The role of cholesterol metabolism in multiple sclerosis: from molecular pathophysiology to radiological and clinical disease activity

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Abstract:

Multiple sclerosis (MS) is a chronic inflammatory disease of the CNS leading to demyelination and axonal degeneration. An increasing body of evidence suggests that lipid metabolism is associated with adverse clinical and MRI outcomes in MS. In this review we summarize the findings of association between low-density lipoproteins (LDL), high-density lipoproteins (HDL), their apolipoproteins and oxysterols with clinical and radiological disease activity in MS. However, the link between causality and lipid metabolism is still to be elucidated after more research is conducted and underlying MS pathophysiology is explained.

Introduction:

MS is a chronic inflammatory disease of CNS leading to myelin and axonal loss associated with consequent neurological disability. There is increasing evidence showing the important role of lipid metabolism in the pathogenesis and disease activity of MS[1-3]

Cholesterol ($C_{27}H_{46}O$) is a sterol lipid that plays an essential role in immune regulation and signalling[4], it is an important of constituent of myelin[5], and precursor to steroid hormones and vitamin D[6] and most importantly an essential structural component of all cell membranes. Therefore it is not surprising that dysregulation of cholesterol metabolism and associations with disease progression have been reported in diverse autoimmune and inflammatory disorders, such as rheumatoid arthritis[7], systemic lupus erythematosus (SLE) [8], inflammatory bowel disease[9] and multiple sclerosis (MS).

Cholesterol is essential for physiological functioning of neurons[10]. It is one of the main structural components of cell membranes and myelin and is required for dendrite and synapse formation[11]. Cholesterol depletion in the brain contributes to dendritic spine and synapse degeneration[12]. Cholesterol is hydrophobic and insoluble in water. It is transported in the circulation by lipoprotein particles comprising a hydrophobic lipid core and an apolipoprotein molecule. Apolipoproteins are essential lipoprotein components and play a structural role as well as mediate lipoprotein function including interactions with other lipoproteins, receptors, transport proteins and cells. Cholesterol in peripheral circulation is transported by multiple lipoprotein particles which are unable to cross the blood-brain-barrier (BBB). Therefore, cholesterol in the CNS is produced de novo[13] and its transport within the CNS is mediated by high density lipoprotein-like particles containing apolipoprotein E (ApoE)[14].

Cholesterol is metabolised into oxysterols, oxidised forms of cholesterol which are able to cross the BBB[15]. n the peripheral circulation the main oxysterol is 27-hydroxycholesterol

(27-OHC) produced in the liver by cytochrome CYP27A1, whereas in the CNS the major oxysterol is 24S-hydroxycholesterol (24-OHC) produced by cytochrome CYP46A1[16]. Indeed, 24-OHC is the major route for cholesterol elimination in the brain as 99% of 24-OHC crosses the BBB and enters the peripheral circulation; but only 1% of 24-OHC enters the cerebrospinal fluid[16]. Oxysterols in the peripheral circulation are then converted into bile acids in the liver and eliminated.

Liver X receptors (LXRs) are nuclear transcription factors with an important role in maintaining lipid homeostasis in the brain [17]. There are two isoforms of LXRs, LXRα and LXRβ, which are encoded by the genes NR1H3 and NR1H2[18]. Interestingly, some mutations of the NR1H3 gene encoding one of the isoforms of LXR are associated with greater risk of developing progressive MS[19]. LXRs are activated by oxysterols, such as 24-OHC[20] and regulate the expression of multiple lipid metabolism genes including reverse cholesterol transporters, ATP-binding cassette family-A1 and G1 (ABCA1/ABCG1) and APOE[3, 21]. Importantly, LXR activation by 24-OHC upregulates expression of ABCA1 transporters[22], which actively promote efflux of 24-OHC from neuronal cells: this is a key mechanism for cholesterol elimination from the brain helping to prevent its toxic accumulation in neuronal tissue [23]. A recent comprehensive study shows that LXR activation is triggered as a general response to myelin-laden microglia and macrophages after acute demyelination[24]. This suggests that acute demyelinating events may cause increased cholesterol efflux from the CNS. We summarize the above-mentioned metabolic pathways in Figure 1.

MS is characterized by BBB dysregulation caused by inflammatory chemokines and cytokines[25]. Increased BBB permeability allows transport of larger molecules (e.g., albumin and immunoglobulins) and increased extravasation of immune cells into the CNS[26]. Under physiological conditions, peripheral blood lipoproteins cannot pass through the BBB. However, when the BBB disrupted, low density lipoprotein (LDL) can enter the CNS and

undergo oxidisation and promote production of proinflammatory cytokines such as interleukin-6 and 8 (IL-6, IL-8), or tumour necrosis factor-α (TNF-α) aggravating the neuro-inflammatory injury[27]. Moreover, the destruction of myelin, subsequent release of cholesterol and increased cholesterol metabolism into 24-OHC as described above [16, 24] can promote disruption of redox homeostasis further increasing oligodendrocyte cell death and forming positive feedback loops[28].

The aim of this short review is to summarize the current knowledge, and discuss future directions in the research of the role cholesterol metabolism in pathogenesis of MS.

Methods:

We performed a review of literature about lipid metabolism research and its connection to clinical and radiological disease activity in MS using PubMed. No restrictions were placed on country or publication date. We used the search terms: high-density lipoprotein (HDL), low-density lipoprotein, apolipoprotein, LXR AND multiple sclerosis AND MRI. We included publications published between 1995 and 2021. We also assessed the references of the articles identified. Selection criteria included English language, focus on disease activity measures and qualitative data.

Results:

HDL and apolipoprotein A1

Apolipoprotein A-1 and A-2 (ApoA-1 and ApoA-2) are the characteristic apolipoproteins of HDL particles. HDL promotes the efflux of cholesterol from cells into peripheral circulation, has antioxidant enzymes such as paraoxonase[29], and has anti-inflammatory functions by inhibiting the expression of adhesion molecules on the endothelial cell membrane and by inhibiting the recruitment of monocytes[29].

Relative increased HDL-cholesterol (HDL-C) is associated with decreased grey matter volume loss[30]. This is confirmed over long-term follow-up when a relative increase in HDL-C and ApoA1 levels over 5-years correlates with lower grey matter and cortical volume loss and with lower conversion rate to secondary progressive MS[31]. Interestingly, higher HDL-C levels correlate with lower mean transit time on perfusion weighted MRI indicating higher blood flow (e.g., higher perfusion of blood in the brain vessels)[32]. Conversely, high total cholesterol (TC)/HDL-C ratio is associated with faster disability progression[33, 34]. Construction of cumulative genetic risk scores show that in patients with more than four risk alleles in lipid related single-nucleotide polymorphisms (SNPs) and lower HDL-C levels – or higher TC/HDL-C ratio – have significantly higher annual Expanded Disability Status Scale (EDSS) progression rate suggesting a gene-environment interaction between cholesterol metabolism and disability progression[35]. Higher HDL-C levels are associated with lower number and volume of contrast-enhancing lesions (CEL)[36]. In addition, higher HDL-C levels are associated with higher vitamin D3 levels[36] possibly due to connected biochemical pathway of cholesterol and vitamin D3 through 7-dehydrocholesterol. There is also a connection between higher HDL-C levels and better BBB integrity as assessed by lower albumin quotient[37] supporting its neuroprotective properties. Importantly, in autoimmune disorders, such as SLE[38] is observed that systemic inflammation, oxidative stress, and autoimmunity in SLE patients induce changes in HDL size distribution and proteomic and lipidomic signatures resulting in the formation of proinflammatory, dysfunctional HDL. Also, MS patients have increased levels of smaller LDL, increased levels of small HDL, larger VLDL, and a higher lipoprotein insulin resistance (LP-IR) index. These alterations are suggested to be associated with modifications of HDL main protein component ApoA-I and an impaired ability of HDL to suppress inflammatory activity [39].

LDL and apolipoprotein B

As mentioned above, LDL particles can only pass through dysfunctional areas of the BBB, caused for example by ongoing inflammation[26]. Influx of particularly oxidized LDLs (oxLDL) through the disrupted BBB, promotes the production of proinflammatory cytokines such as IL-6, IL-8, TNF-α in CNS[27].

Indeed, MS patients have increased serum oxLDL levels and very low-density lipoprotein (VLDL) lipid subfractions compared to healthy controls[33, 40], both are positively correlated with increased EDSS[41]. In this context, it is not surprising that that higher serum LDL-cholesterol (LDL-C) levels positively correlate with a higher number of CELs[42], higher number of T2 lesions, higher number of active (e.g., new or enlarging) T2 lesions[31, 43], and with EDSS progression[36]. Also, ApoB (the main apolipoprotein of LDL particles) levels correlate with higher number of active T2 lesions[44]. Surprisingly, one study (not replicated yet) showed that adverse lipid profiles, including high LDL-C level are not associated with higher risk of clinical relapse[45]. The authors proposed that altered lipid profile might drive progression of disability independent of relapse activity by other, not yet fully understood mechanisms.

Oxysterols

Oxysterols are oxidised derivatives of cholesterol. Many oxysterols are enzymatically produced by specific mitochondrial or microsomal oxidation systems involving cytochrome P-450 enzymes and some are produced by oxidation by reactive oxygen species[46]. One of the most important functions of oxysterols is the regulation of cholesterol homeostasis by binding to different types of receptors such as LXR nuclear orphan receptor, oxysterol binding protein or LDL receptors[46].

The principal oxysterol resulting from cholesterol metabolism in the brain is 24-OHC[16]. Myelin sheets are the major source of cholesterol in the brain, hence increased 24-OHC levels are considered a proxy for increased myelin destruction[47]. Patients with CELs have elevated levels of 24-OHC and surprisingly also 27-OHC in serum, although the mechanism of 27-OHC elevation is not clear[48]. Whether the increase of 27-OHC is associated with peripheral demyelination[49], or peripheral immune activation or other pathophysiological processes remains to be elucidated. Higher 24-OHC correlates with lower normalized brain volume[50]. Surprisingly, higher EDSS and higher age correlate with decreased serum 24-OHC[51, 52]. This may be explained by the decreased turnover of cholesterol in elderly patients due to loss of neurons over time as well as considerably lower number of demyelinating events by increasing age. Another important property of 24-OHC is the ability to disrupt redox homeostasis in neuronal cells[53]. This disruption causes lipid accumulation in neuronal cells leading to increased levels of fatty acid degeneration products which in turn leads to increased oxidative stress[53]. Furthermore, 24-OHC induces lipid peroxidation leading to cholesterol autooxidation[53]. Both lipid accumulation and peroxidation are potent inducers of oligodendrocyte cell death[54]. These results agree with findings of increased levels of 24-hydroxylase – the enzyme needed to produce 24-OHC – found in perivascular macrophage infiltrates which represent a major and early histopathological hallmark of MS pathogenesis[55].

Effect of treatment on lipid metabolism

Interferon-β1A (INF-β1A) decreases not only total cholesterol, and HDL-C, but also LDL-C during the first four weeks of treatment[56]. However, the levels of these lipoproteins gradually increase back to their baseline levels over a 4-year follow-up period[56]. A decrease of HDL-C levels (by 6.1% in average) following INF-β1A initiation correlates with decreased whole-

brain and grey matter volume loss over following 4-years[56]. Alteration of lipid metabolism after interferon treatment initiation is observed in other diseases as well, such as breast carcinoma or hepatitis[57, 58]. Interestingly differential lipid profiles at baseline or early after the initiation of INF-beta treatment were able to distinguish between patients who went on to develop anti-drug antibodies to interferon after 12 months of treatment. In particular, elevated HDL-triglyceride content and elevated VLDL subsets in patients that went on to develop anti-drug antibodies suggesting that distinct lipoprotein signatures could be involved in the mechanisms driving immunogenicity[59].

Other treatments including, fingolimod and dimethyl fumarate treatment lead to increased HDL-C levels[60]. Before treatment initiation only 26% of patients show the recommended HDL-C levels, while 3 months after treatment initiation this number increases to 43% in case of fingolimod and 47% in case of dimethyl fumarate. Hence, it appears that response of HDL-C levels is specific to different disease modifying treatments.

When looked at oxysterol levels, natalizumab treatment decreases CSF concentration of 24-OHC and 27-OHC[61], suggesting its major anti-inflammatory effect including an improvement of BBB integrity.

As for future treatment alternatives, a recent study shows higher remyelination rate in squalene – a cholesterol precursor - treated mice models after a demyelinating event[24]. Furthermore, the same study shows better longitudinal clinical outcome in experimental autoimmune encephalomyelitis (EAE) mice models, who are prophylactically fed with squalene. This suggest that remyelination after/during the acute demyelinating event might be enhanced by external support of de-novo cholesterol production. However, if dietary squalene gets across the BBB and serve as a precursor for de-novo cholesterol synthesis in the brain is yet to be confirmed.

Statin treatment in MS patients

In the context of the previously discussed lipid profile changes in MS patients it is not surprising that a disease modifying and neuroprotective effect of statins has been suggested[62]. In EAE mice models, atorvastatin shows an immunomodulatory effect suggesting a potential benefit also in humans[63]. However, several clinical trials show no effect of high dose statin treatment on disease progression or relapse rate in patients with relapse-remitting form of MS[64]. This might be explained by the fact that statins inhibit HMGCR (3-hydroxy-3-methylglutaryl-coenzymeA reductase) - a rate limiting enzyme in cholesterol production - which prevents cholesterol formation and remyelination. On the other hand secondary progressive patients treated with simvastatin show slower disability progression implicating its possible neuromodulatory effect[64]. Further research on larger cohorts and using wider range of statins is needed to properly evaluate their effect on disease activity. At the moment a phase III, double blind, randomised, placebo controlled trial (1:1) in patients with secondary progressive MS (SPMS) is being conducted to evaluate the effectiveness of simvastatin (80 mg) in slowing disability progression over a 3-year follow-up[65].

Discussion

Cholesterol is essential for the physiological functioning of the immune and nervous systems [4-6, 10, 66]. Relatively strong correlation between lipid metabolism and MS activity measures provoked research of pathophysiological mechanisms involved in this association. Lipid metabolism measures are consistently associated with increased clinical and lesional radiological disease activity[31, 42, 43, 50] resulting neurodegeneration[30, 37]. Immunomodulatory MS treatments induce treatment-specific changes of cholesterol levels and its metabolites (Figure 2)[56, 60, 61].

Although, there is consistent association between altered cholesterol metabolism and MS disease activity, there are still a lot of unanswered questions essential for untangling this relationship. Firstly, we do not know the exact pathophysiology involved in this relationship limiting our ability to assess causality. It is unknown whether dyslipidaemia drives MS pathophysiology, or MS associated inflammation affects cholesterol metabolism. Therefore, large sample size population-based studies confirming higher frequency of altered cholesterol homeostasis in MS patients are lacking. It is also not known whether frequently observed increased LDL-C levels in MS patients are associated with atherosclerosis and higher cardiovascular risk or not [33, 40]. In this context, there is a need of further mechanistic[19] research to deepen our understanding of biochemical mechanisms behind the role of lipids in MS pathogenesis. As most studies in this area are either cross-sectional or retrospective, we suggest that prospective interventional studies might provide other information about causality, bring new insight into MS pathophysiology and identify new treatment targets[67]. Finally, some contradictory findings o previous research may be caused by the lack of distinction between different lipoprotein subclasses as well as missing data on impaired functionality of different lipoprotein species, hence future research is needed to address this challenge(add 39).

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