Recent update on LUTS/BPH  
(Korean experience of basic field)

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LUTS

- Intravesical instillation of human urine after oral administration of trospium, tolerodine and oxybutinin in a rat model of detrusor overactivity (Kim Y et al, BJU Int 2006;97:400–3)
- Nerve growth factor and prostaglandins in the urine of female patients with overactive bladder (Kim JC et al, J Urol 2006;175:1773–6)
- Antimuscarinic agents exhibit local inhibitory effects on muscarinic receptors in bladder–afferent pathways (Kim Y et al, Urology 2005;65:238–42)
- Alterations in voiding frequency and cystometry in the clomipramine induced model of endogenous depression and reversal with fluoxetine (Lee KS et al, J Urol 2003;170:2067–71)
- Effect of (+/−)-epibatidine, a nicotinic agonist, on the central pathways controlling voiding function in the rat (Lee SJ et al, Am J Physiol Regul Integr Comp Physiol 2003;285:R34–90)
BPH

- Effect of 5alpha-reductase inhibitor in expression of transforming growth factor-$\beta$1 in benign prostatic hyperplasia patients
  (Kim HW et al, Kor J Urol 2006;47:1178–84)

- Induction of apoptosis by $\alpha$1-adrenoceptor antagonists in benign prostatic hyperplasia
  (Lee HI et al, Kor J Urol 2003;44:643–48)

- Expression of neuroendocrine cells in benign prostatic hyperplasia and the effect of dihydrotestosterone
  (Hogn SJ et al, Kor J Urol 2003;44:267–71)

- Effects of hOGG1 associated with the aging process on the development of benign prostatic hyperplasia
  (Eom MS et al, Kor J Urol 2002;43:502–7)

- Induction of prostate apoptosis by low dose terazosin in benign prostatic hyperplasia
  (Jeon SS et al, Kor J Urol 2000;41:1051–6)
LUTS/BPH

Effect of partial outlet obstruction and its relief on type I and III collagen and detrusor contractility in the rat
(Kim JC et al, Neurouro and Urodyn 2000,19:29–42)

▪ In vivo hepatocyte growth factor gene transfer to bladder smooth muscle after bladder outlet obstruction in the rat: A morphometric analysis
(Ku JH et al, J Urol 2006;176:1230–5)

▪ Nerve growth factor and vanilloid receptor expression and detrusor instability after relieving bladder outlet obstruction in rats
(Kim JC et al, BJU Int 2004;94:915–8)

▪ Changes of urinary growth factor and prostaglandins in male patients with overactive bladder symptoms

▪ Relative proportions of tissue components in the prostate: Are they related to the development of symptomatic BPH in Korean men?
(Byun SS et al, Urology 2005;66:593–6)

▪ Changes in the expression of smooth muscle heavy chain mRNA following partial bladder obstruction or spinal cord injury in rat: A preliminary study
(Byun Y et al, Kor J Urol 2007;48:522–6)

▪ Effects of connexin expression on detrusor overactivity in male patients with bladder outlet obstruction caused by benign prostatic hyperplasia
(Cha SH et al, Kor J Urol 2004,45:897–902)

▪ The change of detrusor collagen according to the detrusor contractility in benign prostatic hyperplasia
(Kim YJ et al, Kor J Urol 2004,45:535–42)

▪ Three-dimensional arrangement of muscle fibers, collagen and elastin fibers of the proximal prostatic urethra in benign prostatic hyperplasia
(Lee KS et al, Kor J Urol 2002;43:508–12)
Effect of partial outlet obstruction and its relief on type I and III collagen and detrusor contractility in the rat

(Kim JC et al, Neurouro and Urodyn 2000, 19:29-42)

Introduction

- A partial bladder outlet obstruction (BOO)
  - increased bladder mass and collagen deposition
  - rapid hypertrophy
  - decreased contractile property of detrusor muscle

- Collagen is the major constituent of the extracellular matrix in bladder.
  - influence of the passive property of bladder wall
  - role in intercellular transmission of active force
  - Type I collagen provides the tensile strength of the tissue.
  - Type III collagen is associated to the striated fibrils of stromal matrix.
Objectives

- Effects of BOO and its relief on type I and type III collagen
- Relationship between contractility and changes in collagen types
Materials and Methods

- 40 Sprague–Dawley female rats

- Five groups – control
  - 6 wks of BOO
  - 2 wks after relief of BOO
  - 4 wks after relief of BOO
  - 6 wks after relief of BOO

- Bladder mass
  - Muscle strip study
  - Collagen determination
  - Immunohistochemical staining
  - RNA isolation
  - Northern Blot analysis
Results

Bladder mass
- Bladder mass increased after 6 weeks of BOO.
- Bladder mass decreased after relief.
- Bladder mass did not return to the preobstruction level.

Fig. 1. Changes of bladder weight in control, obstruction (obst) and 2, 4, and 6 weeks (6 weeks) after the relief of obstruction. *P < 0.05, compared with control value. +P < 0.05, compared with obstruction value.
Muscle strip study

- Contractility decreased after 6 weeks of BOO.
- Contractility did not change 2 wks after relief.
- Contractility changed 4,6 weeks after relief. However, it did not return to the preobstruction level.

![Graph showing contractile response](image-url)
Collagen

- Total amount
  - increased 6 weeks after BOO
  - decreased after relief
  - Contractility decreased with increasing total amount.

- Concentration
  - decreased after 6 weeks after BOO
  - increased after relief
  - Contractility increased with increasing concentration.

### TABLE I. Total Collagen and Collagen Concentration in Control, Obstruction and 2, 4 and 6 Weeks After the Relief of Obstruction

<table>
<thead>
<tr>
<th>Group</th>
<th>Total collagen (mg)</th>
<th>Collagen concentration (µg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 8)</td>
<td>5.2 ± 0.2</td>
<td>44.0 ± 16</td>
</tr>
<tr>
<td>Obstruction (n = 8)</td>
<td>7.3 ± 0.4*</td>
<td>34.6 ± 1.5*</td>
</tr>
<tr>
<td>2 weeks after relief</td>
<td>6.0 ± 0.7</td>
<td>37.0 ± 2.0</td>
</tr>
<tr>
<td>4 weeks after relief</td>
<td>6.2 ± 0.5</td>
<td>37.2 ± 1.3</td>
</tr>
<tr>
<td>6 weeks after relief</td>
<td>5.4 ± 0.5**</td>
<td>40.9 ± 1.2**</td>
</tr>
</tbody>
</table>

*P = 0.05, compared with control value.

**P < 0.05, compared with obstruction value.

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![Graph A](image1.png)

**Fig. 3** Regression analysis for the effect of collagen content on the bladder muscle contractility. A. The effect of total collagen amount on the contractility to 32 Hz field stimulation. B. The effect of collagen concentration on the contractility to 32 Hz field stimulation.
Immunohistochemical staining

- Normal bladder
  - Types I, III collagen are located in lamina propria.
- BOO bladder
  - Types I, III collagen are located between smooth muscle bundles.
- After relief
  - Deposition of types I, III collagen returned to control.
Analysis of type I and III collagen mRNA

- Upregulation of pro-α1(I), α1(III) gene expression after BOO
  - After relief, Upregulation converted to downregulation.
- Contractility decreased with increasing pro-α1(I), α1(III) gene expression.
Conclusions

- BOO and its relief affect on changes of localization, quantity of collagen types, content of collagen.

- The changes in the collagen types has an impact on the detrusor contractility.
In vivo hepatocyte growth factor gene transfer to bladder smooth muscle after bladder outlet obstruction in the rat: A morphometric analysis (Ku JH et al, J Urol 2006;176:1230–5)

Introduction

- Accumulation of ECM after BOO
  - Deposition of collagenous proteins, matrix constituents
  - Effects on smooth muscle function, capacity of bladder

- HGF
  - Angiogenic factor that shows antiapoptotic properties
  - Activation of matrix degradation
  - Antifirotic effect
Objectives

HGF gene transfer after 2 weeks of BOO
- decreasing TGF-β expression in BOO rat bladder
- decreasing collagen deposition in BOO rat bladder
Material and Methods

- **Animals**
  - 10 week old male SD rats

- **Group 1** – sham operation
  - Group 2 – BOO for 4 weeks
  - Group 3 – HGF gene transfer 2 weeks after BOO

- Recombinant human HGF

- Histology and histomorphometric image analysis

- RNA extraction and RT–PCR

- Western blot analysis
Results

Bladder weight
- BOO effected on increase of bladder weight.
Morphological analysis

- **Group 1** – collagen fiber predominance in LP, interfascicular area
- **Group 2** – muscle hypertrophy, increased concentration of collagen fiber
- **Group 3** – decreased in collagen fiber in LP, interfascicular area

Fig. 2. A, in sham operated rat bladder collagen (arrow) fiber staining pattern was predominant in lamina propria and interfascicular region rather than in pericellular area. B, in partial BOO rat bladder note detrusor muscle thickening and increased collagen content in lamina propria and interfascicular area. C, in partial BOO rat bladder treated with BFG gene transfer technique bladder smooth muscle hypertrophy was not normalized by BFG gene transfer treatment but collagen content was markedly decreased in lamina propria and interfascicular area. Red arrows indicate smooth muscle. Blue areas indicate connective tissue. Left, H & E, reduced from ×40. Right, Masson’s trichrome stain, reduced from ×200.
RT-PCR

- Increased expression of HGF and c-met mRNA in group 3

- Increased expression of TGF-β1 mRNA expression in group 2
  - Decreased expression of TGF-β1 mRNA expression in group 3

![RT-PCR Image]

Fig. 4. HPG, TGF-β1 and c-met mRNA in detrusor smooth muscle of sham operated (Lane 1), partial BOO (Lane 2) and HPG gene transfer treated (Lane 3) bladders. Specific primers were used to amplify cDNA fragments by RT-PCR. Protected RNA fragments were analyzed on denaturing polyacrylamide gels and dried gels were exposed to autoradiography film. Bands represent specific cDNA amplification products of mRNA. All PCR products were sequenced to confirm identity. Lane M, 1 µg of 100 bp DNA ladder used as molecular size standard.
Western blot analysis

- Increased expression of HGF, c–met in group 3

- Increased expression of TGF–β1 in group 2
  - decreased expression of TGF–β1 in group 3

Fig. 5. Protein analysis of HPG, TGF–β1 and c-met in detrusor smooth muscle of sham-operated (Lane 1), partial BOO (Lane 2) and HPG gene transfer treated (Lane 3) bladders. Total protein extracts from each tissue sample were loaded onto highly porous polyacrylamide gels and α-smooth muscle actin was control gene product. Lane M, 200 kDa marker.
Conclusions

HGF may be one of the more promising candidates as an agent for preventing bladder fibrosis after BOO.
Nerve growth factor and vanilloid receptor expression and detrusor instability after relieving bladder outlet obstruction in rats (Kim JC et al, BJU Int 2004;94:915–8)

Introduction

- Irritative symptoms persist after relief of BOO.
- Alterations in the intrinsic properties of bladder smooth muscle
- Changes in the neuronal control of the bladder
- Changes in the afferent nerves
  - NGF is essential protein in the development of the PNS.
  - Neuronal interactions leading to neural plasticity
- TRPV1 is expressed by the both of afferent nerves and urothelial cells.
- Alterations in NGF and TRPV1 expression
  - They influence the persistent irritative symptoms after relief of BOO.
Objectives

- NGF and TRPV1 expression in the bladder after relief of BOO
- Functional changes of the bladder
Materials and Methods

- BOO was induced in 40 male Wistar rats.
- Sham operation was performed in male Wistar 15 rats.
- Obstructuion relieved 3 weeks after BOO.
- Cystometry was performed 3 weeks after relief.
- Measurement of weight, NGF, TRPV1 of bladder
Results

- CMG of BOO – 10/40 unstable bladder
  30/40 normal bladder
- Bladder weight in BOO was higher than in the control group.
- Bladder contraction pressure was similar in control and BOO.
- Contraction interval was markedly shorter in the unstable group.

FIG. 1.
Cystometrograms from the control (top), normal (middle) and unstable (bottom) bladder groups. The contraction interval was shorter in the unstable group (arrows indicate unhibited contraction).
mRNA expressions of NGF and TRPV1 increased in the unstable group.

FIG. 2: RT-PCR measurements in bladder of a. NGF mRNA expression and b. TRPV1 mRNA expression. In control (Lane 1), normal (Lane 2), and unstable (Lane 3) groups. GAPDH was used as the reference gene. M: 100 bp marker. NGF and TRPV1 mRNA expressions were higher in the unstable than in the control and normal groups.
Conclusions

NGF and TRPV1 were increased in unstable bladder tissue after relieving BOO.
- These changes may be related to irritative symptoms after correction of BOO.

Introduction

- High prevalence of LUTS in Korean men with a small prostate
- Proportions of histologic components of prostatic adenoma in patients with symptomatic BPH.
Objectives

Investigate whether the histologic components of prostatic adenoma is related to symptomatic BPH in Korean men
Materials and Methods

- Group I  20–40cc
  Group II  41–80cc
  Group III  ≥80cc

- Immunohistochemistry and computer aided morphometric analysis

- The proportion of stroma and smooth muscle, collagen I,II,III,V, fibronectin, laminin in stromal tissue were determined.
Results

- Proportions of the smooth muscle, collagen, fibronectin, laminin were not significantly different in three groups.

- Proportion of stroma and relative smooth muscle of the prostatic adenoma in group 1,2 was significantly greater than in group 3.

<table>
<thead>
<tr>
<th>Component</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroma in prostatic adenoma</td>
<td>79.0 ± 8.9</td>
<td>77.7 ± 9.9</td>
<td>63.2 ± 6.4</td>
<td>0.008</td>
</tr>
<tr>
<td>Smooth muscle in stroma</td>
<td>38.4 ± 4.3</td>
<td>37.5 ± 5.2</td>
<td>36.1 ± 2.8</td>
<td>0.45</td>
</tr>
<tr>
<td>Total collagen</td>
<td>17.70 ± 5.57</td>
<td>21.64 ± 8.41</td>
<td>16.76 ± 7.89</td>
<td>0.06</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>4.90 ± 4.66</td>
<td>4.73 ± 4.15</td>
<td>6.52 ± 4.80</td>
<td>0.48</td>
</tr>
<tr>
<td>Laminin</td>
<td>10.84 ± 6.33</td>
<td>9.72 ± 6.94</td>
<td>7.28 ± 5.17</td>
<td>0.25</td>
</tr>
<tr>
<td>Relative smooth muscle proportion in prostatic adenoma</td>
<td>30.3 ± 5.3</td>
<td>29.1 ± 5.3</td>
<td>22.8 ± 3.9</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Conclusions

Dynamic component of bladder outlet obstruction, which is mediated by smooth muscle tone, may play a more important role in the development of symptomatic BPH in Korean men.
Changes of urinary growth factor and prostaglandins in male patients with overactive bladder symptoms

Introduction

- **NGF**
  - Increased expression in the bladder may contribute irritative symptoms in patients with LUTS

- **PG**
  - promotion by the expansion of detrusor muscle at the time of bladder inflammation, damage to bladder mucosa
  - decreasing the threshold of stimuli necessary to trigger the bladder contraction
Objectives

To evaluate the alterations of urinary NGF and PG level in male patients with OAB
Materials and Methods

- Patients
  - 75 male patients with OAB
  - 20 control male patients

- Urine collection

- Patients evaluation

- ELISA for NGF and PG
Results

- Increased NGF

- Increased PG$_2$
  - PG$_{2\alpha}$, PGI$_2$ were not changed significantly.
  - PGE$_2$ has more important role in the lower urinary tract function

<table>
<thead>
<tr>
<th>Group</th>
<th>NGF (ng/mL)</th>
<th>PGE$_2$ (ng/mL)</th>
<th>PGF$_{2\alpha}$ (ng/mL)</th>
<th>PGI$_2$ (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.03 ± 0.43</td>
<td>1.15 ± 0.20</td>
<td>0.99 ± 0.18</td>
<td>1.83 ± 0.52</td>
</tr>
<tr>
<td>OAB patients</td>
<td>7.67 ± 7.50*</td>
<td>1.91 ± 0.18*</td>
<td>1.40 ± 0.22</td>
<td>1.49 ± 0.18</td>
</tr>
</tbody>
</table>

*$P < 0.05$ as compared with control. NGF, nerve growth factor; PG, prostaglandin.

Fig. 2 Correlation between urinary PGE$_2$ level and maximum cystometric capacity in OAB patients. The urinary level of PGE$_2$ was negatively correlated with maximum cystometric capacity in OAB patients. $r = -0.321$; $P < 0.05$. OAB, overactive bladder.
Conclusions

Elevated level of urinary NGF and PGE2 can be associated with clinical symptoms of BPH.
Changes in the expression of smooth muscle heavy chain mRNA following partial bladder obstruction or spinal cord injury in rat: A preliminary study (Byun Y et al, Kor J Urol 2007;48:522–6)

Introduction

- **SMMHC** isoforms combination has been researched in BOO model.
  - C-terminal proteins
    - SM1
    - SM2: mainly found in bladder
  - N-terminal proteins (ATP coupling area for SM contraction)
    - SM–A: tonic contraction mainly found in bladder
    - SM–B: phasic contraction mainly found in urethra
Materials and Methods

- 15 female SD rats
  - Control (n=5)
  - BOO induced (n=5)
  - SCI (n=5)

- 6 weeks after BOO, bladder were excised.

- RT-PCR
  - Expression of SMMHC isoforms at C-terminal (SM1, SM2)
  - Expression of SMMHC isoforms at N-terminal (SM-A, SM-B)
Results

BOO: decreased SM1 expression

Fig. 2. Representative reverse transcriptase-polymerase chain reaction (RT-PCR) amplification and density analysis for the SM1 and SM2 expression in the rat urinary bladders of the control (C), the spinal cord injured group (S), and the partial obstruction group (P). The SM1 expression in the partial obstruction and spinal cord injury groups is significantly lower when compared to the controls (*p < 0.05). The SM1 expression in the spinal cord injury rats is significantly lower when compared to the partial obstruction group (**p < 0.05). The density data is given in means ± SEMs.
- BOO: decreased SM-A expression

**Fig. 3.** Two representative reverse transcriptase-polymerase chain reaction (RT-PCR) amplifications for the SM-A and SM-B expression in the rat urinary bladders of the control (C), the spinal cord injured group (S), and the partial obstruction group (P).
Conclusions

Changes of expression of SMMHC isoforms