Quantitative assessment of lymphatic function by near infrared imaging technique

Y. Abdulla, P. Jobling, D.F. van Helden and M.S. Imtiaz, School of Biomedical Sciences and Pharmacy, The University of Newcastle, Callaghan, NSW 2308, Australia.

The lymphatic system maintains tissue fluid homeostasis and plays an important part in immune cell trafficking. Near infrared (NIR) imaging techniques have been used as a tool to assess lymphatic pumping in both basic medical sciences and clinical medicine. In this study we developed a near infrared imaging system to evaluate lymphatic pumping in the rat leg under controlled conditions. Non-recovery experiments were performed on Wistar male rats (n = 6, 405 ± 63.55 g) deeply anaesthetized with *i.p.* urethane (1-1.5 g/kg) before exposure of the femoral vascular bundle for imaging. All procedures were approved by the University of Newcastle Animal Care and Ethics Committee. Indocyanine green (ICG) 0.8 mM pre-mixed with bovine serum albumin (BSA) was injected a hind foot, measurements performed and then the experiment repeated for the other leg. Leg temperature was controlled using a custom designed fluid circulation system. A near infrared system consisting of laser diode with biconcave lens, and CCD camera with 830 nm band pass filter, was used to image the ICG in the foot-injected hind limb up to the groin area. ICG was visible as a bright signal, indicating transit through the lymphatic system. Matlab and Labchart were used to extract the transit time and pumping frequency from the video recordings. All data was calculated as mean ± SEM. The following parameters were measured: lymph transit (T1: first arrival time; T5: time for response to achieve 5 fluorescence intensity units (*i.e.*, 5 FIU); T50: time to achieve 50% of maximal intensity), contraction frequency and relative ejection fraction.

The first arrival time (T1) was 3.4 ± 1.6 min and 7.3 ± 1.5 min in left and right leg respectively (P = 0.10). The fluorescence intensity rose to value of 5 FIU more quickly compared to right leg (left leg 2.1 ± 0.8 min, right leg 8.4 ± 1.9 min, $P < 0.05$). Lymphatic contraction frequency was 6.5 ± 1.5 per min and the relative ejection fraction was 24 ± 4 % (n = 6). Lymph transit in the rat is faster in the left leg. The reasons for this are not clear and will be investigated further. We have developed a preparation that allows us to quantify both lymph transit time and contraction frequency of lymphatic pumping.