Distribution of therapeutic proteins into thoracic lymph after intravenous administration is protein size-dependent and primarily occurs within the liver and mesentery

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Purpose: The lymphatic system is a primary site for cancer metastases, proliferation of infectious diseases and the immune response to inflammatory diseases and organ transplantation (Trevaskis *et al*. 2015). These conditions have in the past been treated with small molecule drugs that display limited lymphatic affinity, which in turn can restrict therapeutic efficacy. Therapeutic proteins can better target and treat lymphatic diseases since they are cleared *via* the lymphatics following interstitial (SC or IM) administration. However, therapeutic proteins are often administered intravenously (IV). Recently therapeutic proteins have been shown to access the thoracic lymph in surprisingly high quantities after IV administration (Kaminskas *et al*. 2013). The aim of this study was to determine, for the first time, the major sites of thoracic lymph access of therapeutic proteins, and the protein properties that enhance lymph access, after IV administration.

Methods: In order to achieve this, novel methods were developed or optimised to collect hepatic, mesenteric or thoracic lymph from male SD rats (Bollman *et al*. 1948). For anaesthetized studies, rats were anesthetised by SC injection (1.5 ml/kg) of cocktail I (56 mg/ml ketamine, 15 mg/ml xylazine, 0.6 mg/ml acepromazine), and subsequent doses (0.44 ml/kg) were given hourly with cocktail II (100 mg/ml ketamine, 1 mg/ml acepromazine). Four different sized PEGylated or non-PEGylated therapeutic proteins (native interferon α2b (IFN, 19kDa), PEGylated interferon α2b (IFN-PEG12, 31kDa), PEGylated interferon α2a (IFN-PEG40, 60 kDa) or trastuzumab (150 kDa)) were then administered *via* short IV infusion, and plasma and lymph concentrations of the proteins determined *via* ELISA.

Results: The recovery of the therapeutic proteins in the thoracic lymph duct, which collects lymph from most of the body, was significantly greater for trastuzumab, IFN-PEG40 and IFN-PEG12 (all >3% dose over 8 h) when compared to native IFN (0.9% dose). Conversely, the thoracic lymph/plasma (L/P) concentration ratio and thus efficiency of extravasation and transport through the interstitium to lymph was highest for the smaller proteins IFN and IFN-PEG12 (at 90-100% *vs* 15-30%). The lower total recovery of IFN and IFN-PEG12 in thoracic lymph reflected more rapid systemic clearance and shorter plasma circulation half-life. For all therapeutic proteins, the majority (>80%) of lymph access occurred *via* the hepatic and mesenteric lymphatics

Conclusions: Optimising the properties of IV administered therapeutic proteins represents a viable approach to better target and treat pathological states involving the lymphatics, particularly in the liver and mesentery. This includes cancer metastases, infectious and inflammatory diseases. Successful development of the novel technique to collect hepatic lymph will also enable future work to evaluate tissue-specific lymph transport in health and disease.

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Kaminskas LM, Ascher DB, McLeod VM, Herold MJ, Le CP, Sloan EK, Porter CJ. (2013) *J. Control. Release*, **168(2)** 200-8.

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