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COLLAGEN CONTENT IN DETRUSOR MUSCLE AND RATIO OF BLADDER WEIGHT TO BODY WEIGHT

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The urinary detrusor muscle is chiefly composed of smooth muscle and collagen fiber, with a fraction of elastin fiber. In the normal bladder, the collagen consists of approximately 27% dried fat-free detrusor muscle in humans and 17.3% in dogs. The administration of a sclerosing agent into the detrusor muscle causes bladder fibrosis, which can hardly be detected by the conventional histological staining methods. Overdistension of the bladder also induces interstitial fibrosis and thickening of the protoplasmic membrane. Bladder fibrosis and disrupted protoplasmic connection will make a barrier to propagation of depolarization into adjacent cells; a failure of the syncytial contraction of detrusor muscle. Bladder fibrosis also disturbs the physical property of detrusor muscle, that is, the viscoelasticity is elevated and the bladder becomes stiffer and smaller. It is consequently obvious that the collagen content is of prime factor which influences the bladder function.

It is a usual finding that a bladder wall in chronic diseases becomes very thick and trabeculated. This is frequently accompanied with VUR which requires the plastic operation in some cases. Few articles deal with the quantitative study of thickened bladder tissue under these circumstances.

The aim of present study is to evaluate through the experimentally formed bladder model (i) the amount of collagen in the dried fat-free detrusor muscle quantitatively and (ii) the ratio of wet bladder weight to body weight.

MATERIALS AND METHODS

Mongrel dogs of 15 to 25 kg were used for the present investigation.

(i) Three experimental models.

The amount of collagen in the dried fat-free bladder specimen was studied in 20 normal dogs (7 females and 13 males). The following 3 pathologic groups were prepared in 20 female dogs. (i) In 8 female dogs, neurogenic bladder dysfunction (NB group) was made, i.e. bilateral sacral rhizotomy, S1 to S3, of both anterior and posterior roots in 7 dogs and bilateral pelvic nerves were severed close to ureterovesical junction in 1 dog. The average duration was 12.8 weeks. (ii) A sclerosis of the bladder muscle was formed in 5 females by injecting 10 to 35 ml (Mean=23 ml) of 3% sodium tetradecyl sulfate (Elkins-Sinn Inc.) (STS group). Through an abdominal incision, this agent was infiltrated to all over the bladder muscle layer from neck to vertex with a No. 25G needle. The average observation period was 10.0 weeks. (iii) A bladder neck obstruction (BNO group) was made in 7 female dogs. The bladder was exposed through an abdominal incision. About 2 cm below the bladder neck, several stitches were sewn around the urethra with 00 silk thread. Similar stitches were made 1 cm proximally to the previous one. These

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Table 1. Schematic presentation of procedure for determination of collagen content. HOP stands for L-hydroxyproline (British Drug House Ltd).

Whole bladder homogenized in normal saline with blender.

\[ \text{3 to 5 times} \rightarrow \text{Centrifugation, 5 min at 5,000 RPM} \]

\[ \text{Precipitate} \rightarrow \text{Supernatant discarded} \]

Lipid extracted by agitation and filtration

(i) 1:3 of methanol and chloroform, 3 to 4 times

(ii) anhydrous ether, 3 to 4 times

\[ \text{Precipitate} \rightarrow \text{Drying, 22 hours at 120°C} \]

100 mg specimen into 15 ml 0.1 N NaOH

Placed in boiling water, 2 to 3 hours, then cooled

\[ \text{Centrifugation, 3 min at 1,500 RPM} \]

\[ \text{Supernatant} \rightarrow \text{Precipitate discarded} \]

0.75 ml of supernatant (5.0 mg specimen)

Hydrolysis of protein

5 ml 6 N HCl

Autoclave, 22 hours at 120°C

Cooled and pH adjusted to 8.3 to 8.5 with KOH

\[ \text{Filtration} \]

Total volume adjusted to 15.0 ml with H$_2$O

\[ \left(0.5 \text{ ml} + \frac{3.5 \text{ ml}}{2}\right) \times 2 \]

\[ \left(0.25 \text{ ml} + \frac{3.75 \text{ ml}}{2}\right) \times 2 \]

\[ \left(4.0 \text{ ml} + \frac{10 \text{ ml}}{4}\right) \times 2 \]

\[ \left(\frac{10 \text{ ml}}{4}\right) \times 2 \]

\[ \left(\frac{4 \text{ ml}}{2}\right) \times 2 \]

\[ \left(\frac{2 \text{ ml}}{4}\right) \times 2 \]

\[ \left(\frac{1 \text{ ml}}{4}\right) \times 2 \]

\[ \left(\frac{0.5 \text{ ml}}{2}\right) \times 2 \]

Blank, pH adjusted to 8.3 to 8.5

Approx. 4 g KCl

0.5 ml 10% dl-alanine sol (pH 8.7)

1.0 ml potassium borate buffer (pH 8.7)

Agitation 25 min

Oxidation of HOP into pyrrole-2-carboxylic acid

1.0 ml 0.2 M chloramine T sol

Agitation 25 min

3.0 ml 3.6 M sodium thiosulfate and quickly mixed

5.0 ml toluene

Agitation 5 min

\[ \text{Centrifugation, 3 min at 1,500 RPM} \]

\[ \text{Toluene layer discarded} \]

Placed in boiling water for 30 min, then cooled

Pyrrole formed
### (2) Biochemical analysis of collagen

The whole urinary bladder was resected and trimmed 1 cm below the ureteral orifices. After the fat was removed from the bladder surface, its weight and volume, by means of the water displacement method, were measured and the specimen was stored in a freezer at -15°C until the analysis was performed. The whole specimen was homogenized, and then fat and other substances soluble in physiological saline were extracted, i.e. (i) mixing with normal saline and centrifuged at 5,000 rpm for 5 min, (ii) agitation and filtration of precipitate with mixture of methanol and chloroform (1:3) and then with anhydrous ether. The obtained solid specimen was dried at 120°C for 22 hours. The succeeding procedures followed the method reported by Kivirikko et al. The principle of this method is to assay, by the colorimetric method, the hydroxyproline amount, which is specifically contained in collagen (Table 1). Elastin is found 1 to 2% in dried fat-free bladder specimen.

Since hydroxyproline is contained 1.5 to 2.3% in elastin, which is resistant to solution of 0.1 N NaOH, elastin is excluded in the procedure marked with two asterisks in Table 1. Hydroxyproline is known to consist of 13.5% of collagen in mammalian organs. Then the percentage of collagen present in the dried fat-free specimen can be calculated as follows:

\[
\text{Hydroxyproline (mcg) in 1 ml hydrolysate} \times \frac{100}{13.5} \times 100(\%)
\]

### (3) Ratio of bladder weight to body weight

The weight and volume of the wet whole bladder were previously measured. Subsequently, the specific gravity can be ob-

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### Table 2. The overall results are demonstrated for both normal and pathological groups. Numbers in parentheses indicate standard deviation. Statistic significance was studied compared to the normal group (Student's t-test).

<table>
<thead>
<tr>
<th>Dog group</th>
<th>A Bladder Weight (Mean)</th>
<th>B Specific Gravity of Bladder</th>
<th>C Body Weight (Mean)</th>
<th>D Collagen in dried fat-free specimen</th>
<th>E Ratio of A/C (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (N=20)</td>
<td>16.5 g</td>
<td>1.03 (0.06)</td>
<td>21.2 kg</td>
<td>30.2% (5.1)</td>
<td>0.78 (0.30)</td>
</tr>
<tr>
<td>NB (N=8)</td>
<td>40.3 g</td>
<td>1.06 (0.02)</td>
<td>20.4 kg*</td>
<td>42.6% (4.1) P&lt;0.01</td>
<td>1.88 (0.74) P&lt;0.01</td>
</tr>
<tr>
<td>STS (N=5)</td>
<td>20.6 g</td>
<td>1.04 (0.02)</td>
<td>18.5 kg*</td>
<td>50.8% (11.5) P&lt;0.02</td>
<td>1.12 (0.12) P&lt;0.01</td>
</tr>
<tr>
<td>BNO (N=7)</td>
<td>22.6 g</td>
<td>1.06 (0.01)</td>
<td>20.7 kg*</td>
<td>33.6% (8.0) P&gt;0.05</td>
<td>1.10 (0.39) P&gt;0.05</td>
</tr>
</tbody>
</table>

* Body weight measured on the day of operation.
tained. The normal ratio was established in 18 female dogs, sacrificed for the another experimental purpose. Dogs of NB group lost weight during the observation period, while the others gained weight. The urinary infection seemed to be the prime reason for the former. Consequently the body weight measured on the day of operation was used to calculate this ratio in these 3 groups.

RESULTS

Table 2 summarizes the results. Collagen was found to consist of 30.2% in the dried fat-free specimen of the normal bladder tissue. This value increased significantly in NB and STS groups. However, in the BNO group the significant difference was not observed \( (P>0.05) \) with Student’s \( t \)-test. The ratio of bladder weight to body weight was also elevated in the former, but the increase in the latter was similarly not significant. The specific gravity became larger in all 3 groups as compared to the normal, which suggested that an edematous change was not present.

DISCUSSION

Figure 1 schematically demonstrates the result of Table 2. It is clearly shown that the bladder has become extremely enlarged and that collagen component has occupied almost half of the solid bladder tissue in NB group. The enlarged bladder wall is caused by increase in amount of both collagen and smooth muscle, not by the edematous change. In BNO group, it is likely that a much longer duration and/or a smaller urethral caliber is necessary so as to induce a similar change found in NB and STS groups.

Bradley and his associates\(^5\), with a different biochemical technique, found an average of 17.3% of collagen in 5 normal dog bladders, which is much less than that observed here, 30.2%. The differing sensitivity of the technique and/or the loss of globular protein during our first stage of purifying procedure of bladder (one asterisk in Table 1) might account for this discrepancy. In 7 dog bladders with a sclerosing agent, they observed an increased value of an average of 43.7%, in good accord with the present study.

The ratio of wet bladder weight to body weight was found to be 0.78 g/kg in the normal dog, confirming the value of 0.84 g/kg (24 dogs) as reported by Veenema et al.\(^10\). They, however, did not observe an increase in this ratio with 7 dogs which underwent a sacral neurectomy. The shorter observation period of their experiment, only 2.5 weeks, seems to be responsible. We found this ratio surprisingly elevated in NB group, being 2.4 times as high as the normal control.

Collagen fiber has a higher elastic modulus, \( 1.3 \times 10^9 \) dynes/cm\(^2\), than that of smooth muscle, \( 6 \times 10^4 \) dynes/cm\(^2\).\(^11\). The increased collagen component renders the urinary bladder less elastic and contracted, subsequently bladder capacity becomes smaller. When a cystometric study is performed on this type of bladder, the curve has shown the loss of flat portion, showing a steep upward climb\(^5\). That is, the bladder wall has become hypertonic. It is conceivable that the same process could happen sooner or later in the clinical subject, implying the importance of early management of bladder hygiene for neurogenic bladder dysfunction, chronic infection and overdistended bladder.
SUMMARY

The collagen content of bladder and the ratio of bladder weight to body weight were studied in the dog. Three pathologic models were experimentally formed. In the normal dog, 30.2% of dried fat-free bladder wall accounted for collagen and the ratio of weight was found to be 0.78 g/kg. In neurogenic dysfunction and sclerosis groups, increase in collagen content and elevation of ratio of weight were significantly observed.

REFERENCES


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