>
<td>6% (n = 20)</td>
<td></td>
<td>0% (n = 1)</td>
<td>6% (n = 18)</td>
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<tr>
<td>None</td>
<td>27% (n = 120)</td>
<td>28% (n = 95)</td>
<td>0.0969</td>
<td>27% (n = 79)</td>
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</tr>
<tr>
<td>Mild</td>
<td>60% (n = 267)</td>
<td>54% (n = 184)</td>
<td></td>
<td>59% (n = 174)</td>
<td>52% (n = 154)</td>
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<tr>
<td>Mod-sev</td>
<td>13% (n = 59)</td>
<td>18% (n = 63)</td>
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<td>15% (n = 43)</td>
<td>20% (n = 58)</td>
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<tr>
<td>None</td>
<td>58% (n = 258)</td>
<td>42% (n = 142)</td>
<td>&lt;0.0001</td>
<td>57% (n = 170)</td>
<td>41% (n = 120)</td>
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<tr>
<td>Mild</td>
<td>37% (n = 164)</td>
<td>42% (n = 143)</td>
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<td>38% (n = 111)</td>
<td>43% (n = 127)</td>
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<td>5% (n = 23)</td>
<td>16% (n = 56)</td>
<td></td>
<td>5% (n = 15)</td>
<td>17% (n = 49)</td>
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We conclude that these data support a revised concept of histologic injury after renal transplantation in which moderate-to-severe fibrosis and arteriolar hyalinosis may be avoided in the majority of renal allografts in the first 5 years after transplantation. In this view of chronic injury, mild fibrosis present at 1 year does not portend the development of more severe fibrosis by 5 years. Chronic, progressive histologic injury does indeed occur. However, these cases appear to be the result of specific incidents such as acute rejection, recurrent disease or polyoma virus infection. Thus, we suggest that efforts to improve long-term renal allograft function should focus on identifying and preventing specific causes of renal allograft injury rather than a general overhaul of our entire approach to patients and their immunosuppression. Although we are encouraged by these 5-year results, longer follow-up will be needed to determine whether these improved histologic trends will be maintained and will lead to increased long-term graft survival.

Stegall et al, AJT 2011; 11: 698–707
The Discrepancies between Mayo and Australian Studies

- SPK vs. Solitary Kidneys
  - Infections
  - Volume Contraction
- CsA vs. TAC
  - Different rejection rates and dosing
- Mayo
  - Low Risk
  - 95% Caucasian
  - 80% LD
  - DD better GFR than LD
  - No ECD

% lesions in protocol biopsies at 5 years

- Nankivell
- Stegall

66% 17% 19%
90%
Kidney Allograft Fibrosis and Atrophy Early After Living Donor Transplantation

Banff 97 scores of 0 (open bar), 1 (stippled bar), 2 (stripped bar), 3 (black bar). Top: LD kidney recipients; Bottom: DD kidney recipients

Cosio et al, AJT 2005; 5: 1130–1136
Recent studies suggest that the striking reduction in the incidence of acute rejection achieved in recent years has not been followed by an equally significant improvement in long-term graft survival (2). The results of this study suggest that the recent advances in immunosuppression have not controlled the development of kidney allograft fibrosis and atrophy thus may not improve graft survival much further. The identification of markers of fibrosis risk and the use of protocol biopsies may allow us to address this issue directly and early enough after transplantation when remedies may be effective.
Chronic Kidney Disease Stage Progression in Liver Transplant Recipients

Summary Part-I

• Chronic CNI nephrotoxicity may be less prevalent in the new era of immunosuppression
  – TAC > CsA
  – Lower doses
  – Alternative immunosuppressants

• However, CNIs are still the backbone of Immunosuppression
Calcineurin Inhibitor Nephrotoxicity

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*Department of Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium; †Department of Pediatrics, Stanford University School of Medicine, Stanford, California
Working Hypothesis

CNI

Nox2

Intrarenal Hypoxia

Fibrosis
Nox2
Oxidative stress as a common pathway to chronic tubulointerstitial injury in kidney allografts

Arjang Djamali
Department of Medicine, Nephrology Section, University of Wisconsin, Madison School of Medicine and Public Health, Madison, Wisconsin
Submitted 19 January 2007; accepted in final form 23 April 2007
Nox-2 Is a Modulator of Fibrogenesis in Kidney Allografts

Mycophenolic Acid May Delay Allograft Fibrosis by Inhibiting Transforming Growth Factor-β1-Induced Activation of Nox-2 Through the Nuclear Factor-κB Pathway

Arjang Djamali,1,2,3 Aparna Vidyasagar,1 Gokhan Yagci,1,2 Ling-Jin Huang,1,2 and Shannon Reese1
Cell culture Studies
Calcineurin inhibitors increased Nox2 mRNA in NRK52E cells

*\textit{p}<0.05 compared to no treatment

CsA increased Nox2 and TGF-β1-related proteins in NRK52E cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>Nox2</th>
<th>p-p38-MAPK</th>
<th>p-smad3</th>
<th>p-NFκB</th>
<th>α-SMA</th>
<th>β-actin</th>
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<td>TGF-β1 20 ng/mL</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CsA 1 μM</td>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CsA 10 μM</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*b-Protein:β-actin normalized ratios

* p<0.05 compared to Rx

Calcineurin inhibitors dedifferentiated epithelial NRK52E cells

No treatment                                  CsA 1 μM                               CsA 10 μM

TAC 1 μM                                   TAC 10 μM

Spindle-shaped cells

CsA-induced EMT and Nox2 protein expression were TGF-β1 dependent

Nox2 and CsA nephrotoxicity: animal models

Fisher 344

- Vehicle
- CsA 15 mg/kg/d
- CsA + Apocynin 16 mg/kg/d
- CsA + DPI 0.5 or 1 mg/kg/d

1 month

Renal Function

Fisher 344

n=8-10 in each group

Olive Oil

CsA (15mg/kg/24h)

Scr (mg/dL) at 1 month

p<0.05

Characteristics of chronic CsA nephrotoxicity

No CsA

High dose CsA

PAS

Trichrome

Afferent arteriole

Arteriolar hyalinosis

“Striped” fibrosis

Fibrosis and Nox2 in the Rat Model of CsA Nephrotoxicity

Vehicle vs CsA

Trichrome

(a) Vehicle

(b) CsA

Striped Fibrosis

α-SMA and Nox2 double-stain

(c) Vehicle

(d) CsA

Nox2

α-SMA

Inhibition of Nox2 was associated with reduced CsA-induced Fibrogenesis

(a) Immunoblots

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NoRx</th>
<th>CsA 15mg/kg/24h</th>
<th>Apo 16mg/kg/24h</th>
<th>DPI 0.5mg/kg/24h</th>
<th>DPI 1mg/kg/24h</th>
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<tbody>
<tr>
<td>Nox2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>α-SMA</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrotyrosine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>P-smad3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>T-smad3</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>GAPDH</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

(b) Protein levels normalized to GAPDH

* p < 0.05 CsA compared to all other groups
** p < 0.05 CsA compared to all except Apocynin-treated group

Inhibition of Nox2 was associated with reduced picrosirius staining

CsA Nephrotoxicity was reduced in Nox2−/− mice

a-Wild Type + CsA 30mg/kg/24h  b-Nox2−/− + CsA  c-Trichrome Staining

Interstitial fibrosis

Nox2 HNE α-SMA

WT KO

* p<0.05

## Liver Transplant Recipients with Biopsy-Proven Chronic CNI Toxicity

<table>
<thead>
<tr>
<th>Number of Patients</th>
<th>15</th>
</tr>
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<tbody>
<tr>
<td>Year of Transplant</td>
<td>1995-2005</td>
</tr>
<tr>
<td>Age at transplant (years)</td>
<td>54.5±2.6</td>
</tr>
<tr>
<td>White Race</td>
<td>13</td>
</tr>
<tr>
<td>Male Gender</td>
<td>8</td>
</tr>
<tr>
<td>Time to Biopsy (years)</td>
<td>5.4±0.8</td>
</tr>
<tr>
<td>Diabetes</td>
<td>8</td>
</tr>
<tr>
<td>HTN</td>
<td>14</td>
</tr>
<tr>
<td>Serum creatinine at biopsy</td>
<td>1.5±0.08</td>
</tr>
<tr>
<td>Proteinuria on UA &gt; 1+</td>
<td>5</td>
</tr>
<tr>
<td>Last serum creatinine</td>
<td>1.8±0.1</td>
</tr>
<tr>
<td>Follow-up after biopsy (years)</td>
<td>2.5±0.3</td>
</tr>
</tbody>
</table>

Nox2 was increased in human kidneys with CNI-induced fibrosis

a-Control Human Kidney
b-Chronic TAC Nephrotoxicity
c-Chronic CsA Nephrotoxicity

Nox2 is a Mediator of Chronic CsA Nephrotoxicity

A. Djamali\textsuperscript{a,b,*}, S. Reese\textsuperscript{a}, O. Hafez\textsuperscript{a}, A. Vidyasagar\textsuperscript{a}, L. Jacobson\textsuperscript{a}, W. Swain\textsuperscript{a}, C. Kolehmainen\textsuperscript{a}, L. Huang\textsuperscript{b}, N. A. Wilson\textsuperscript{a} and J. R. Torrealba\textsuperscript{c}
Summary Part-II

• TAC and CsA increased Nox2 expression in nonphagocytic cells
• Nox2 expression increased in human and animal model of chronic CsA-induced fibrosis
• Inhibition of Nox activity associated with
  – Reduced CsA-induced fibrosis
  – Prevention of CsA-induced hypoxia/hypoperfusion
• Nox2 is involved in pathogenesis of CNI-induced renal injury
Conclusions

• There is no specific biomarker of chronic CNI nephrotoxicity
• TAC and CsA at high dose can be nephrotoxic
• Animal studies support this evidence
• However, in transplant kidneys
  – The specific role of chronic CNI nephrotoxicity has yet to be defined
  – Balancing act: avoid chronic rejection vs. chronic “drug toxicity”
  – TAC better than CsA for GFR and maybe fibrosis
Acknowledgements

• Nephrology Lab
  – Nancy Wilson Schlei, PhD
  – Shannon Reese, MS
  – Lynn Jacobson, BS
  – Omeed Hafez
  – Zaheer Akhtar
  – Pierre Emmanuel Chammas
  – Ling-jin Huang

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  – Liz Sadowski, MD
  – Sean Fain, PhD

• Funding
  – R01 NIDDK 2011-16