Commentary

"Blow my mind(in)" - Mindin neutralization for the prevention of atherosclerosis?

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Abstract

The hallmark features of atherosclerosis include accumulation of low-density lipoprotein (LDL) carrying cholesterol in the vessel wall, formation of lipid laden foam cells and the creation of a pro-inflammatory microenvironment. To date, no effective treatments are clinically available for increasing cholesterol efflux from vascular macrophages and inducing reverse cholesterol transport. In a recent article in Clinical Science, Zhang and colleagues identify the extracellular matrix protein mindin/spondin 2 as a positive regulator of atherosclerosis. Genetic knockout of mindin in apolipoprotein-E (apoE)$^{-/-}$ mice attenuated atherosclerosis, foam cell formation and inflammation within the vessel wall. Conversely, selective overexpression of mindin in macrophages in apoE$^{-/-}$ mice was sufficient to promote a greater severity of atherosclerosis. Interestingly, foam cell formation was closely associated with expression of cholesterol transporters (ABCA1 and ACBG1) that facilitate cholesterol efflux. Liver X receptor-β (LXR-β) is a key modulator of cholesterol transporter expression and formed direct interactions with mindin. Furthermore, the protective effects of mindin deficiency on foam cell formation were blocked by inhibition of LXR-β. This article highlights a novel role for mindin in modulating foam cell formation and atherosclerosis development in mice through direct regulation of LXR-β. Thus far, direct targeting of LXR-β via pharmacological agonists has proven problematic due to the lack of subtype selective inhibitors and associated adverse effects. Indirect targeting of LXR-β, therefore, via mindin inhibition offers a new therapeutic strategy for increasing LXR-β induced cholesterol efflux, reducing foam cell formation and preventing or treating atherosclerosis.

Atherosclerosis is a disease associated with accumulation of cholesterol within macrophages, resulting in the formation of macrophage foam cells, which form the key innate component of the chronic non-resolving inflammatory response that characterises the evolving atherosclerotic plaque [1].

Disruption of normal cholesterol homeostasis resulting in an excess of cholesterol inside macrophages within the vessel wall promotes the formation of foam cells [2]. Foam cells display a pro-inflammatory phenotype and release local inflammatory mediators such as interleukin (IL)-1β, inducible nitric oxide synthase (iNOS) and IL-6 that facilitates additional leukocyte recruitment and contributes to the pro-inflammatory status of a growing atheroma. Accumulation of intracellular cholesterol depends on the balance between cholesterol influx and efflux. Cholesterol efflux from macrophages relies on the ATP-binding cassette transporters, ABCA1 and ABCG1 which transfer cholesterol to high-density lipoprotein (HDL) [3, 4]. HDL carries excess cholesterol to the liver and eventually intestine for excretion, thus completing the process of reverse cholesterol transport (RCT). Therefore, macrophage lipid metabolism and foam cell formation represent an important target for therapeutic research in atherosclerosis.

To date, strategies aimed at modulating RCT such as cholesteryl ester transfer protein inhibitors have performed poorly in clinical trials. Another therapeutic target that has had a troubled development path is apolipoprotein A-1 Milano, a naturally occurring mutant form of apolipoprotein A-1 that is expressed in heterozygous form by a small population of people from Limone sul Garda in Italy, who have remarkably low levels of cardiovascular disease despite marked reductions in HDL [5]. Carriers of this particular mutant form display increased cholesterol efflux which may, in part, explain their reduced cardiovascular risk [6]. However, with the discontinuation of MDCO-216 (synthetic recombinant apolipoprotein A-1 Milano) in 2016 (http://www.themedicinescompany.com/investors/news/medicines-company-discontinues-development-mdco-216-its-investigational-cholesterol), alternative approaches are required if RCT is to become a viable therapeutic means by which to normalise cholesterol homeostasis in cardiovascular patients.

Each stage of the RCT process is mediated by Liver X receptors (LXRs) through their modulation of cholesterol transporters. LXRs are members of the nuclear receptor family of transcription factors and are composed of two isoforms: LXR-α and LXR-β. Both isoforms, to some degree, have overlapping tissue distribution, with LXR-α the dominant isoform in the liver and other metabolic organs but with LXR-β displaying the more widespread distribution. LXRs can act as cholesterol sensors with physiological concentrations of modified cholesterol such as 22(R)-, 24(S)- and 27-hydroxycholesterol acting as activators of LXRs with subsequent transcription of genes that carry an LXR response element (LXRE) in their

promoter region. In several cell types including macrophages, ABCA1 and ABCG1 carry LXREs [7, 8]. When intracellular cholesterol concentrations rise, LXR ligands can induce upregulation of ABCA1 and ABCG1, thus promoting cholesterol efflux and thereby acting to preserve cholesterol homeostasis in macrophages [9, 10]. LXR ligands are atheroprotective in animal models and macrophages are thought to be the primary target cell through which LXR ligands act [10] by initiating the transfer of cholesterol to HDL.

A direct correlation between the extent of cholesterol efflux from macrophages and cardiovascular disease risk has been established in humans with subjects displaying the highest level of macrophage cholesterol efflux showing a reduction in adverse cardiovascular events compared with the cohort with the lowest levels [11]. Therefore, as key mediators for promoting cholesterol removal from macrophages and the vessel wall, LXR ligands have been the target of therapeutic interest. A limiting factor in the search for therapeutic LXR ligands is their ability to induce hyperlipidaemia and hypertriglyceridemia, contraindicating their use in cardiovascular disease patients. Studies in animal models of atherosclerosis have identified selective activation of LXR-β as being protective in atherosclerosis with the hyperlipidaemic effects being ascribed to LXR-α; however, LXR-β selective drugs have thus far failed to translate from animal models to humans [12].

In the study by Zhang and colleagues [13], recently published in Clinical Science, a potential new upstream modulator of LXR-β is described. The authors investigate the extracellular matrix protein, mindin (spondin 2), a member of the mindin/F-spondin family of extracellular matrix proteins. Mindin has regulatory roles in diseases including cancer, diabetes and cardiometabolic disorders. Evidence also exists for an immunoregulatory role [14] but a specific role in modulating atherosclerosis has not been investigated until now.

Zhang and colleagues describe a role for mindin as a positive regulator of atherosclerosis. Using apolipoprotein-E (apoE)$^{-/-}$ mice deficient in mindin in addition to apoE$^{-/-}$ mice that display overexpression of mindin exclusively in macrophages, the authors employed a series of experiments to investigate the effect of mindin on plaque burden and foam cell formation before investigating the mechanisms underlying these phenotypic changes. Following initiation of high-fat diet (HFD) feeding, apoE$^{-/-}$ mice displayed increased levels of mindin in serum from as early as 6 weeks on HFD, with serum levels steadily rising to a peak at 28 weeks HFD (the end point of the study). Crossing mindin$^{-/-}$ mice with apoE$^{-/-}$ mice yielding mindin$^{-/-}$apoE$^{-/-}$ double knockouts resulted in a 60% decrease in plaque burden following 28 weeks HFD compared with apoE$^{-/-}$ control mice and the remaining plaques displayed a more stable plaque phenotype with reduced lipid concentration and greater vascular smooth muscle cell content. The latter data is in line with previous results demonstrating that mindin deletion increased smooth muscle cell proliferation in a wire-injury mouse model [15].

Importantly, these results were not dependent on circulating lipid levels, which were equivalent between the two groups. Immunofluorescence staining of plaques revealed localisation of mindin was mostly confined to lesional macrophages with mindin deficient arteries displaying reductions in several key inflammatory mediators: IL-6, iNOS and the chemokine (C-C motif) ligand 2, also known as monocyte chemoattractant protein 1. To ascertain whether the athero-protective effect of mindin deficiency is dependent on its function within the aortic wall or in hematopoietic cells, four different groups of chimeras were generated by injecting bone marrow (BM) cells from apoE$^{-/-}$ or mindin$^{-/-}$apoE$^{-/-}$ donors into apoE$^{-/-}$ or mindin$^{-/-}$apoE$^{-/-}$ recipient mice subjected to whole-body irradiation. Results clearly indicated mindin deficiency is more important in BM-derived hematopoietic cells than it is in vascular wall cells. Consistent with this observation was the reduced foam cell formation observed in BM derived macrophages (BMDMs) isolated from mindin$^{-/-}$apoE$^{-/-}$ mice treated with ox-LDL. This result prompted the authors to consider mindin may be having a regulatory role in cholesterol trafficking. Mindin deficiency was associated with enhanced expression of ABCA1 and ABCG1 but not receptors or enzymes associated with cholesterol uptake or processing, confirming mindin was specifically influencing the efflux of cholesterol from macrophages. Utilizing apoE$^{-/-}$ mice with macrophage-specific overexpression of mindin (Lyz2-mindin-TG) resulted in a worsening of atherosclerosis, increased lipid accumulation and increased macrophage derived inflammatory mediators. A similar increase in foam cell formation was observed in BMDMs from Lyz2-mindin-TG *in vitro*. Given the importance that LXR-β has in regulating ABCA1 and ABCG1 expression, the authors investigated a mechanistic link between mindin and LXR-β on cholesterol efflux. LXR-β was increased in the aorta and ox-LDL treated BMDMs derived from mindin$^{-/-}$apoE$^{-/-}$ mice with co-immunoprecipitation confirming direct interactions between mindin and LXR-β. Finally, to establish a direst mechanistic link between mindin and LXR-β, foam cell formation and ABCA1 and ABCG1 expression in BMDMs derived from apoE$^{-/-}$ and mindin$^{-/-}$apoE$^{-/-}$ mice were assessed following gene silencing of LXR-β. The inhibition of foam cell formation generated by loss of mindin was reversed by LXR-β inhibition with comparable results observed for ABCA1 and ABCG1 expression.

Considering the important role LXR-β has in modulating cholesterol efflux, a word of caution is warranted regarding the lack of data on LXR-α which is also expressed in macrophages with ABCA1 also being a direct target of LXR-α. Indeed, there is some controversy as to which LXR subtype has the most relevance when it comes to modulating atherosclerosis in mouse models [16]. Due to the high homology between the two LXR isotypes (only one amino acid difference in the ligand binding site), subtype specific ligands are challenging to create and those LXR-β subtype selective ligands that have been tested in animals and

humans have been associated with increased lipid levels [12]. Nevertheless, LXR-β activation remains an attractive therapeutic option, particularly if macrophages are the primary cell type targeted. The relative scarcity of LXR-β in the liver compared with LXR-α may reduce the risk of hepatic steatosis, a side effect of non-selective LXR ligands in animal models [12]. Future studies will be required to ascertain if mindin inhibition has a modulating influence on LXR-α and the relevance, if any, this effect has on the pathology and potential adverse effects. Lipid metabolism is markedly different in rodents than in humans and while the lack of increase in circulating lipids noted in mindin$^{-/-}$apoE$^{-/-}$ mice is encouraging, extrapolating the effects of LXR activation on lipid metabolism from animal models to humans has previously proved unreliable. For example, BMS-852927, an LXR-β selective partial agonist that increased RCT in cynomolgus monkeys was associated with hyperlipidaemia in humans, effects not observed in the primate model, despite similar efficacy in inducing RCT [12]. It is very possible such effects could be avoided by specific targeting of macrophage LXR-β thus avoiding adverse effects on hepatic liver metabolism.

In summary, the data published by Zhang and colleagues reveal a new role for mindin as a pro-atherogenic factor. This role is due to attenuation of foam cell formation and promotion of cholesterol efflux following mindin deficiency. That the effects of mindin ablation on ABCA1 and ABCG1 expression and foam cell formation could be reversed by inhibition of LXR-β suggests mindin directly inhibits LXR-β in an atherosclerotic mouse model thus reducing cholesterol transporters and cholesterol efflux. Considering the difficulties in designing high potency specific LXR-β ligands and the adverse events associated with pharmacological LXR agonists to date, this study offers a rationale for designing mindin inhibitors as a novel mechanism for enhancing macrophage LXR-β activation and promoting cholesterol efflux as a therapeutic strategy in cardiovascular disease.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ApoE, apolipoprotein-E; BM, bone marrow; BMDMs, bone-marrow derived macrophages; HDL, high-density lipoprotein; HFD, high-fat diet; iNOS, inducible nitric oxide synthase; IL, interleukin; LXR, Liver X receptor; LDL, low-density lipoprotein; LXRE, LXR response element; ox-LDL, oxidised low-density lipoprotein; RCT, reverse cholesterol transport.

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