High density lipoprotein promotes targeted delivery into lymph and lymph nodes: A viable carrier for immunotherapies and vaccines

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Background: The lymphatic system is a network of vessels, nodes and tissues that circulate immune cells and provide a site for antigen presentation and immune activation. Promoting the delivery of immunotherapies and vaccines to target cells within the lymphatics has the potential to enhance therapeutic efficacy and lower off-target side effects. This can be achieved through the use of nano-sized carriers, which, upon interstitial administration (SC, IM), are too large to access blood capillaries and therefore gain access to lymphatic capillaries draining the injection site (Trevaskis, Kaminskas & Porter 2015). Recent studies have demonstrated that an endogenous nanostructure, high density lipoprotein (HDL), is returned from peripheral tissues to the systemic circulation *via* the lymphatic system (Martel *et al.*, 2013; Lim *et al*., 2013). This intrinsic lymph-targeting property suggests the potential to use HDL as biocompatible drug delivery carrier for immunotherapies and vaccines. However, there are various subclasses of HDL that exist in nature and it is not clear whether there is preferential lymph access for specific types of HDL.

Aim: To characterize various types of endogenous and synthetic HDLs and determine the impact of HDL properties on lymph uptake and lymph node (LN) retention profiles.

Methods: Endogenous HDLs were isolated from biological fluids (rat lymph, rat plasma and human plasma) by density gradient ultracentrifugation and separated into two subclasses (D-HDL of density ~1.10-1.18 g/ml and L-HDL of density ~1.06-1.10 g/ml). Synthetic HDLs (rHDLs) were prepared by lipids and apolipoprotein A-I (the main protein component of HDL). All HDLs were characterized for physical properties (size, shape, surface charge). To assess lymph uptake, HDLs were radiolabelled and SC administered into the hind leg of thoracic lymph-cannulated female Sprague-Dawley rats.

Results: Endogenous HDLs of the same density range had comparable size (~10 or ~20 nm for D-HDLs and L-HDLs, respectively) regardless of source and all appeared spherical. The sizes were successfully mimicked by the prepared rHDLs which appeared discoidal in shape. Overall, all HDLs and rHDLs had slightly negative surface charge. When lymphatic transport was assessed, all HDLs were directly transported into the lymph and not blood circulation as suggested by the high lymph to plasma concentration ratios (up to 100: 1) of the associated radiolabels during the first 12 hours post-dose. This was further confirmed by specific accumulation of the radiolabel dose in the LN draining the injection side of the leg but not on the opposite side. Comparing the various HDL types, certain endogenous HDLs displayed significantly higher lymphatic transport (~10-20% *vs* ~5-10%) and LN retention when compared to the other HDLs despite similarity in size, shape and surface charge. Further studies will investigate whether this was due to variation in the composition of the HDLs, which may alter their interaction with surrounding cells at the injection site or within LN.

Conclusion: This study has characterized HDL from various sources and confirmed their lymph and LN targeting properties. There is variation in lymph uptake and LN retention across different HDLs, which appeared to be independent of HDL physical properties. A better understanding of the HDL characteristics that enhance lymph and LN uptake is relevant for optimizing the design of HDL as a carrier for immunotherapies and vaccines.

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