ORIGINAL ARTICLES

Development of mannose functionalized dendrimeric nanoparticles for targeted delivery to macrophages: use of this platform to modulate atherosclerosis

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Dysfunctional macrophages underlie the development of several diseases including atherosclerosis where accumulation of cholesteryl esters and persistent inflammation are 2 of the critical macrophage processes that regulate the progression as well as stability of atherosclerotic plaques. Ligand-dependent activation of liver-x-receptor (LXR) not only enhances mobilization of stored cholesteryl ester but also exerts anti-inflammatory effects mediated via trans-repression of proinflammatory transcription factor nuclear factor kappa B. However, increased hepatic lipogenesis by systemic administration of LXR ligands (LXR-L) has precluded their therapeutic use. The objective of the present study was to devise a strategy to selectively deliver LXR-L to athero-sclerotic plaque-associated macrophages while limiting hepatic uptake. Mannose-functionalized dendrimeric nanoparticles (mDNP) were synthesized to facilitate active uptake via the mannose receptor expressed exclusively by macrophages using polyamidoamine dendrimer. Terminal amine groups were used to conjugate mannose and LXR-L T091317 via polyethylene glycol spacers. mDNP-LXR-L was effectively taken up by macrophages (and not by hepatocytes), increased expression of LXR target genes (ABCA1/ABCG1), and enhanced cholesterol efflux. When administered in-travenously to LDLR−/− mice with established plaques, significant accumulation of fluorescently labeled mDNP-LXR-L was seen in atherosclerotic plaque-associated macrophages. Four weekly injections of mDNP-LXR-L led to significant reduction in atherosclerotic plaque progression, plaque necrosis, and plaque inflammation as assessed by expression of nuclear factor kappa B target gene matrix metalloproteinase 9; no increase in hepatic lipogenic genes or plasma lipids was observed. These studies validate the development of a macrophage-specific delivery platform for the de-livery of anti-atherosclerotic agents directly to the plaque-associated macrophages to attenuate plaque burden. (Translational Research 2018;193:13–30)

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AT A GLANCE COMMENTARY

Background

No modalities are currently available to directly deliver therapeutic agents to atherosclerotic plaque-associated dysfunctional macrophages to modulate plaque size or characteristics. Herein, we describe the development of a mannose-functionalized dendrimeric nanoparticle platform for specific delivery of LXR ligand to plaque-associated macrophages. In contrast to increased hepatic lipogenesis associated with systemic delivery of LXR ligands, no dyslipidemia was noted while atherosclerotic plaque size as well as plaque inflammation or necrosis was reduced.

Translational Significance

Development of a macrophage-specific delivery platform represents a significant advance in direct delivery of pharmaceutical agents to atherosclerotic plaque-associated macrophages to beneficially modulate disease process and improve outcomes.

INTRODUCTION

Macrophages are effector cells that not only play a major role in innate and adaptive immunity, but also play an important role in tissue repair and homeostasis. However, normal physiological functions of macrophages are perturbed in several diseases including metabolic diseases such as atherosclerosis, diabetes, and obesity. Therefore, targeting the recruitment, activation, or regulation of dysfunctional macrophages represents a promising therapeutic strategy. With the advancement of nanotechnology, development of nanomedicines to efficiently target dysfunctional macrophages can strengthen the effectiveness of therapeutics and improve clinical outcomes.[1] Several macrophage-specific surface receptors have been described including mannose receptor,[2] folate receptor,[3] and TIM-4 or BAI-1[4,5] that can potentially be used for effective targeting. Although folate-functionalized nanomedicine to target cancerous tissue-associated macrophages has been described[6]; strategies to target dysfunctional macrophages in other chronic diseases such as atherosclerosis are very limited.

Among the vast repertoire of nanomedicine options, polyamidoamine (PAMAM) dendrimers offer several distinct advantages for the development of multifunctional dendrimeric nanoparticles (DNP). More than 120 terminal amine groups on PAMAM dendrimer generation 5.0 permit accessible surface modifications and also offer high buffering capacity for the unique "proton-sponge" effect desirable for endosomal escape. Furthermore, PEGylation of DNP prevents agglomeration, decreases the highly positive charge on the surface, and increases the high steric exclusion, thus extending the circulation time in the blood.[7-9] We have successfully developed several PAMAM-derived nanoparticles and demonstrated their use for enhanced delivery of drugs[10] as well as for gene delivery.[11] Furthermore, functionalized PAMAM dendrimer-triglycine-Epidermal growth factor nanoparticles were demonstrated to successfully deliver drugs or nucleic acids via specific receptor binding.[12] Thus, PAMAM dendrimer-based DNPs represent a viable starting point to develop nanomedicines to target dysfunctional macrophages.

Atherosclerosis is one of the many metabolic diseases where dysfunctional macrophages play a causal role and are involved in all stages of its development. The 2 main and causally related characteristics of the dysfunctional macrophages in atherosclerosis are accumulation of cholesteryl esters (CE) and inability to appropriately resolve inflammation.[13,14] Intracellular CE accumulation in macrophages can be reduced by enhancing the removal of unesterified or free cholesterol (FC), a process rate-limited by intracellular CE hydrolysis catalyzed by neutral CE hydrolase (CEH),[15,16] and earlier studies from our laboratory have demonstrated CEH overexpression-mediated increase in CE mobilization.[17-19] FC generated by CEH-mediated hydrolysis of CE becomes available for ApoA1 or High density lipoprotein-dependent efflux through FC transporters ABCA1/G1[20] and carried to the liver for final elimination from the body. Although overexpression of ABCA1 attenuates atherosclerosis,[21,22] deficiency of ABCA1 in macrophages enhances plaque progression.[23] Therefore, a strategy to enhance FC removal from dysfunctional macrophage foam cells by simultaneously increasing CEH activity[24] and ABCA1/G1 expression[25] is likely to attenuate foam cell formation. Activation of liver-X-receptor (LXR) is one such strategy. LXR activation in macrophages is athero-protective,[25,26] and the underlying mechanism is thought to be increased expression of ABCA1/G1 resulting in increased FC efflux