Investigating the impact of liposome properties on lymphatic distribution following intraperitoneal delivery

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Background: The lymphatic system is a primary site for cancer metastases, infectious disease proliferation and inflammatory disease response. Additionally, our lab has identified the important role of gut-lymph as a source of factors that promote organ failure in acute and critical illnesses. These lymphatic related diseases have in the past been treated with small molecule drugs that display limited lymphatic affinity, which in turn restricts therapeutic efficacy (Trevaskis *et al.*, 2015). Therapeutic proteins and nano-sized drug carriers (*e.g.* liposomes) better target and treat some lymphatic diseases since they are cleared *via* the lymphatics following interstitial (SC, IM) administration (Kaminskas *et al.*, 2013; Trevaskis *et al.*, 2015). However, interstitial administration results in specific access to the lymphatics draining the injection site and limited access to deep lymphatics draining the abdominal and thoracic organs.

Aims: To determine 1) the potential to deliver drugs to the deep lymphatics *via* intraperitoneal (IP) administration in liposomes, 2) the impact of liposome properties (size, charge, surface derivatisation) on lymphatic uptake after IP administration, and 3) the major sites of lymphatic access from the peritoneal cavity.

Methods: Liposomes of varying size, surface charge and derivatisation (PEGylation) are loaded with radiolabelled lipid and/or sucrose was prepared. Liposome properties were confirmed by dynamic light scattering (DLS) and Cryo-TEM. Stability was assessed in buffer, plasma and lymph fluid collected from anaesthetized Wistar rats from other studies. Anaesthetised male Wistar rats were IP administered 1 mL of the radiolabelled liposome formulations. Isoflurane (2.5-1.5%) delivered *via* a face mask, maintained the anaesthesia for the duration of the study. Lymph and blood samples were collected periodically for 8 h. Lymph was collected from the thoracic lymph duct at either the abdomen or just prior to entry into the blood circulation at the jugular-subclavian junction. At the conclusion of the study, peritoneal fluid and lymph nodes were collected. Radiolabel concentrations in all samples were measured *via* scintillation counting.

Results: Stable liposomes ranging in size from 50-150 nm with positive, negative and neutral surface charges or that were PEGylated on the surface were prepared. Peritoneal retention was highest for the PEGylated liposomes. Cumulative uptake into the lymph at the intra-abdominal junction was highest for negatively charged, conventional liposomes (1.6% of dose) and least for the PEGylated liposomes (0.47% of dose) consistent with the increased peritoneal retention. Comparing the two sites of cannulation, a substantially higher percentage of the dose of radiolabelled liposome was recovered in lymph collected from the jugular-subclavian junction when compared to within the abdomen for the negative liposomes (11.2% *vs* 1.6%). This suggests the liposomes predominantly enter the lymphatics at the diaphragm.

Conclusion: The results confirm the potential to deliver drugs to the deep lymphatics *via* IP administration in liposomes, particularly liposomes that are <150 nm in size and negatively charged. The major site of lymphatic entry following IP administration appears to be *via* the diaphragmatic lymphatics. Overall this suggests that diseases involving the deep lymphatics that drain the abdominal and thoracic organs such as cancers, infections, inflammatory diseases and acute and critical illness may be better targeted and treated by IP administration of nanocarriers.

Kaminskas LM, Ascher DB, McLeod VM, Herold MJ, Le CP, Sloan EK, Porter CJ. (2013) *J Control Release* **168**: 200-208.

Trevaskis NL, Kaminskas LM, Porter CJ. (2015) *Nat Rev Drug Discov* **14**: 781-803.