Diabetes and Inflammasomes in the Bladder

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Disclosures: None
Introduction

• Diabetic Bladder Dysfunction (DBD) is the most common complication seen in diabetic patients.

• DBD is a progressive complication
  – Early DBD = irritative voiding symptoms  
    (diminished sensation, frequency, urge incontinence)
  – Chronic DBD = decompensated bladder  
    (insensate bladder, poor compliance, overflow incontinence)
Endocrine referral-DONE!!(?)

- Normalize blood sugars; stop diuresis
- 58% of patients achieve ADA goals\(^1\)
- DCCT found strict glycemic control decreased retinopathy, nephropathy. DBD not so much! \(^2,3,4\)
- What else can we do?

\(^2\) Genuth S Endocr Pract 2006; 12 Suppl 1:34-41
\(^3\) Sarma AV et al. Urology 2009; 73(6): 1203-9
Bladder Damage from Diabetes

- Neuropathy
- Smooth muscle dysfunction
- Urothelial (barrier) dysfunction
### DBD as manifestation of Peripheral Neuropathy

- **PN correlates with OAB in diabetic women**

<table>
<thead>
<tr>
<th>Variable</th>
<th>OAB syndrome</th>
<th>Without OAB syndrome</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>26 (32.5)</td>
<td>54 (67.5)</td>
<td></td>
</tr>
<tr>
<td>Polyneuropathy</td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>With</td>
<td>22 (27.5)</td>
<td>27 (33.8)</td>
<td></td>
</tr>
<tr>
<td>Without</td>
<td>4 (5.0)</td>
<td>27 (33.8)</td>
<td></td>
</tr>
<tr>
<td>Retinopathy</td>
<td></td>
<td></td>
<td>0.763</td>
</tr>
<tr>
<td>With</td>
<td>5 (6.3)</td>
<td>9 (11.3)</td>
<td></td>
</tr>
<tr>
<td>Without</td>
<td>21 (26.3)</td>
<td>45 (56.3)</td>
<td></td>
</tr>
<tr>
<td>Nephropathy</td>
<td></td>
<td></td>
<td>0.572</td>
</tr>
<tr>
<td>With</td>
<td>7 (8.8)</td>
<td>11 (13.8)</td>
<td></td>
</tr>
<tr>
<td>Without</td>
<td>19 (23.8)</td>
<td>43 (53.8)</td>
<td></td>
</tr>
</tbody>
</table>

DBD as manifestation of Peripheral Neuropathy

- **PN correlates with OAB in diabetic men**

<table>
<thead>
<tr>
<th>Variable</th>
<th>OAB syndrome</th>
<th>Without OAB syndrome</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>14 (35.0)</td>
<td>26 (65.0)</td>
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</tr>
<tr>
<td>Polyneuropathy</td>
<td></td>
<td></td>
<td>0.022*</td>
</tr>
<tr>
<td>With</td>
<td>11 (27.5)</td>
<td>10 (25.0)</td>
<td></td>
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<tr>
<td>Without</td>
<td>3 (7.5)</td>
<td>16 (40.0)</td>
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</tr>
<tr>
<td>Retinopathy</td>
<td></td>
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<td>0.115</td>
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<tr>
<td>With</td>
<td>3 (7.5)</td>
<td>1 (2.5)</td>
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</tr>
<tr>
<td>Without</td>
<td>11 (27.5)</td>
<td>25 (62.5)</td>
<td></td>
</tr>
<tr>
<td>Nephropathy</td>
<td></td>
<td></td>
<td>0.089</td>
</tr>
<tr>
<td>With</td>
<td>8 (20.0)</td>
<td>7 (17.5)</td>
<td></td>
</tr>
<tr>
<td>Without</td>
<td>6 (15.0)</td>
<td>19 (47.5)</td>
<td></td>
</tr>
</tbody>
</table>

## Rodent Studies

- **Decrease in myelinated nerve density**

<table>
<thead>
<tr>
<th>Time point</th>
<th>Group</th>
<th>Muscle NF200-IR area, mm²</th>
<th>Muscle NF200-IR/muscle area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>Control</td>
<td>0.110 ± 0.013</td>
<td>0.017 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.112 ± 0.005</td>
<td>0.013 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>Diuretic</td>
<td>0.114 ± 0.023</td>
<td>0.011 ± 0.002</td>
</tr>
<tr>
<td>9 week</td>
<td>Control</td>
<td>0.129 ± 0.007</td>
<td>0.019 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.112 ± 0.013</td>
<td>0.012 ± 0.001*</td>
</tr>
<tr>
<td></td>
<td>Diuretic</td>
<td>0.128 ± 0.013</td>
<td>0.012 ± 0.001*</td>
</tr>
<tr>
<td>20 week</td>
<td>Control</td>
<td>0.128 ± 0.001</td>
<td>0.021 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>0.108 ± 0.008</td>
<td>0.010 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>Diuretic</td>
<td>0.129 ± 0.004</td>
<td>0.011 ± 0.001</td>
</tr>
</tbody>
</table>

Rodent Studies

- Afferent nerve conduction velocity is decreased

DM and Smooth Muscle Dysfunction

- Smooth muscle contractility changes according to compensated versus de-compensated state

DM effects on urothelial barrier

Etiology of Diabetic Bladder Dysfunction

- Osmotic diuresis (polyuria)
- Hyperglycemia
Polyuria and hyperglycemia

- Diuresis causes bladder hypertrophy

Polyuria and hyperglycemia

- Diabetes/hyperglycemia, but not diuresis alone, leads to oxidative stress

What is the molecular mechanism?

• Brownlee’s Unified Theory

• Inflammation
Brownlee’s Unified Theory of Diabetic Complications
Inflammation

Diabetic metabolites
- uric acid
- lipids

ROS
- mitochondrial dysfunction

K+ cellular efflux

NLRP3 Activation

INFLAMMATION
The NLRP3 Inflammasome

Canonical Pathway - Activation

Activating DAMPs and PAMPS

ROS

K⁺ efflux

NLRP3 Inflammasome

NEK7

Ca²⁺

IP₃

ER

Ca²⁺

NLRP3 mediates DM complications

- Retinopathy
- Nephropathy
- Cardiomyopathy
- Neuropathy
- Endothelial dysfunction
Inflammasomes in the bladder?

- NLRP3 found in human bladder (Tschopp 2007)
- Rat urothelium (Hughes 2014)

And in the mouse!!

Wild Type

Diabetic

NLRP3
NLRP3 in urothelium
Inflammasomes and sterile cystitis

- NLRP3 mediates inflammation in cyclophosphamide induced cystitis and BOO

- NLRP3 inhibitor (glyburide) prevents inflammation→preserves tissue and function

Does NLRP3-induced inflammation lead to DBD?
Diabetic metabolites activate NLRP3
Akita Mouse Model

- Type 1 DM mouse
- Heterozygous Ins2 mutation
Materials and Methods

• Female Ins2 (Akita) mice were compared to age-matched controls for the following end-points:
  
  • Voiding Dysfunction: Cystometry
  
  • Active Caspase-1 Activity: FAM-FLICA assay
    o Surrogate for activated NLRP3 inflammasome
  
  • Inflammation: Evans Blue extravasation assay
DBD Occurs by Week 15 in Akita Mice

Wildtype Mouse Bladder Pressure Tracing

Diabetic Mouse Bladder Pressure Tracing
DBD Occurs by Week 15 in Akita Mice

**Void Volume**

- Wildtype: 160 ± 10 µL
- Diabetic: 60 ± 5 µL

**Frequency**

- Wildtype: 8 ± 2 voids/hr
- Diabetic: 10 ± 2 voids/hr

*Significant difference (p < 0.01)
Increased Caspase-1 Activity at 15 Weeks

**Active Caspase-1**

<table>
<thead>
<tr>
<th></th>
<th>Mean Fluorescence Intensity (RFUs)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wildtype</strong></td>
<td><img src="wildtype_bar_chart.png" alt="Red Bar Chart" /></td>
</tr>
<tr>
<td><strong>Diabetic</strong></td>
<td><img src="diabetic_bar_chart.png" alt="Blue Bar Chart" /></td>
</tr>
</tbody>
</table>

*Significant difference between Wildtype and Diabetic groups.*
Increased Bladder Inflammation at Week 15

Evans Blue Extravasation

Concentration in Bladder (ng/mg)

Wildtype

Diabetic

*
DBD in Akita Mice

- Early DBD appears at 15 weeks in Akita mice

- Early DBD associated with
  - Inflammation
  - Activation of Caspase-1
So what happens without NLRP3?

Akita diabetic mouse with NLRP3 knocked out

Akita/NLRP3 KO
Akita/NLRP3 KO
- No change in hyperglycemia
**Akita/NLRP3 KO**
- DBD is attenuated

### Void Volume

- NLRP3: wt KO wt KO
- Diabetic

### Frequency

- Micturitions/hr

### PVR

- wt KO wt KO
- Diabetic
Akita/NLRP3 KO
- Nerve density is preserved
NRP3 inflammation and DBD

High Glucose

DAMPs

NLRP3

IL-1β

Neuropathy

Smooth muscle hypertrophy

Urothelia dysfunction

DBD
NLRP3 and Neuropathy

• Rat BOO model
• BOO (pressure, stretch, hypoxia) activates NLRP3
• Decrease in bladder nerve density
• IL1β causes neuronal apoptosis
NLRP3 Mediates Denervation during BOO

Conclusion

The NLRP3/IL-1β pathway mediates denervation during BOO

# Nerves

Total number of Nerves

Bladder Area

IL-1β (ng/ml)

Pelvic Ganglion Nerves

Apoptosis

Con

Nerve Density

DAMPs (ATP)

Inflammasome

Pyroptosis

Pro-IL-1β

Casp-1

Adaptor

IL-1β

Pelvic Ganglion Nerv

Control

Sham

Veh

Gly

Ana

IL-1β

Apoptosis

High pressure

Stretch

Hypoxia/reperfusion

NLR

IL-1β Induces Apoptosis in Pelvic Neurons in vitro
Future Work

• NLRP3 impact on:
  – Neuropathy
  – Smooth muscle dysfunction
  – Urothelium/barrier function

• Natural course: DM vs. DM/NLRP3^-/

• NLRP3 inhibition as adjunct to Glycemic control
Acknowledgements