Practical Lessons in Murine Thoracic Lymph Duct Cannulations: Observations in Female and Male Mice Across Four Different Strains That Impact on "Cannulatability"

SUZANNE M. CALIPH,[1] DAVID M. SHACKLEFORD,[1] DAVID B. ASCHER,[2] LISA M. KAMINSKAS[1]

[1]Drug Delivery Disposition Dynamics Group, Monash Institute of Pharmaceutical Sciences, Parkville, Victoria 3052, Australia
[2]Department of Biochemistry, University of Cambridge, Cambridge CB2 1GA, UK

Received 22 October 2014; revised 16 November 2014; accepted 18 November 2014

Published online 23 December 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24312

ABSTRACT: Cannulation of the thoracic lymph duct in experimental animals allows direct measurement of the lymphatic exposure of lymph-targeted drugs. When coupled with recent advances in genetically modified and diseased mouse models, this presents further opportunities to define changes in biological processes and disease in response to drug treatment. Although cannulation of the thoracic lymph duct in mice is inherently challenging because of the small size and delicate nature of the duct, it can be further confounded by anatomical variations between animals. In this communication, we present our observations on the anatomical features of the thoracic lymph duct between mice of different strains and genders, and discuss the impact of these features on the "cannulatability" of the duct. We also provide some technical tips to help guide other investigators to deliver higher experimental success rates. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:1207–1209, 2015

Keywords: lymphatic transport; pharmacokinetic/pharmacodynamic models; drug targeting; gender; transgenic

Lymph-cannulated mice present pharmaceutical scientists with the opportunity to define the exposure of lymph-targeted drugs at the site of action. This provides more conclusive insight than would be gained through surrogate measures based on plasma or lymph node sampling, particularly given examples where lymph node retention poorly reflects the kinetics of lymphatic exposure.[1] Furthermore, significant advances in genetically modified and disease mouse models enable scientists to couple these direct measurements of exposure at the site of action with information on biological changes in response to drug treatment. Experience tells us, however, that interanimal variability in the structure of the lymphatic system is significant, and there is a paucity of information in the literature describing the influence of rodent strain and gender on the anatomic features and "cannulatability" of the lymph duct. The intention of this communication is therefore to present our practical experiences in cannulating the thoracic lymph duct of mice, highlighting observed differences in the anatomy of the thoracic lymph duct in four different strains of mice (and between genders in two strains). Our intention is not to provide a comprehensive précis of the field, but rather to provide some practical guidance to other investigators with the aim of increasing experimental success rates and reducing animal usage and cost.

The mice examined included 3–6-month-old C57Bl/6J mice (11 female, 10 male; one of the most common strains used in biomedical research), transgenic C57Bl/J-B6DBA mice (8 female, four male), FVB/N mice (4 female; commonly used background strain for transgenic animals), and athymic nude mice (8 female; common in cancer research). This work was approved by the institutional animal ethics committee.

The thoracic lymph duct was exposed and cannulated where possible in isoflurane-anaesthetized mice via a modification to the method described by Ionac[2] and is depicted diagrammatically in Figure 1. Polyvinyl cannula (0.28×0.61 mm^2 inner and outer diameter) with a 90° bend 3 mm from a beveled tip was used. Note that unlike in the rat,[3] this is the only surgical approach by which the thoracic lymph duct of mice can be exposed. In contrast to the suggestion by Ionac to isolate the lymph duct from the abdominal aorta, we found that this often led to rupture of the duct and failed cannulation. We found that the best approach was to remove the thin layer of connective tissue covering the duct by gentle separation with a pair of microforceps on its lateral side. This clarified the duct and the retained connections with the surrounding tissue and aorta provided stability during cannulation.

Although the thoracic lymph duct in male mice was located adjacent to the aorta and was easily identified in all cases, the lymph duct in female mice was partially or completely concealed beneath the aorta that increased the risk of damage to the duct or aorta during isolation and cannulation. This was most problematic in female C57Bl/6J mice, where the lymph duct was located primarily beneath the aorta (Fig. 2), but less so in other mouse strains and in female transgenic mice created by crossing the C57Bl/J strain with the B6DBA strain, where thoracic ducts were only partially concealed. Although not definitive, it alludes to the possibility that this anatomical feature can be corrected by appropriate cross-breeding.

In addition, female mice generally displayed multiple lumbar arteries and smaller vessels that emerged from the aorta and crossed the thoracic lymph duct (Figs. 3 and 4). The ease with which the thoracic lymph duct could be isolated and cannulated between these sections of blood vessels also differed significantly with mouse strain. Isolation of a small section of

Correspondence to: Lisa M. Kaminskas (Telephone: +61-3-99039522; Fax: +61-3-99039583; E-mail: lisa.kaminskas@monash.edu)

Journal of Pharmaceutical Sciences, Vol. 104, 1207–1209 (2015)

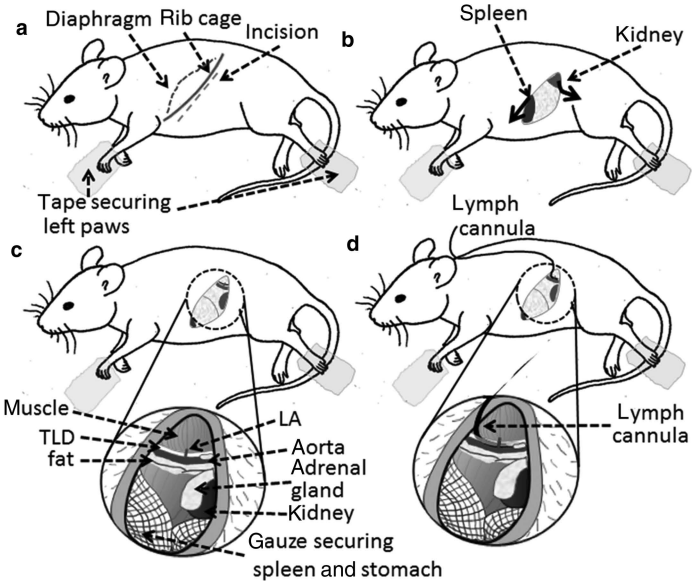

Figure 1. Schematic representation of the surgical procedure to isolate and cannulate the thoracic lymph duct in mice. (a) Location of the incision site relative to the rib cage and dorsal/ventral aspects of the mouse. (b) Position of the left kidney and spleen and direction to move each organ (solid arrows). (c) Surgical field once organs and fat have been moved away from the abdominal aorta and thoracic lymph duct (TLD), showing the positioning of the lumbar artery(s) (LA), adrenal gland, kidneys, and stomach/intestinal contents (which are held in position with sterile gauze). (d) Placement of the lymph cannula prior to sealing in place with a drop of superglue and small piece of muscle from the abdominal wall.

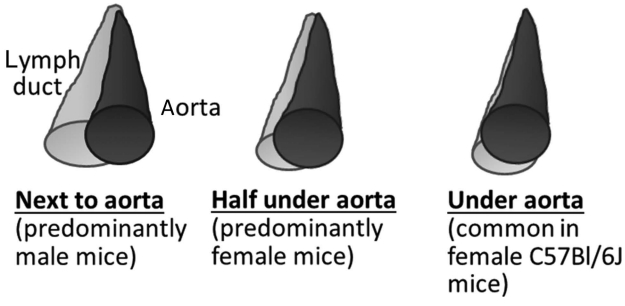

Figure 2. Diagrammatic representation of the location of the thoracic lymph duct relative to the abdominal aorta in mice when viewed from the left and dorsal side of mice.

the duct for insertion of the cannula was either impossible or extremely difficult in female C57Bl/6J mice without cutting these vessels and causing significant bleeding (~10% cannulation success). Isolation or cauterization of these vessels was met with an increased risk of rupturing the lymph duct. Female C57Bl/J-B6DBA and FVB/N mice displayed two lumbar arteries that were connected in the center of the surgical field, which provided a small "window" through which the thoracic duct could be cannulated (~50% cannulation success). Female nude mice also displayed several lumbar arteries that were generally present in a more anterior direction within the surgical field, enabling good access and "cannulatability" (~70% success). Encouragingly, this suggests that there is the significant potential to correlate the lymphatic exposure of chemotherapeutic drugs with therapeutic activity against primary and secondary

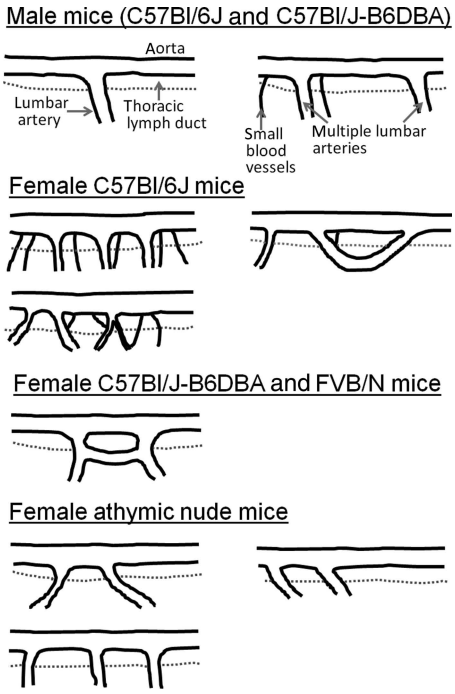

Figure 3. Diagrammatic representation of the anatomy of blood vessels crossing the thoracic lymph duct in male and female mice.

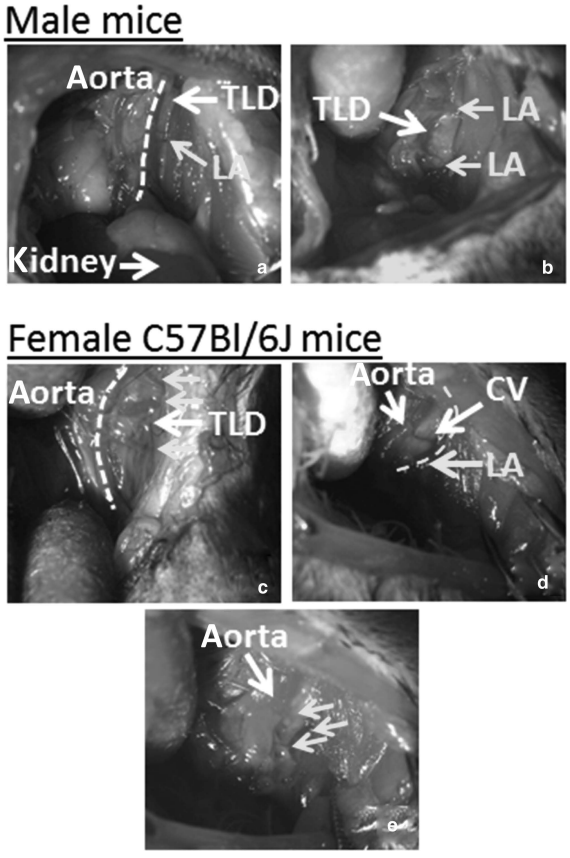

Figure 4. Images of the anatomy surrounding the thoracic lymph duct (TLD) in male and female mice, showing the varying architecture of blood vessels (lumbar arteries, LA, solid yellow arrows; small connecting blood vessels, CV) crossing the duct from the abdominal aorta. (a and c) A broken white line shows the position of the abdominal aorta.

DOI 10.1002/jps.24312

lymphatic tumors within the same model.[3] In general, however, male mice displayed only one lumbar artery within the field (~70% of mice), but a small proportion (~30%) contained two. Together with the adjacent position of the thoracic lymph duct compared to the aorta, male mice presented the best visibility and "cannulatability" (~80% success).

ACKNOWLEDGMENTS

L.M.K. was supported by an NHMRC Career Development fellowship (APP1022732). D.B.A. was supported by a NHMRC CJ Martin fellowship (APP1072476). This work was supported by an Australian NHMRC grant.

REFERENCES

1. Edwards GA, Porter CJH, Caliph SM, Khoo SM, Charman WN. 2001. Animal models for the study of intestinal lymphatic drug transport. Adv Drug Deliv Rev 50:45–60.

2. Ionac M. 2003. One technique, two approaches, and results: Thoracic duct cannulation in small laboratory animals. Microsurgery 23:239–245.

3. Kaminskas LM, Ascher DB, McLeod VM, Herold MJ, Le CP, Sloan EK, Porter CJ. 2013. PEGylation of interferon alpha2 improves lymphatic exposure after subcutaneous and intravenous administration and improves antitumour efficacy against lymphatic breast cancer metastases. J Control Release 168:200–208.