REVIEW ARTICLE

Brain cholesterol and the role of statins in neuroprotection

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Abstract

The class of drugs known as statins is attracting great interest for their efficacy in the treatment of obesity and cardiovascular disease. However, they may be effective in the treatment of certain neurodegenerative diseases as well. Thus, interest in the interaction of circulating and brain cholesterol has significantly increased. Recent research has raised the question whether statins are able to affect the metabolism of brain cholesterol. Actually, defects in brain cholesterol metabolism have been shown to be implicated in certain neurodegenerative diseases. Despite considerable efforts, there is a lack of information concerning the basic pharmacokinetics and pharmacodynamics of statins in the brain due to poor drug permeability across the blood-brain barrier. The purpose of this review is to examine biosynthesis of cholesterol in the brain, distribution of statins and their possible neuroprotective actions. Moreover, review aims to examine types of transporters of statins across the blood-brain barrier.

Introduction

Statins are generally recognized for their efficacy in the treatment of obesity and cardiovascular disease (Taylor *et al* 2012). But there is growing evidence to support the hypothesis that statins may act as neuroprotectants in several neuropathological conditions or as potential agents in the prevention of neurodegenerative diseases, particularly Alzheimer´s disease (Kandiah & Feldman 2009; Barone *et al* 2014; Zvěrová *et al* 2014). The association between Alzheimer´s disease and cholesterol levels has been highlighted in the last decade. Since dysregulation of cholesterol homeostasis in the brain has been linked to chronic neurodegenerative disorders (Vance 2012), the treatment of neurodegenerative disease with statins was proposed by several authors as an effective emerging therapy to stop or delay the neurodegenerative process (Jick *et al* 2000; Wolozin *et al* 2000; Hajjar *et al* 2002; Silva *et al* 2013). One of the biggest problems and challenges in the development of new drugs and treatment strategies for neurodegenerative (CNS) diseases is the difficulty of passing the drugs across the blood-brain barrier (BBB). Mechanisms for statin uptake into the brain include diffusion and active transport across the BBB depending on the acid or lactone form of statins (Wood *et al* 2014).

Cholesterol in the CNS

Cholesterol is the major lipid compound of the brain and the brain is the most cholesterol-rich organ (Dietschy & Turley 2001; Björkhem *et al* 2004). Approximately one quarter of the total amount of human cholesterol and its derivatives is found in the brain while the whole body cholesterol makes only up 2% of total body weight (Dietschy & Turley 2001; Dietschy & Turley 2004). This sterol is critically important for the maintenance of physiological functions in the brain such as synaptic transmission (Mauch *et al*

2001), axon guydance and growth factor signaling. A study of Koudinov & Koudinova (2001) indicated that the lack of cholesterol supply in hippocampal neurons caused the failure of synaptic plasticity and neurotransmission. Thus, defects in cholesterol metabolism lead to structural and functional CNS disorders (Di Paolo & Kim 2011). In the brain generally, cholesterol is produced by both astrocytes and oligodendroglia (Dietschy & Turley 2001; Dietschy & Turley 2004; Bjorkhem & Meaney 2004; Bjorkhem & Meaney 2015). Within the cells, the biggest reservoirs of cholesterol are found in the plasma membrane (Mesmin & Maxfield 2009). In CNS, cholesterol is unesterified and it represents the major sterol in the adult brain. Neurons, like all other body cells, must be continuously supplied by unesterified cholesterol (Cibičková 2011). About only 1% of the total cholesterol remains as esterified form, as lipid droplets (Bryleva *et al* 2010).

Majority of cholesterol present in the brain is in the form of myelin that surrounds axons and facilitates the transmission of electrical signals (Snipes & Suter 1998; Dietschy & Turley 2001; Quan *et al* 2003).

An excess or deficiency of cholesterol in the brain might be expected to have profound consequence, but cellular cholesterol homeostasis is tightly regulated (Brown & Goldstein 1986). This can be explained by an efficient recycling of brain cholesterol. The metabolism of brain cholesterol differs from that of other tissues. The BBB effectively prevents uptake from the circulation (Dietschy & Turley 2001; Abad-Rodrigue *et al* 2006). Thus, there is a highly efficient apolipoprotein-dependent recycling of cholesterol in the brain, with minimal loses in the circulation (Bjorkhem & Meaney 2015). Whereas efflux of plasma lipoproteins across the intact BBB is limited, the majority of brain cholesterol is derived by *de novo* synthesis from the endoplasmatic reticulum (ER) within the nerve cells (Goldstein & Brown 1990; Di Paolo & Kim 2011). Newly synthesized cholesterol is transferred from ER to plasma membrane rapidly (DeGrella & Simoni 1982). Cholesterol is synthesized via the isoprenoid biosynthetic pathway, which starts with acetyl-CoA as substrate. For cholesterol production, at least 20 enzymes are involved (Waterham 2006). The half-life of cholesterol in the adult rat brain has been estimated to be 6 months (Andersson & al 1990), while half-life of plasma cholesterol is only a few days (Dietschy & Turley 2004). Cholesterol is necessary for brain development and its synthesis continues at a lower rate in the adult brain (Dietschy 2009). Thus, as the CNS matures and cholesterol pools in the brain become constant, the rate of *de novo* cholesterol synthesis in the brain probably decline (Thelen *et al* 2006). Thelen *et al* (2006) discovered that during aging, cholesterol synthesis is decreased in the hippocampus, while absolute cholesterol content remains at a stable level.

According to some studies, cholesterol in the intact brain is synthesized in such extent that physiological statin concentrations may have only low cholesterol reducing effect, if any (Dietschy & Turley 2001; Abad-Rodrigue *et al* 2006).

Export of Cholesterol from the Brain

In spite of the efficacy of the cholesterol recycling in the brain, a small efflux of cholesterol into the circulation is needed to maintain the steady state. Mechanisms to export cholesterol into the circulation are required to maintain homeostasis, because a sufficient availability of cholesterol is necessary for normal neuronal function. The brain has lipoprotein transport system independent of that in the peripheral circulation. There are two different pathways to efflux of cholesterol from the brain. Under steady-state conditions, excretion of apolipoprotein E (ApoE)-bound cholesterol is mediated via the cerebrospinal fluid (CSF) (Pitas *et al* 1987a). ApoE is expressed in the brain in high concentrations, such that the brain is the organ with the second highest ApoE expression after the liver (Linton *et al* 1991). This apolipoprotein is one of the major apolipoproteins in plasma (Bojar *et al* 2012) and it is the quantitatively the most important transport protein for cholesterol in the brain. The stability of ApoE in the brain requires the association with lipids (Wahrle *et al* 2004). ApoE and cholesterol are produced by astrocytes (Boyles *et al* 1985) and it is shuttled from astrocytes to neurons (Mauch *at al* 2001; Michikawa *et al* 2000; Vance & Hayashi, 2010). The capacity of this pathway is very limited and can export only 1–2 mg cholesterol per day. The interaction between ApoE-containing lipoproteins and neuronal receptors seem to be crucial for normal neuronal function (Pitas *et al* 1987b; Vance 2012).

Another, more important mechanism to efflux of cholesterol from the brain involves conversion of cholesterol into its brain specific metabolite 24S-hydroxycholesterol (24S-OH-CHOL) by cholesterol 24-hydroxylase (Lutjohann *et al* 1996; Bjorkhem *et al* 1997). This side-chain oxidized oxysterol is able to cross the BBB at a much faster rate than cholesterol itself (Pitas *et al* 1987a; Bjorkhem *et al* 1997; Bjorkhem & Meaney, 2015). The introduction of a hydroxyl group in the side chain of oxysterols (Figure 1) leads to a local reordering of membrane phospholipids (Kessel *et al* 2001). The flux of 24S-OH-CHOL through the BBB is limited to about 6–7 mg per day (Lutjohann *et al* 1995; Bjorkhem *et al* 1998).

In humans, the efflux of 24S-OH-CHOL corresponds to the uptake of a similar amount of 24S-OH-CHOL by the liver, which indicates the proprietary production of 24S-OH-CHOL in the brain (Bjorkhem *et al* 1998).

Statins and Their Effect on Brain Cholesterol

There is a growing evidence to support the hypothesis that statins may act as neuroprotectants in several neuropathological conditions (Shitara & Sugiyama 2006). Statins are well tolerated and have relatively few side

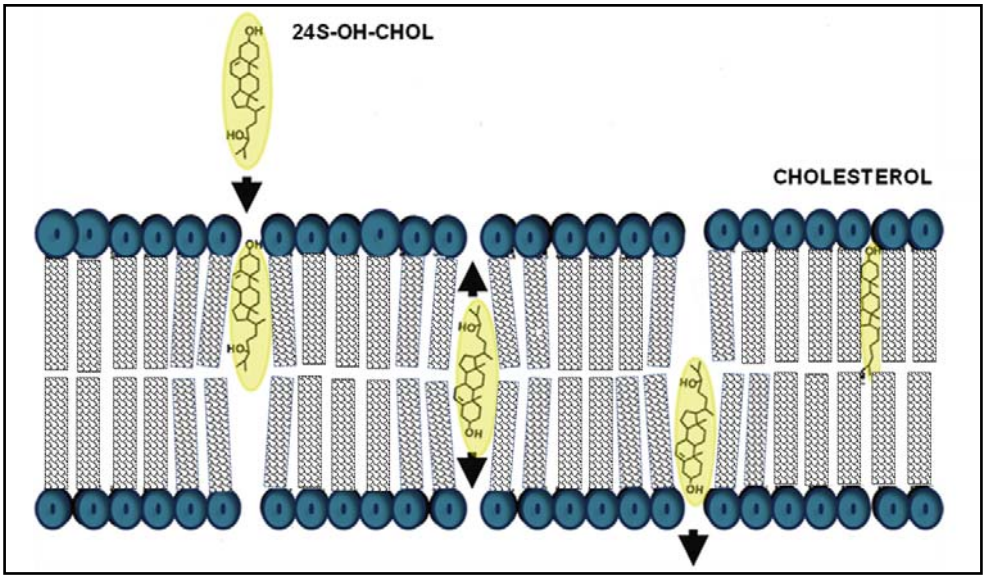

Fig. 1. The efflux of cholesterol from the brain by 24S-hydroxycholesterol. Oxysterol 24S-hydroxycholesterol (24S-OH-CHOL) is able to traverse the blood-brain barrier due to the introduction of an hydroxyl group in the side chain of oxysterols. It leads to a local reordering of membrane phospholipids**.**

effects. The primary action of statins is to inhibit cellular cholesterol synthesis. However, the cholesterol synthesis pathway has different by-products, the non-sterol isoprenoids that are important in normal cellular function (Van der Most *et al* 2009). Thus, except of cholesterol reducing effect, statins may perform their neuroprotection via modulation of isoprenoid levels.

Statins directly inhibit the first step in the biosynthesis of cholesterol, which is the conversion of the hydroxyl-methyl-glutaryl-CoA (HMG-CoA) into L-mevalonate by inhibiting HMG-CoA reductase (Shitara & Sugiyama 2006). In the CNS, HMG-CoA reductase is an enzyme which is expressed with high transcript levels in cortical, cholinergic and hippocampal neurons (Korade *et al* 2007). This enzyme catalyzes the production of L-mevalonate from HMG-CoA. L-mevalovate is the precursor of a number of different lipids such as farnesyl pyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) (Goldsetin & Brown 1990; Schonbeck & Libby 2004; Wood *et al* 2010). Statin-induced neuroprotection in some cases has been proposed to be therefore due to a reduction in FPP and/or GGPP levels (Cole & Vassar 2006; Hooff *et al* 2010; Li *et al* 2012). However, short-term statin treatment does not alter cholesterol levels in the brain (Botti *et al* 1991). Only long-term treatment can affect the cholesterol level in the brain. It was reported that more than 6 months of statin treatment reduces cholesterol level in the CSF (Fassbender *et al* 2002).

Some data suggest that statins activate a general neuroprotective mechanism. It has been demonstrated that the intake of statins is associated with a decreased incidence of Alzheimer´s disease (AD). Higher serum levels of cholesterol seem to stimulate beta-secretase, which acts on amyloid precursor protein (APP) and arise beta-amyloid (Aβ). Cholesterol facilitates deposition of Aβ into plaques, that is important for development of AD (Cibičková & Palička 2005). Several studies reported that statins reduce the production of the Aβ peptide *in vitro* (Wolozin *et al* 2006; Hoglund & Blennow 2007). Statins are a family of drugs with pleiotropic functions. They are able to decrease oxidative stress, glial activation and they could *up*-regulate endothelial nitric oxide synthase expression. In several studies, statins maintained the number of Purkinje cells and their networks in the AD cerebellum (Cibičková 2011; Kozuki *et al* 2011).

Statins can activate several neuroprotective signalling pathways. The neuroprotective effect correlate roughly with the efficacy of blocking HMG-CoA and neuroprotective impression could be reversed by addition of mevalonate or cholesterol (Zacco *et al* 2003). Some findings also suggest that statins induce neuroprotection by promoting the release of neurotrophic factors. For example, simvastatin has been demonstrated to induce expression of brain-derived neurotrophic factor (BDNF) following traumatic brain injury. The mechanism how statins yield this effect is however unclear (Wu *et al* 2008).

Statins could act as neuroprotectants through several another mechanisms (Elkid 2006). They can contribute to the neuroprotection by reduction of oxidative damage via the inhibition of endothelial $O_2^{-\bullet}$ formation (Wallerath *et al* 2003) and the increase of Cu/Zn superoxide dismutase (SOD3) activity as well as the number of functionally active endothelial progenitor cells (Landmesser *et al* 2005). Statins can reduce the production of reactive oxygen species (ROS) by inhibiting the formation and activation of nicotinamide adenine dinucleotide phosphate (NADPH)-complex (Wassmann *et al* 2001). In addition, they decrease oxidative damage by the increase of expression and activation of endothelial nitric oxide synthase (eNOS) (Kureishi *et al* 2000; Laufs *et al* 2005). Statins modulate endothe-

lial function by enhance nitric oxide production (NO) (Bellosta *et al* 2000). eNOS is inhibited by the presence of oxidized low-density lipoprotein (LDL), so statins improve eNOS expression (Laufs *et al* 1998; Liu *et al* 2009). The production of NO may affect cerebrovascular disease by enhancing vascular smooth muscle relaxation and by increasing cerebral blood flow (Sterzer *et al* 2001). To better understand the effects of statins on cholesterol metabolism in the brain it is necessary to find out both direct and indirect effects of these drugs (Beziaud *et al* 2011). Some effects of statins, described as pleiotropic effects, including positive influence on vascular injury and NO production might affect the integrity of the BBB (Banks & Erickson 2010).

All statins share the same main mechanism of action, but their pharmacokinetic profile is quite different (Shitara & Sugiyama 2006). In the pharmacodynamic and pharmacokinetic behavior of statins, hydrophilic or lipophilic nature plays a very significant role. Hydrophobic statins can easily cross the BBB, whereas hydrophilic statins are thought not to cross the barrier. There is clinical evidence that the dosage of statins can cause lower or higher permeability (King *et al* 2003).

Transport of Statins Across the Blood-Brain Barrier

The most important factor limiting statins transport into the CNS is BBB. The BBB limits the brain penetration of most CNS drug candidates. Drug transport in the CNS is highly regulated by the BBB, the CSF barriers, and by brain parenchyma. The cellular membranes of parenchyma cells act as a second „barrier" to drug permeability. The anatomical basis of the BBB is the brain microvascular endothelial barrier. The microvascular cells of the brain include endothelial cells, the pericyte, astrocyte, and nerve endings that end directly on the vascular surface (Pardridge 2007). The endothelial barrier is specifically tight at the interface with the brain astrocytes and in normal conditions can be passed using endogenous BBB transporters resulting in carrier mediated transport, active efflux transport and receptor mediated transport. This barrier exists at the level of endothelial cells of brain vasculature and maintains the brain homeostasis (Koziara *et al* 2006).

The BBB prevents diffusion of large molecules into the brain (Reese & Karnovsky 1967), therefore BBB dysfunction is assumed to contribute to brain injury. After ischemic stroke and traumatic brain injury, statins have been shown to provide neuroprotection with beneficial effects on the neuronal and neurovascular systems (Chen *et al* 2003; Wu *et al* 2008; Wible & Laskowitz 2010). The BBB can be disrupted by different ways. Disruption may be accompanied by leakage of plasma proteins into the brain. Since albumin is toxic to astrocytes (Nadal 1995) this process may be followed by vascular pathology (Lossinsky *et al* 1995) and chronic neuropathologic changes (Salahiddin *et al* 1988). The pharmacological access for inducing neuroprotection after an injury due to cerebral ischemia includes blocking of signalig pathways that initiate cell death (Mantz *et al* 2010; Moskowicz *et al* 2010). Similarly, several epidemiological evidences on the beneficial effect of statins were found in lowering the risk of developing dementia (Jick *et al* 2000; Wolozin *et al* 2000; Hajjar *et al* 2002). A few studies have shown that statins pass the BBB. The BBB can be traversed due to multiple endogenous transporters within the barrier. Mechanisms for statin uptake into the brain include diffusion and active transport across the BBB depending on the acid or lactone form of statins (Wood *et al* 2014). Hydrophobic statins (atorvastatin, simvastatin, fluvastatin, lovastatin, cerivastatin) can easily cross the BBB, whereas hydrophilic statins (rosuvastatin, pravastatin) are thought not to cross the barrier. There is clinical evidence that the dosage of statins can cause lower or higher permeability (King *et al* 2003, Tsuji *et al* 1993).

The mechanism of brain drug delivery is based on knowledge of endogenous BBB transporters and on reformulating drug structures. The main types of transporters are the adenosine triphosphate (ATP)-binding cassette (ABC) (Willyerd *et al* 2015) and solute carrier (SLC) transporters (Lin *et al* 2015). SLC is a family of membrane-bound proteins and it comprises facilitated and ion-coupled transporters (Lin *et al* 2015). ABC transporters, namely ABCA1, ABCG1 and ABCG4 are expressed by neurons to mediate cellular sterol efflux at the plasma membrane (Kim *et al* 2008). ABC transporters rely on ATP to actively pump substrates across cell membranes (Willyerd *et al* 2015).

Statins are metabolized in the brain or actively transported out of the brain. There is the evidence that hydrophobic statins interact with monocarboxylic acid transporters-4 (MCT4) to a greater extent than hydrophilic statins (Kobayashi *et al* 2006). Monocarboxylic acid transporters (MCT1, MCT2, MCT3, MCT4) have been identified in the brain. They are associated with BBB and reside in astrocytes and neurons to varying degrees (Pierre & Pellerin 2005). Atorvastatin acid, simvastatin acid and lovastatin acid were found to be transported into cells by organic anion transporter polypeptide (OATP) family, specifically OATP2 (Hsiang *et al* 1999). This polypeptide has been identified in the BBB of rats and in the choroid plexus (Lee *et al* 2013). Some studies have demonstrated the existence of both efflux and influx transporters within glial cells what highlight the complexity of drug distribution within the CNS (Decleves *et al* 2000; Hong *et al* 2000; Hong *et al* 2001; Dallas *et al* 2001; Lee *et al* 2001).

A different mechanism which could contribute to the elimination of statins from the brain is the permeability glycoprotein (P-gp, encoded by *ABCB1* gene) transporter. It is an efflux transporter and one of the most intensively studied cellular multidrug transporters associated with drug removal from cells. P-gp (also known as ABCB1 or MDR1) mediates the export of

drugs from cells located in the blood-brain barrier, in the luminal membrane of the small intestine, hepatocytes and kidney proximal tubules in many organisms (Juliano & Ling 1976; Giacomini *et al* 2003; Lin & Yamazaki 2003; Girardin 2006; Sharom 2006), serving a protective function for the body against foreign substances. P-gp is a member of the ATP-binding cassette (ABC) superfamily. It is one of the ATP dependent efflux transporters that have an important physiological role in limiting drug entry into the brain. Because P-gp is ATP dependent, it is able to transport a variety of chemical compounds against a concentration gradient (Girardin 2006; Sharom 2006). P-gp exports structurally diverse hydrophobic compounds from the cells, driven by ATP hydrolysis. The protein also plays an important physiological role in limiting drug uptake in the gut and entry into the brain (Sharom 2006). P-gp is involved in the excretion, absorption and distribution of lipophilic and amphipathic drugs (Sharom 2011).

Tight junctions reduce drug transfer between blood and the cerebrospinal fluid (CSF). P-gp is transporter that removes drugs from the brain interstitial fluid to blood or into the CSF (Eyal *et al* 2009). P-gp expression in the BBB plays an important role in limiting the entry of various drugs into the CNS. Inhibition of the P-gp transporter may lead to increased drug delivery to the brain (Eyal *et al* 2009). Several statins, particularly atorvastatin and lovastatin, have been shown to interact with P-gp at the molecular level (Holtzman *et al* 2006). Results of several studies with *in vitro* models have shown that certain statins (lovastatin, simvastatin, atorvastatin) are inhibitors for P-gp or may represent the substrates for this transporter as well (Boyd *et al* 2000; Bogman *et al* 2001; Wang *et al* 2001; Sakaeda *et al* 2002; Hochman *et al* 2004; Bogman *et al* 2001; Hirrlinger *et al* 2002). The lipid bilayer plays an important role in P-gp function, and may regulate both the binding and transport of drugs (Sharom 2006).

Conclusion

Despite well-known beneficial effects of statins in the treatment of obesity and cardiovascular disease their role in neuroprotection is just starting to study. In the brain, statins may affect cholesterol level by different biochemical pathways including direct inhibitory effect on cholesterol and isoprenoids or indirectly e.g. by modulating NOS activity. Mechanisms of statin delivery to the brain include diffusion and active transport across the blood-brain barrier. Thus, different types of statin transporters start to be a hot topic worth to study.

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