Long-Distance Transportation of Live Isolated Lymphatic Vessels

Anatoliy A. Gashev, M.D., Ph.D., DMedSci¹ and Michael J. Davis, Ph.D.²

Abstract

Background: We performed experiments to test whether isolated lymphatic vessels would remain viable after overnight long-distance shipment.

Methods and Results: Freshly isolated segments of rat mesenteric lymphatic vessels were placed into tubes filled by chilled D-MEM/F12 supplemented with antibiotics and shipped via overnight by express mail from College Station, TX to Columbia, MO. Standard physiological and pharmacological tests were performed to investigate the influence of the long-distance transport procedure on the spontaneous contractility of isolated, cannulated, and pressurized rat mesenteric lymphatic vessels. The results demonstrate that normal contractile function of isolated lymphatic vessels can be preserved with long-distance shipping and subsequent overnight recovery if the proper precautions are taken.

Conclusions: The method of lymphatic vessel transportation described in this report opens up the opportunity to perform *in vitro* functional tests on lymphatic tissues harvested and initially processed in a remote location. In addition, the described procedures may expand the options for potential sources of fresh human tissue, harvested during surgery or autopsy and allowed to be available to lymphatic researchers in remote locations.

RECENTLY, WE DEMONSTRATED THE EFFECTIVENESS OF newly developed experimental protocols for the maintenance of isolated rat mesenteric lymphatic vessels and thoracic duct in culture for 3–12 days without significant impairment of the parameters of their pumping.¹ We also described methods for effective adenoviral/GFP transfection of lymphatic endothelial and muscle cells in the same vessels.¹ After obtaining positive results in the first set of functional tests on lymphatic segments cultured for 3–4 days, we performed subsequent experiments to test whether isolated lymphatic vessels would remain viable after overnight express shipment. The successful development of such a procedure would facilitate collaborations between laboratories.

Mesenteric lymphatic vessels were isolated from male Sprague-Dawley rats (weighing between 300 and 400 g). All animal procedures performed for this study were reviewed and approved by our respective Institutional Animal Care and Use Committees and adhered to the regulations, policies, and principles detailed in the Public Health Service Policy for the Humane Care and Use of Laboratory Animals

(PHS Policy, 1996). To isolate mesenteric lymphatic vessels, a rat was anesthetized with a solution containing a combination of fentanyl/droperidol (0.3 ml/kg IM) and diazepam (2.5 mg/kg IM). A 4-cm long midline abdominal incision was made through the skin, underlying fascia and muscle layers. A small loop of intestine, 6–7 cm in length, was exteriorized through the incision. A section of the mesentery containing lymphatic vessels was positioned in a dissection chamber within the field of view of a dissecting microscope and continuously suffused with standard Dulbecco's phosphate-buffered saline (DPBS) (Invitrogen Corp., Carlsbad, CA; Cat. # 14040-133). Suitable mesenteric lymphatic vessels were identified and cleared of all surrounding tissue. Sections of mesenteric lymphatic vessels 2–3 cm in length were carefully dissected for use in the subsequent protocol. After isolating mesenteric lymphatic vessels, the rat was euthanized with pentobarbital (120 mg/kg body weight IP).

Freshly isolated segments of rat mesenteric lymphatic vessels were gently double-rinsed ablumenally in sterile DPBS. This procedure was performed in two sterile 35-mm

¹Department of Systems Biology and Translational Medicine, College of Medicine, Cardiovascular Research Institute Division of Lymphatic Biology, Texas A&M Health Science Center, Temple/College Station, Texas.

²Department of Medical Pharmacology and Physiology, University of Missouri, Columbia, Missouri.

This work was supported in parts by Texas A&M Health Science Center College of Medicine & Department of Systems Biology and Translational Medicine, and by the National Institutes of Health (NIH RO1 HL-070308, HL-089784 and AG030578).

plastic Petri dishes under a dissecting microscope with sterilized forceps, to minimize possible contamination. Subsequently, each lymphatic vessel was placed into a separate 0.65 ml clear plastic tube (VWR Cat. # 53550-100) completely filled with $\sim 4^{\circ}\text{C}$ D-MEM/F12 (Invitrogen Corp., Cat.# 11039) supplemented with antibiotic mixture (Invitrogen, Cat.# 15140) to achieve a concentration of 100 units of penicillin and $100\mu\text{g}$ of streptomycin per ml of D-MEM/F12. Care was taken to ensure that the vessel segment was completely submerged under the medium in the tube. The cap of the tube was tightly closed to eliminate trapped air, and then additionally secured by tape. Multiple tubes were placed in between pre-chilled wet ice packs into an insulated container and shipped via overnight express mail from College Station, TX, to Columbia, MO. The isolation/packaging procedures for a set of 2–3 vessel segments usually required approximately 2 h and shipment time from pickup to delivery was approximately 16–18 h.

On our first test of this method of transportation, some vessels were unpacked and used for functional tests 1–4 h after arrival. The success rate for the redevelopment of

spontaneous contractions was $\sim 50\%$ in vessels immediately cannulated and pressurized. However, overnight (second night after shipment) recovery of vessels at 37°C in a cell culture incubator with 10% CO_2 increased the success rate for the redevelopment of spontaneous contractions and other functional tests to $\sim 90\%$. Therefore, for subsequent experiments each individual lymphatic vessel segment was removed from the shipment tube, placed in a Petri dish filled with room-temperature D-MEM/F12 media supplemented with antibiotics, pressed to the bottom of the dish by one end of vessel segment to have it completely submerged in media, and stored overnight in a 10% CO_2 , 37°C cell culture incubator. To test the viability of shipped/overnight-recovered isolated lymphatic segments, we performed standard functional tests² and compared the data to that from control experiments on rat mesenteric lymphatic vessels acutely isolated from animals in Missouri (nonshipped control group). After cannulation, pressurization, and equilibration at 37°C , 8 of 9 transported vessels developed spontaneous contractions after overnight incubation. Within each 2–3 cm vessel segment, only the nonconstricted regions were selected

FIG. 1. Examples of the contraction patterns of acute and shipped/overnight recovered vessels and their responses to elevated pressure and substance P. **(A) Left:** An acutely isolated lymphatic vessel had a basal contraction FREQ of $\sim 8/\text{min}$ when pressure was $1\text{ cm H}_2\text{O}$. FREQ increased as pressures (in both input and output pipettes) were slowly ramped from 1 to $15\text{ cm H}_2\text{O}$. **Right:** After pressure was returned to $1\text{ cm H}_2\text{O}$, bath application of substance P evoked ~ 2.5 -fold increase in FREQ, which increased even further in response to an increasing pressure ramp. Exchange of the bath for Ca^{2+} -free solution at an intermediate pressure level resulted in immediate dilation and cessation of spontaneous contractions. **(B)** Response of a different lymphatic vessel subjected to similar protocols after long-distance transportation and overnight incubation. The vessel exhibited qualitatively similar responses to elevated pressure, substance P, and Ca^{2+} -free bath. Note also the retention of rate-sensitive contraction inhibition (at time = 244–246 min) when pressure was rapidly lowered, which also occurred in the acutely isolated vessel.

for subsequent cannulation and study. One vessel developed only intermittent contractions and one vessel was severely constricted; we referred these cases to potential dissection damage, neither vessel was used for further analysis. At 3 cm H₂O, the level of basal tone and frequency of spontaneous contractions were very similar between transported/overnight-recovered vessels, and acutely isolated (nontransported) vessels. During the slow pressure ramps from 1 to 10 cm H₂O (lasting ~10 min, and in the absence of imposed flow) and the substance P activation, the transported/overnight-recovered vessels responded in a manner very similar to what has been observed and described previously for acutely isolated rat mesenteric lymphatic vessels.^{2,3} Figure 1 demonstrates the examples of the contraction patterns of acute and shipped/overnight-recovered vessels and their responses to elevated pressure and substance P. For statistical analysis, the continuous frequency of contractions (FREQ) and contraction amplitude (AMP) data for each of 13 ramps from 11 segments of 8 shipped/overnight-recovered vessels, and 18 ramps from 18 acute vessels were binned according to pressure (in 40 bins from 1 to 10 cm H₂O). Subsequently, ANOVAs with multiple comparison tests on each bin were performed (JMP 7.1).

Figure 2 demonstrates comparison of parameters of lymphatic phasic contractility in isolated rat mesenteric lymphatic

vessels studied acutely and after long-distance transportation and subsequent overnight incubation. There was a tendency for basal contraction frequency to be lower (~20%) and contraction amplitude to be higher (~25%) at all almost all pressures in the transported/overnight-recovered vessels such that the AMP*FREQ product (AFP; AFP = amplitude*frequency)² as a function of pressure was very similar for both transported/overnight-recovered and acutely isolated vessels. Over the lower pressure range (1–4.5 cm H₂O), there were no significant differences in AFP; from 4.5–10 cm H₂O, AFP in the shipped/overnight-recovered vessels was significantly (~10%) higher at most pressures.

In conclusion, these results demonstrate that overnight, long-distance transportation of isolated lymphatic vessels in D-MEM/F12 media supplemented with antibiotics does not result in substantial impairment of contractile function. We conclude that transported vessels are able to maintain normal pump activity after 2 days, especially when overnight express shipment is combined with subsequent overnight recovery at 37°C before functional tests are performed. The method of lymphatic vessel transportation described in this report opens up the opportunity to perform *in vitro* functional tests on lymphatic tissues harvested and initially processed in a remote location. We believe that such ability may extend

FIG. 2. Comparison of parameters of lymphatic phasic contractility in isolated rat mesenteric lymphatic vessels studied acutely (acute; $n=18$) and after long-distance transportation and subsequent overnight incubation as described in text (shipped; $n=11$). Amp*Freq = amplitude-frequency product, an index of the minute pumping ability of the vessels. + indicates a significant difference ($p < 0.05$) from the value within the same group at 3.0 cm H₂O (for panels A, C) and from the value within the same group at 10 cm H₂O for panel B (Dunnett's). * indicates a significant difference between the acute and shipped groups at the same pressure.

the set of available options for collaborating laboratories, when some sophisticated experimental models or techniques to influence the regulatory mechanisms of lymphatic function exist on one side, but specific analytical tools are available only on other side. As an example, for potential joint collaborative efforts, our newly developed transportation technique may eliminate the necessity to perform costly gene transfection experiments¹ at all locations; the distribution of transfected vessels might facilitate substantial savings in time and resources. However, considering the wider potential of our transportation approach, we believe that the procedures described above may expand the options for potential sources of fresh human tissue, harvested during surgery or autopsy and allow it to be available to lymphatic researchers in remote locations.

Authors Disclosure Statement

The authors have no conflicts of interest or financial ties to disclose.

References

1. Gashev AA, et al. Methods for lymphatic vessel culture and gene transfection. *Microcirculation* 2009;16:615–628.
2. Zhang R, et al. Length-dependence of lymphatic phasic contractile activity under isometric and isobaric conditions. *Microcirculation* 2007;14:613–625.
3. Davis MJ, et al. Modulation of lymphatic muscle contractility by the neuropeptide substance P. *Am J Physiol Heart Circ Physiol* 2008;295:H587–597.

Address correspondence to:

Anatoliy A. Gashev

Department of Systems Biology and Translational Medicine

College of Medicine

Cardiovascular Research Institute

Division of Lymphatic Biology

Texas A&M Health Science Center

702 SW H.K. Dodgen Loop Temple, TX 76504-7145

E-mail: gashev@tamu.edu