Visualization of the Thoracic Duct with Injections of Dyes or Contrast Media into the Testicular Parenchyma in the Rabbit

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ABSTRACT. The thoracic duct drains lymph from the caudal part of the body to the venous system. The visualization of the thoracic duct is important for diagnosis of chylothorax, which may be caused by the damage of the duct. However, it is not easy to visualize the thoracic duct by injecting dyes and/or contrast media into peripheral lymph nodes and mesenteric lymphatics. In the present study, we examined whether the thoracic duct can be visualized by injecting dyes and contrast media directly into the testicular parenchyma. Under deep pentobarbital anesthesia, 14 male Japanese White rabbits were percutaneously injected with dyes (India ink or methylene blue) or contrast media (iohexol 240, 300, or Lipiodol) into the testicular parenchyma. Then, we grossly observed the stained thoracic duct or took radiographs and CT images of the duct. In all cases with dyes injections, the thoracic duct was successfully visualized. We observed stained lymphatic vessels from the testis to the duct. In case of contrast media injections, the thoracic duct was visualized with X-ray and CT imaging, when 1.5–2.0 ml per one testis of iohexol 240 at 37°C were injected into the right or both testes. The duct was most clearly visible, 3–5 min after the injections. The results show that the thoracic duct is reliably visualized simply by injecting dyes or contrast media into the testicular parenchyma. Our visualization method may also be applicable to the diagnosis of chylothorax in male animals.

KEY WORDS: rabbit, testis, thoracic duct, visualization.

The thoracic duct is the main lymphatic channel that drains lymph from the caudal part of the body to the venous system. The damage of the thoracic duct causes chylothorax that is characterized by accumulation of chyle in the pleural cavity. In such cases, the visualization of the thoracic duct is important for diagnosis and treatment of chylothorax in veterinary practice [3, 4].

To visualize the thoracic duct, dyes such as India ink (for anatomical purpose) and methylene blue (MB) or X-ray contrast media have been injected into the mesenteric, inguinal, popliteal, and metatarsal lymph vessels [1, 3, 8] or the mesenteric, popliteal, and axillary lymph nodes [5, 9, 12]. However, except for the popliteal lymph node, surgery is required to approach these nodes and vessels, before injections of dyes or X-ray contrast media. Furthermore, technical skills are also needed, when injections are made into these thin lymphatics especially in small dogs and cats. An alternative, less invasive method is injections of dyes or contrast media into the popliteal lymph nodes. However, it has been reported that popliteal injections visualize the thoracic duct less reliably than mesenteric injections [5].

Recent studies have shown that the testicular efferent and abdominal lymphatics were visualized by injections of dyes such as patent blue violet and India ink or X-ray contrast media such as ethyl ester of iodinated poppy-seed oil fatty acid into the testicular parenchyma in the rat [2, 10]. The results of these studies suggest the possibility that the thoracic duct could also be visualized, if adequate dyes or contrast media are selected and injected into the testis under an appropriate condition. Therefore, in the present study, we tried to find a noninvasive, simpler and more reliable method to visualize the thoracic duct by injecting various dyes and X-ray contrast media into the testicular parenchyma in the rabbit, which is one of appropriate experimental animals with large testes relative to its body size [6].

MATERIALS AND METHODS

Fourteen male Japanese White rabbits (Saitama Experimental Animal Supply Co., Saitama, Japan and Funabashi Farm Co., Chiba, Japan) weighing 3.0–4.0 kg were used. During the experiments, the rabbits were anesthetized with intraperitoneal injections of pentobarbital sodium (35.0–50.0 mg/kg body weight). All the experimental procedures complied with the guidelines of the National Institutes of Health, and were approved by the Ethical Committee for Animal Experimentation of Tokyo University of Agriculture and Technology. The rabbits were supine positioned, and percutaneously injected with dyes or X-ray contrast media directly into the parenchyma of the right, left, or both testes using a 25G butterfly or a 23G, 26G, or 27G standard needle connected to a syringe. Injections were made by syringe pumps over 180 sec or by hand (Table 1). The dyes injected were commercially available India ink and MB (1% aqueous), whereas the X-ray contrast media used were iohexol 240 mgI/ml (Omnipaque™ 240, Daiichi Sankyo Co., Tokyo, Japan),
iohexol 300 mg/ml (Omnipaque™ 300, Daiichi Sankyo Co.), and ethyl ester of iodinated poppy-seed oil fatty acid (Lipiodol™ Ultra-Fluide; 480 mg/ml, Guerbet Japan Co., Tokyo, Japan) (Table 1). In rabbits 9–14 (see below), iohexol 240 and 300 were warmed up to 37°C before injections.

Rabbits 1–5 were injected with India ink or MB (Table 1). Ten minutes (rabbits 1 and 3–5) or 24 hr (rabbit 2) after the injections, they were perfused through the carotid arteries or heart with saline under deep pentobarbital anesthesia to observe the thoracic duct more easily. In rabbits 3 and 4, 10% formalin was also perfused for fixation following saline perfusion. Then, we opened the thoracic and abdominal cavities to observe the thoracic duct.

Rabbits 6–14 were injected with iohexol 240, 300, or Lipiodol (Table 1). In rabbits 6–10, 13, and 14, right lateral thoracic and abdominal radiographs were taken just and 3–40 min after the completion of contrast media injections (Table 1).

In rabbits 9 and 10, which were used for the X-ray lymphography 51 days ago, and in rabbits 11 and 12 (Table 1), computed tomography (CT) scans of the trunk were performed to observe the thoracic duct according to the same injection procedures as described above. The images were obtained by a scanner (SOMATOM Emotion Duo, Siemens Co., Munich, Germany) at 130 kV, 240 mA and slice thickness of 1–1.25 mm.

In cases with dye injections, images were taken with a D70 digital camera (Nikon Co., Tokyo, Japan). In cases with contrast media injections, OsiriX version 2.7.5 (OsiriX Foundation, Geneva, Switzerland) was used to read the images in DICOM files to export TIFF files. For CT data, OsiriX was also used to obtain cross-sectional images. Then, all the images were digitally trimmed and adjusted to obtain optimal brightness, contrast, and sharpness with Adobe Photoshop Elements (Adobe Systems, San Jose, CA).

### RESULTS

In all cases with injections of India ink or MB (Table 1), the thoracic duct was successfully visualized running along the right side of the aorta (Fig. 1A). Stained lymphatic vessels were traced to the thoracic duct from the testis through the abdominal lymphatics and cisterna chyli. The thoracic duct in rabbit 4 was most clearly visible. The thoracic duct in rabbit 2 was still visible even 24 hr after India ink injections, although its coloration was much fainter than that in the other rabbits. The differences of coloration in the thoracic duct were little between injections of India ink and MB.

In rabbits 9, 10, and 13, an X-ray contrast medium iohexol 240 at 37°C was injected into the right or both testes (1.5–2.0 ml per one testis) over 180 sec (Table 1). Among these three rabbits, the thoracic duct was most clearly visible. The thoracic duct in rabbit 2 was still visible even 24 hr after India ink injections, although its coloration was much fainter than that in the other rabbits. The differences of coloration in the thoracic duct were little between injections of India ink and MB.

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### Table 1. Summary of methods

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>Body weight (kg)</th>
<th>Injected substance</th>
<th>Temperature of substance</th>
<th>Volume injected into one testis (ml)</th>
<th>Injected testis</th>
<th>Duration of injection (sec)</th>
<th>Interval between injection and observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.5</td>
<td>India ink</td>
<td>RT</td>
<td>0.15</td>
<td>Both</td>
<td>3</td>
<td>60 min</td>
</tr>
<tr>
<td>2</td>
<td>4.0</td>
<td>India ink</td>
<td>RT</td>
<td>0.15</td>
<td>Both</td>
<td>3</td>
<td>24 hr</td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>MB</td>
<td>RT</td>
<td>2.0</td>
<td>Both</td>
<td>60</td>
<td>60 min</td>
</tr>
<tr>
<td>4</td>
<td>3.0</td>
<td>MB</td>
<td>RT</td>
<td>1.0</td>
<td>Both</td>
<td>60</td>
<td>60 min</td>
</tr>
<tr>
<td>5</td>
<td>3.5</td>
<td>MB</td>
<td>RT</td>
<td>0.15</td>
<td>Both</td>
<td>3</td>
<td>30 min</td>
</tr>
<tr>
<td>6</td>
<td>3.5</td>
<td>Iohexol 300</td>
<td>RT</td>
<td>0.35</td>
<td>Right</td>
<td>3</td>
<td>5, 15, 25, 40 min</td>
</tr>
<tr>
<td>7</td>
<td>3.5</td>
<td>Lipiodol</td>
<td>RT</td>
<td>0.5</td>
<td>Right</td>
<td>5</td>
<td>0, 10, 20, 35 min</td>
</tr>
<tr>
<td>8</td>
<td>4.0</td>
<td>Iohexol 240</td>
<td>RT</td>
<td>0.6</td>
<td>Both</td>
<td>60</td>
<td>0, 5, 10, 15, 25 min</td>
</tr>
<tr>
<td>9</td>
<td>4.0</td>
<td>Iohexol 240</td>
<td>37°C</td>
<td>2.0</td>
<td>Both</td>
<td>180(a)</td>
<td>0, 3, 5, 10, 15 min</td>
</tr>
<tr>
<td>10</td>
<td>3.0</td>
<td>Iohexol 240</td>
<td>37°C</td>
<td>1.5</td>
<td>Both</td>
<td>180(a)</td>
<td>3 min</td>
</tr>
<tr>
<td>11</td>
<td>3.5</td>
<td>Iohexol 240</td>
<td>37°C</td>
<td>1.75</td>
<td>Right</td>
<td>180(a)</td>
<td>3.5 min</td>
</tr>
<tr>
<td>12</td>
<td>3.5</td>
<td>Iohexol 240</td>
<td>37°C</td>
<td>1.75</td>
<td>Right</td>
<td>180(a)</td>
<td>0, 3, 5 min</td>
</tr>
<tr>
<td>13</td>
<td>3.5</td>
<td>Iohexol 240</td>
<td>37°C</td>
<td>1.75</td>
<td>Left</td>
<td>180(a)</td>
<td>0, 3, 5, 7, 10, 15, 20, 30 min</td>
</tr>
<tr>
<td>14</td>
<td>3.5</td>
<td>Iohexol 300</td>
<td>37°C</td>
<td>1.75</td>
<td>Left</td>
<td>180(a)</td>
<td>0, 3, 5, 7, 10, 15, 20, 30 min</td>
</tr>
</tbody>
</table>

a) CT scans were also performed.

b) Only CT scans were performed.

c) Injected by syringe pumps.

MB: methylene blue. RT: room temperature.
In rabbits 9–12, the thoracic duct was also visualized along the right side of the aorta by CT scans in any transverse planes (Fig. 1C, D). We observed the thoracic duct running just ventral to the vertebral bodies at levels of the 7th to 12th thoracic vertebrae (T7–12) (Fig. 1C) and left ventrolateral to the vertebral bodies at levels of T1–6 (Fig. 1D). However, in all cases with CT scans, the thoracic duct was not completely observed at the level of T10. Furthermore, there were interruptions of imaging at some levels of T9–11 and T1–3, depending on each rabbit. Therefore, the thoracic duct was seen continuously at levels of T4–8 and the level of T12 in all cases with CT scans.

**DISCUSSION**

We found that the thoracic duct was reliably visualized simply and noninvasively by injecting dyes or contrast media into the testicular parenchyma. For successful visualization of the thoracic duct, especially with X-ray and CT imaging, it is essential to select an appropriate X-ray contrast medium, and adjust injection volume and temperature of the media.

Previous studies demonstrated efferent testicular lymphatics and their associated lymph nodes, after injections of 0.01 ml of India ink into each testis in the mouse [7] and 0.25–0.3 ml of India ink or patent blue violet into the unilateral testis in the rat [2, 10]. However, these studies did not attempt to visualize the thoracic duct. In the present study, injections of 0.15–2.0 ml of India ink and MB over 3–60 sec into both testes successfully visualized the thoracic duct, which was most clearly visible 30 or 60 min after injections. The thoracic duct was still observed even 24 hr after injections of India ink.

In contrast media injections, a previous study in the rat showed that the abdominal lymphatics, not the thoracic duct, were visualized 30 min after injections of 0.25 ml of Lipiodol into right or both testes [10]. It is likely that the thoracic duct may have also been visualized if less viscous contrast media were used. Indeed, we found significant differences in visualization of the thoracic duct among injections of iohexol 240, 300, and Lipiodol. The thoracic duct was visualized only after injections of 1.5–2.0 ml of iohexol 240 at 37°C into one or both testes. This may be due to the fact that iohexol 240 contains a smaller amount of iodine (240 mg/ml) than iohexol 300 (300 mg/ml) and Lipiodol (480 mg/ml). Furthermore, iohexol 240 is least viscous among these contrast media, as shown by the manufacturers and previous studies [e.g., 11]. Although iohexol 240 has the weakest imaging ability, we sufficiently visualized the thoracic duct even in case of an injection into the unilateral testis.
testis. In lymphography by X-ray, it should be noted that the thoracic duct is most clearly visible, 3–5 min after injections of the contrast medium; the thoracic duct becomes gradually faint, 5 or more minutes after the injections. Our data also show that the injected contrast medium rapidly moves from lymph to the ureter and urinary bladder.

In lymphography by CT scanning, it is advantageous that the relationship between the thoracic duct and other organs was readily visible especially in any transverse plane. Although the imaging of the thoracic duct was not complete in the present study, the usage of a higher performance CT scanner would overcome this problem.

A previous study has shown that reproductive ability in male rats is unchanged, 50 or more days after Lipiodol injections into the parenchyma of the testes [10]. Furthermore, the present study demonstrates that the thoracic duct was visualized in radiographs, even after an injection into the unilateral testis. Taken together, these data suggest that injections of contrast media into the testis produce little adverse effects on the reproductive ability. Therefore, the present simple and reliable visualization method may be applicable not only to anatomical studies of the thoracic duct but also to clinical diagnosis and treatment for chylothorax in male animals.

REFERENCES