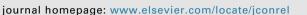
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## Size- and site-dependent distribution of therapeutic proteins into thoracic lymph

Therapeutic proteins include a diverse range of biologics, such as native proteins (monoclonal antibodies, cytokines, growth factors, enzymes, hormones, etc.), and those with complex structures, such as poly (ethylene glycol) (PEG)-conjugated proteins, fusion proteins, antibody fragments and antibody-drug conjugates. Therapeutic proteins have revolutionized the treatment of many diseases, and the number of biologics in the clinic has been steadily increasing, but not fast enough [1]. The process of absorption, distribution, metabolism and excretion (ADME) dictates protein concentrations at target and off-target sites. To date, few studies have attempted to identify the key mechanisms and factors controlling the ADME of therapeutic proteins.

In this issue, two fundamental studies attempt to address the gap in knowledge of key factors that drive therapeutic protein pharmacokinetics, including the impact of protein size. The paper by Dr. Rita Vanbever and her team examined the influence of molecular size, delivery site, and inflammation, on the ADME of PEGylated antibody fragments following pulmonary delivery [2]. Dr. Natalie Trevaskis and her group determined, for the first time, the sites of extravasation and lymphatic distribution of intravenously (IV) administered therapeutic proteins as a function of protein size [3].

Therapeutic proteins < 20 kDa in size are typically cleared via the lymphatics following subcutaneous (SC) or intramuscular (IM) administration [4]. This contrasts to small molecule drugs which are primarily transported from injection sites via the draining blood capillaries, since the flow of blood through vascular capillaries is ~100-500 times faster than the flow of lymph, thereby promoting uptake into the blood. Proteins are transported via the lymphatics, because their transport across the blood vascular endothelium is hindered. Additionally, convective flow from blood to lymph may promote transfer of larger molecules to lymph. Thus, therapeutic proteins display increased lymphatic distribution when compared with small molecule drugs following SC or IM administration. Interstitial administration of therapeutic proteins has been shown to result in better targeting and treatment of diseases involving the lymphatics, such as cancer metastases, infections, and inflammatory diseases [4].

Therapeutic proteins, however, are often IV administered. To determine the sites of lymphatic access of IV administered therapeutic proteins the Trevaskis team developed and optimized unique methods to collect hepatic, mesenteric, and thoracic lymph from rats. Four different sized therapeutic proteins used in their study were: native interferon (IFN)  $\alpha$ 2b (19 kDa), PEGylated IFN  $\alpha$ 2b (IFN-PEG12, 31 kDa), PEGylated IFN  $\alpha$ 2a (IFN-PEG40, 60 kDa), or trastuzumab (150 kDa). They were administered via short IV infusion, and plasma and lymph concentrations of the proteins determined. 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 with native IFN (0.9% dose). Conversely, the thoracic lymph to plasma concentration ratio (i.e., efficiency of extravasation and transport through the interstitium to lymph) was higher for the smaller proteins IFN and IFN-PEG12 (at 90-100% vs 15-30% for trastuzumab and IFN-PEG40). The lower total recovery of IFN and IFN-PEG12 in thoracic lymph reflects more rapid systemic clearance, and thus, lower systemic exposure. Overall, lymphatic distribution may be enhanced by administration of smaller proteins with lower systemic clearance. These findings provide guidance on the optimal protein properties to enhance distribution to lymphatic target sites.

Interestingly, the major sites of lymphatic access of all four administered therapeutic proteins were the liver and mesentery. This is because of the high lymph flow rates in these regions, as well as enhanced extravasation and transfer to lymph due to the permeable nature of the fenestrated and sinusoidal blood capillaries in the mesentery and liver, respectively. The smaller (< 31 kDa) proteins more readily extravasated and accessed the mesenteric lymphatics, whereas the larger proteins gave more specific access to the liver lymphatics. This also provides guidance on optimal protein properties to enhance distribution to target sites in the liver, mesentery and their draining lymphatics.

The studies by the Trevaskis team are important for successful translation of the therapeutic proteins to clinical applications. The fundamental understanding of the pharmacokinetics and distribution of therapeutic proteins is essential in avoiding costly clinical studies. Their finding points to a clear path of optimizing therapeutic protein design and delivery, and thus, how to better target and treat disease conditions involving the deep visceral lymphatics (such as localized cancers, inflammatory diseases, infections and organ transplantation). The work by the Trevaskis team in successful development of the original techniques to collect lymph from specific sites will also enable future work to evaluate the role of regional lymphatics in health and disease.

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