Fetal Obstructive Uropathy in Sheep Model

Hiroaki Kitagawa
St. Marianna University School of Medicine

Kawasaki, Japan
Kawasaki Motor Cycle?
Kawasaki Motor Cycle?
Kawasaki Disease?

- Dr. Tomisaku Kawasaki who discovered KAWASAKI disease in 1967.
- No relationship to Kawasaki prefecture.
Kawasaki Disease?

- Dr. Tomisaku Kawasaki who discovered KAWASAKI disease in 1967.
- No relationship to Kawasaki prefecture.
Long term follow up of antenatally detected hydronephrosis

Kevin C. Pringle2), Hiroaki Kitagawa1), Yasuji Seki1), Shinichiro Fujiwaki3), and Koonosuke Nakada1).

Division of Pediatric Surgery, St. Marianna University School of Medicine 1).
Department of Surgery and Anaesthesia and Obstetrics and Gynecology, Wellington School of Medicine and Health Science, Wellington New Zealand 2).
Department of Obstetrics and Gynecology, St. Marianna Yokohama City Seibu Hospital3).
Materials and Methods:

Babies with Hydronephrosis detected antenatally from 1990 to 1997 were followed up to 2004 at Wellington Hospital. Retrospectively, renal pelvis diameter $\geq 4\text{mm}$ before 33 weeks gestation or $\geq 7\text{mm}$ after 33 weeks gestation are diagnosed as Hydronephrosis.
Follow up Protocol

1. Postnatal U/S
   Four days after birth to avoid false-negative
2. MCU
   megaureter were investigated
3. MAG3 or $^{99m}$Tc-DMSA
   U/S appears deteriorated
Postnatal diagnosis of antenatally detected renal abnormality

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>No organic obstruction</td>
<td>108</td>
</tr>
<tr>
<td>Obstructive uropathy</td>
<td>24</td>
</tr>
<tr>
<td>VUR (primary)</td>
<td>10</td>
</tr>
<tr>
<td><strong>MCDK</strong></td>
<td>9</td>
</tr>
<tr>
<td>Duplex kidney</td>
<td>6</td>
</tr>
<tr>
<td>Megaureter</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>161</strong></td>
</tr>
</tbody>
</table>
# Obstructive uropathy

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ureterocele</td>
<td>9</td>
</tr>
<tr>
<td>PUJ</td>
<td>8</td>
</tr>
<tr>
<td>PUV</td>
<td>5</td>
</tr>
<tr>
<td>VUJ</td>
<td>1</td>
</tr>
<tr>
<td>Urethral atresia</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>24</strong></td>
</tr>
</tbody>
</table>
## Surgical cases

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remove kidney</td>
<td>11</td>
</tr>
<tr>
<td>Cohen reimplant</td>
<td>10</td>
</tr>
<tr>
<td>Unroofing of ureteric orifice</td>
<td>4</td>
</tr>
<tr>
<td>Anderson Hynes Pyeloplasty</td>
<td>8</td>
</tr>
<tr>
<td>Ablation for PUV</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>37</strong></td>
</tr>
</tbody>
</table>
Antenatally diagnosed renal diseases with respect to age at diagnosis. Surprisingly, more than 1/3 can diagnosed before 20 weeks gestation.
Late diagnosed patients have high chance to become normal appearance from this graph.
Outcome of Hydronephrosis without obstruction identified with respect to age at diagnosis

- <20 weeks: 22 (17 Become normal, 22 Still hydronephrosis)
- 21-30 weeks: 14 (14 Become normal, 16 Still hydronephrosis)
- >30 weeks: 16 (11 Become normal, 16 Still hydronephrosis)
Outcome of Hydronephrosis without organic obstruction plotted by year after birth. Seventy percents of patients become normal in appearance within 3 years time. If you focus on blue graph (Become normal group), then 87% become normal in 3 year time.
This is the group of patients still have hydronephrosis 5 year after birth plotted by year after birth. Twenty one patients still have hydronephrosis without organic obstruction. This group might change to normal appearance but the chance is small. The longest duration is 9 year from previous slides.
Hydronephrosis kidneys monitored with serial U/S studies. Open circle whose renal pelvis became normal in 5 years. Triangle patients whose hydronephrosis persisted over 7 years. Then patient increased the size before and after 33 weeks but 7 become normal after birth but 2 from this group had obstructive uropathy. If you have continuous dilatation, some of this group had obstruction but if the diameter decreased in size during pregnancy, this group doesn’t have obstructive uropathy from our data.
Results:

1. One hundred and sixty one patients were diagnosed antenatally and 108 patients (67%) had hydronephrosis without organic obstruction.

2. Nineteen patients had a normal ultrasound after birth and 70 % of the patients with a non-organic hydronephrosis became normal.
Results:

3. Eighty seven percent became normal by 3 years of age. There were four patients whose hydronephrosis took more than 5 years to become normal.

4. The longest a patient took to develop normal kidneys on U/S scan was 9 years. Seven patients whose kidney became normal dilated again, usually at about 5 years of age. All of these 7 have normal renal function on MAG 3.
Results:

5. Some infants with hydronephrotic kidneys were monitored with serial U/S studies before and after 33 weeks gestation.

6. Seven of the ten in whom the diameter of pelvis increased in utero, became normal after birth and two had organic obstruction requiring surgical correction. Only 1 of the 8 in whom the diameter decreased still has hydronephrosis and none had organic obstruction. A total of 11 patients (18%) required an operation.
Results:

7. The longest a patient took to develop normal kidneys on U/S scan was 9 years. Seven patients whose kidney became normal dilated again, usually at about 5 years of age. All of these 7 have normal renal function on MAG 3.
Conclusions:

The majority of hydronephrosis patients without organic obstruction were managed conservatively (70%) and become normal within 3 years time (87%) but difficult to select the surgical cases from antenatal scan.
Introduction

- MCDK is the most common form of renal dysplasia but the pathogenesis is unclear.

- We attempted to determine the origin of the cystic component in the MCDK using immunohistochemical stains and transmission electron microscopy.
Methods
(Creation of Obstructive Uropathy)
Surgical Procedures (Female)

Ligation of Urechus

Ligation of Bladder Neck
Surgical Procedures (60 days lambs)

Ligation of Penile Urethra (Male)

Ligation of Bladder Neck (Female)
Materials and Methods

An obstructive uropathy was created in fetal lambs at 60 days gestation by ligating the urethra and urachus. They were delivered 20-31 days later by caesarian section. The kidneys were processed for histological examination.
Macroscopic findings:

- **Control**: 1 cm
- **Type A**: 1 cm
- **Type B**: 1 cm
- **Type C**: 1 cm
Multicystic Dysplastic Change (MCDK)
Classified as having large cysts, small cysts and no cysts
Early Effects after Urinary Tract Obstruction
Materials and Methods

An obstructive uropathy was created in fetal lambs at 60 days gestation by ligating the urethra and urachus. They were delivered 2-7 days later by caesarian section. The kidneys were processed for histological examination.
Early Effects after Urinary Tract Obstruction

- 48 hours
- 3 Days
- 5 Days
- 7 Days
Conclusion

The first the areas in the developing kidney that suffer damage after obstructive uropathy are the nephrogenic zone and proximal tubule. This change started 3 days after obstruction. We conclude that shunting procedures need to be performed considerably earlier than we had previously thought.
Effect of Vesico-amniotic Shunt for Fetal Obstructive Uropathy in Lambs

St. Marianna University School of Medicine, Division of Pediatric Surgery
Hiroaki Kitagawa

Wellington School of Medicine
Kevin C. Pringle
Materials and Methods

An obstructive uropathy was created in fetal lambs at 60 days gestation. Vesicostomy (female) or urethrostomy (male) were performed 21 days later to release the pressure. Fetuses were sacrificed at term (145 days) and the urinary tract was removed for histology.

60 days  
\[\text{Obstruction}\]  
81 days  
\[\text{Shunt Op.}\]  
145 days  
\[\text{TERM}\]
Shunt Operation

Vesicostomy in a female lamb

Urethrostomy in male
In normal term lambs the right kidneys weighed $16.54 \pm 1.80\text{g} (n=5)$ and the left kidneys weighed $16.52 \pm 2.29\text{g} (n=5)$.

In the oligonephronia type of shunted kidneys, the right kidneys weighed 3g and left kidneys weighed 4.6g.
Shunt model Without Cyst (#71)
Mild pelvic Dilatation and Normal Renal Histology
Mild pelvic Dilatation and Normal Renal Histology
Shunt model Without Cyst (#71)
Conclusions (1)

- In utero urinary tract obstruction causes reduction of numbers of functioning nephrons and produces cysts in the nephrogenic zone, and in the deeper cortex.

- These cysts and dilated proximal tubules suppress new nephron formation.
Results (2)

- Successful shunt with a small bladder (8±5ml).
- The histology demonstrated relatively well-preserved renal architecture with reduced nephron mass (**oligonephronia**) in two lambs and multicycstic dysplastic change (**MCDK**) in three. Six (55%) had **normal nephrogenesis**.
Conclusions (3)

- Twenty days after obstruction there were early features of dysplasia but the **nephrogenic zone** was still present.

- We conclude that **early shunting may salvage renal function.**
Dilated proximal tubules
Shunt

Timing is important!
Shunt

Timing is important!
Obstructive Uropathy

Type A

Type B

Type C
Conclusions

Our shunt results are similar to those achieved in humans.
It is still unclear why different phenotypes result after shunting identical models of obstructive uropathy.
We conclude that shunting prevented development of cystic dysplasia in some lambs but half of the lambs had oligonephronia or MCDK. This group might develop renal failure later in life.
What happen to the Bladder?

Do we able to preserve the bladder function after shunting?

Why the bladder capacity become very small after shunting?

Is shunt operation doing good for the bladder?
Macroscopic Findings of Bladder

Huge Dilated Bladder

Atrophic Bladder after Shunting
Bladder histology after obstructive uropathy

- Control
- Partial Obstruction
- Dilated Bladder
- Shunt Model
Masson’s Trichrome stain of the bladder

A: Control

B: Partial Obstruction

C: Complete Obstruction

D: Shunt Model
Discussion

1. Normal voiding and renal function after birth is important to maintain a good QOL.

2. Do we have to do bladder augmentation after shunting operation?
Discussion

1. Once the bladder becomes severely dilated, the bladder wall becomes severely fibrotic.

2. In our model, the extremely dilated bladders had already developed a very dense layer of submucosal fibrosis.

3. If this bladder is shunted, the submucosal fibrosis becomes even more severe, resulting in a small-volume bladder with extremely poor compliance.
Conclusion:

- Shunt operations after obstructive uropathy fail to preserve bladder function.

- We conclude that the fetus needs the urination cycle for normal bladder development. This requirement exists even when the obstruction is successfully bypassed.
A monitor system using a Pressure Amplifier (Model:PA-100) connected to a 3 French (3F) catheter form of pressure transducer 3French (Model:AS301, Star Medical Inc. Japan, Web:http://www.starmedical.co.jp) through the extension cable from the bladder to produce the pressure-volume curve.
The pressure-volume curve of a normal term control lamb. The pressure began to increase between 3-4 minutes (30-40ml) after commencing the injection of normal saline into the bladder. At five minutes after beginning the infusion, the pressure remained at around 40 mmHg as the size of bladder gradually increased.
A pressure-volume curve of a V-A shunt bladder. The pressure in this bladder began to rise very steeply after the infusion of only 8 ml of saline, reaching 60 mmHg within 30 seconds of commencing the infusion.

The compliance curve of another V-A shunt model. The bladder volume was 8 ml. One minute after commencing the infusion of normal saline into the bladder, the bladder pressure rose to more than 160 mmHg.
The compliance curve of another V-A shunt model. This bladder volume was approximately 20ml. The pressure started to rise at 1 minute after commencing the infusion. The pressure reached to 100 mmHg within 2 minutes (20ml).
Histology of Normal Bladder Wall

The Fig A and B (Masson’s trichrome stain) demonstrate the normal thin bladder wall. Submucosal fibrous connective tissue is visible, but no intramuscular fibrosis is recognized. On immunohistochemistry staining for alpha-smooth muscle actin (SMA), the muscular layer stains positively for SMA (C).
Histology of Shunted Bladder Wall

In the bladder of a shunted lamb (Masson’s trichrome stain), (A,B) there is marked thickening of the bladder wall with fibrosis extending from the submucosal layer into the intramuscular region. On immunohistochemistry staining for SMA (C), myofibroblastic cell proliferation is recognized in the submucosal layer. The muscular layer is also thickened, but the muscular bands are fragmented by thick bands of collagen.
Pressure-limited Vesico-amniotic Shunt Tube for Fetal Obstructive Uropathy

Hiroaki Kitagawa, Kevin C. Pringle¹, Junki Koike², Jane Zuccollo¹,

Division of Pediatric Surgery, St. Marianna University School of Medicine, Kawasaki, Japan

Department of Obstetrics and Gynecology, Wellington School of Medicine, New Zealand¹
Introduction

In-utero shunting (V-A shunt) of obstructive uropathy in fetal lambs produces a shrunken, non-compliant bladder. We hypothesized that using a ventriculo-peritoneal shunt (V-P shunt) for the V-A shunt may preserve the filling/emptying cycle and thus normal bladder development.
Insertion of V-P shunt tube (Group A)
Pressure and Flow Curve

- High pressure valve
- Medium pressure valve
- Low pressure valve

- Flow rate (mL/hr)
- Pressure (mmH2O)
The macroscopic thickness of the bladder wall (HE)

Control Group

Group A: 1.6±0.5mm

Group B: 2.7±0.7mm

0.4±0.2mm
Masson’s Trichrome Stain

- Control
- Non-valve shunt
- Valve shunt
The immuno-histochemical stain for α-SMA

control

Valve shunt

Non valve shunt
## Results

<table>
<thead>
<tr>
<th></th>
<th>Pressure Valve (n=8)</th>
<th>Non-valve (n=11)</th>
<th>Control (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (gram)</td>
<td>4150±753</td>
<td>4236±887</td>
<td>4870±626</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>46.6±4.0</td>
<td>47.6±3.6</td>
<td>49.1±3.2</td>
</tr>
<tr>
<td>Bladder Volume (ml)</td>
<td>57±41*</td>
<td>8.8±4.7*</td>
<td>65±18</td>
</tr>
<tr>
<td>Bladder Thickness (cm)</td>
<td>1.6±0.5*</td>
<td>2.7±0.7*</td>
<td>0.4±0.2</td>
</tr>
</tbody>
</table>

* p<0.01 (Student's t test)
Summary

1. The mean bladder volume in Group A was $57 \pm 41$ ml (n=8) compared with $9 \pm 5$ ml (n=11) in Group B (p<0.05). The mean bladder volume of normal control term lambs was $65 \pm 18$ ml (n=3).

2. Histological examination of the shunted bladders showed that both had increased fibrosis in the sub-mucosa and muscle layers compared with control bladders.
Conclusions

A pressure controlled V-P shunt tube for fetal obstructive uropathy improves bladder volume but doesn’t prevent the development of bladder wall fibrosis. Earlier shunting may prevent this.
Is a vesico-amniotic shunt intrinsically bad?
Shunting a normal fetal bladder

Hiroaki Kitagawa, Kevin C. Pringle ¹, Junki Koike ², Hideki Nagae²

Division of Pediatric Surgery, St. Marianna University School of Medicine, Kawasaki, Japan
Department of Obstetrics and Gynecology, Wellington School of Medicine, New Zealand¹
Introduction

We previously demonstrated that in-utero vesico amniotic shunting (V-A shunt) of obstructive uropathy in fetal lambs produces a shrunken, non-compliant bladder. We hypothesised that the normal fetal bladder filling and emptying cycle is critical to the development of normal bladder function.
Bladder Histology HE(2006)

Control

Control+shunt
There are fibrosis between the muscle layer. Alpha-SMA demonstrated wide space between the muscle.
Bladder Histology α-SMA(2006)

There is a wide space between the muscle. I think this is atrophy because they didn’t use the muscle in fetal stage.
## Results

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Shunt Model</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BW</strong></td>
<td>4733+1016 (g)</td>
<td>3758+606 (g)</td>
<td>p=0.17</td>
</tr>
<tr>
<td><strong>Crown rump length</strong></td>
<td>48+1.5 (cm)</td>
<td>43+6 (cm)</td>
<td>p=0.22</td>
</tr>
<tr>
<td><strong>Kideny weight (R)</strong></td>
<td>15+3.8 (g)</td>
<td>14.5+3.7 (g)</td>
<td>p=0.84</td>
</tr>
<tr>
<td><strong>Kideny weight (L)</strong></td>
<td>15.3+4.3 (g)</td>
<td>13.3+3.0 (g)</td>
<td>p=0.73</td>
</tr>
<tr>
<td><strong>Bladder Volume</strong></td>
<td>62+16.6 (ml)</td>
<td>5+2.8 (ml)</td>
<td>p=0.03</td>
</tr>
</tbody>
</table>
Results

- The mean bladder volume in shunted lambs (Group A) was $4\pm2.8$ ml ($n=4$) compared with $60\pm17$ ml ($n=3$) in control lambs (Group B) ($p<0.05$).
- Bladders in the shunted lambs had very poor compliance compared to normal lambs’ bladders.
- Histological examination of the shunted bladders showed increased fibrosis and distortion of the muscle layers compared with control bladders.
Conclusions

- Even in the absence of obstruction, preventing normal bladder filling and emptying in fetal life produces fibrotic bladders with poor compliance.
What is the appropriate valve shunt pressure for treat the obstructive uropathy in fetal lamb?

Hiroaki Kitagawa
St.Marianna University School of Medicine
Kawasaki Japan
We have previously shown that using a ventriculo-peritoneal shunt (V-P shunt) as a vesico-amniotic shunt (V-A shunt) to preserve the filling/emptying cycle is important for bladder development in fetal lamb. Then, what is the appropriate pressure for bladder development is the next question.
Introduction

In-utero shunting (V-A shunt) of obstructive uropathy in fetal lambs produces a shrunken, non-compliant bladder. We proved that using a ventriculo-peritoneal shunt (V-P shunt) for the V-A shunt preserve the filling/emptying cycle and thus normal bladder development 1).

1) Pressure-limited vesico-amniotic shunt tube for fetal obstructive uropathy
Materials and Methods

1. We created obstructive uropathy in 60-day gestation fetal lambs.
2. V-A shunting was performed 21 days later using the shunt tube and biopsy the bladder wall.
3. We used the high pressure shunt tube and low pressure shunt tube.
4. They were delivered at 130 days.
Pressure and Flow Curve

Flow rate (mL/hr)

Pressure (mmH2O)

- High pressure valve
- Medium pressure valve
- Low pressure valve
Shunt Tube
Models

- Low pressure type (L group) 4(5)
- High pressure type (H group) 3(4)
- Obstruction type (O group) 5(7)

Total 12 /16
survival
Pressure valve shunt
Operation View

Dilated urachus
Gross anatomy
Shunt (High pressure)

No shunt (Obstruction)

MCDK (2/5)

HE stain X40

Shunt (Low pressure)
Shunt (High pressure)

- Rupture of the muscle and thickness
- HE stain X40

No shunt (Obstruction)

- Rupture of the muscle and thickness

Shunt (Low pressure)
## Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight</th>
<th>Length</th>
<th>Average Bladder Volume</th>
<th>Dilated Urachus, Urinary Ascitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>L group (4)</td>
<td>3407</td>
<td>43</td>
<td>115ml</td>
<td>0/4</td>
</tr>
<tr>
<td>H group (3)</td>
<td>3942</td>
<td>44.6</td>
<td>12.5ml (2)</td>
<td>3/3</td>
</tr>
<tr>
<td>O group (5)</td>
<td>3984</td>
<td>48</td>
<td>impossibility</td>
<td>3/5</td>
</tr>
</tbody>
</table>
Summary (H group)

- In High pressure type, dilated urachus and urinary ascitis appeared in high frequency like the obstructed model.
- So, the pressure was avoided in kidney pathology and it was a normal view mostly.
- The bladder wall carried out thickness of H group and the obstruction group, and the bladder was a bladder wall view of low capacity.
Summary (L group)

In low pressure type, dilated urachs and urinary ascites were not carried out in L group.

Although there was some nephric tubule dilated, it is not as MCDK.

The bladder view was almost normal 1).

Conclusions

We were considered that capacity of the urinary bladder is secured and low pressure shunt tube is proper shunt pressure.
Acknowledgements

Thank you very much Professor Millar.