Drug delivery to the brain: How can nanoencapsulated statins be used in the clinic?

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Abstract

Statins are used for the primary and secondary prevention of cardiovascular disease by inhibiting cholesterol synthesis in the liver. Statins have also non-cholesterol-related effects, called pleiotropic effects, that arise from statins anti-inflammatory, immunomodulatory and anti-oxidant properties. These effects are especially attractive for the treatment of various brain diseases ranging from stroke to neurodegenerative diseases. Still, low brain concentrations after oral drug administration hinder the clinical application of statins in these pathologies. Pharmaceutical nanotechnologies may offer a solution to this problem, as local or targeted delivery of nanoencapsulated statins may increase brain availability. This special report rapidly summarises the potential of statins in the treatment of brain diseases and the pharmaceutical nanotechnologies that could provide a viable approach to enable these indications.

Keywords:

Statin
Neuroprotection
Nanomedicine
Drug delivery
Alzheimer's disease
Inflammation
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Stroke
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Statins: a future beyond cholesterol lowering?

In last decades, statins have emerged as one of the most successful class of drug from both a therapeutic and commercial point of view. In fact, since the approval of lovastatin by FDA in 1987, statins have become the election treatment of hypercholesterolemia leading to beneficial effects in terms of cardiovascular (CV) risk reduction. Statins have been demonstrated to be effective in primary prevention and in reducing disease progression with well-demonstrated improvements in terms of CVD morbidity and mortality [1,2]. The mechanism of action of statins is the reduction of the liver cholesterol synthesis by competitively inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, thus preventing the formation of mevalonic acid, a precursor in the *de novo* cholesterol biosynthetic pathway. This induces an increased hepatic expression of low-density lipoprotein (LDL) receptors with subsequent enhanced clearance of circulating LDL-cholesterol. In addition, it has been demonstrated that statins lower triglycerides, although to a smaller extent and increase high-density lipoprotein (HDL) cholesterol plasma levels [3,4].

However, some clinical observations related to the rapidity and magnitude of the efficacy in subgroups of patients have suggested that some cholesterol independent or "pleiotropic" effects were at play beyond the lipid-lowering actions [4]. These properties have been explained by the inhibition, *via* the same mevalonate pathway, of isoprenoid compounds, such as farnesylpyrophosphate and geranylgeranylpyrophosphate, essential for the post-transcriptional modification and subsequent subcellular localization and intracellular trafficking of a number of proteins, including signalling GTPase proteins (G proteins), such as Rho, Ras, Rac, Rap, Ral [5]. These proteins possess a regulatory role in many biochemical processes and their inhibition, affecting cells regulatory pathways, could be the explanation of the non-lipid-lowering effects evidenced for statins.

Anti-inflammatory, anti-oxidant and immunomodulatory effects are believed to be the result of the inhibition of G proteins. In the case of vascular diseases, these actions have been related to improvement of endothelial function by means of the up-regulation of endothelial nitric oxide synthase (eNOS), decrease in vascular smooth cell proliferation, reduction in platelets reactivity and lowering in pro-inflammatory cytokines and reactive oxygen species (ROS) [6,7]. Finally, statins may hinder leukocyte infiltration of inflamed tissue by blocking the β2 integrin leukocyte function antigen-1 (LFA-1). However, the actual clinical relevance of the pleiotropic effects is strongly debated and a clear demonstration of these non-cholesterol related effects appear somewhat elusive [8,9].

Despite this fact, the significance of the pleiotropic effects of statins appears to emerge from a number of new indications for which this class of drugs have been proposed [2]. In fact, many chronic diseases are characterized by dysfunctional inflammatory and/or immune responses; in this context statins have been proposed as an approach to treat the symptoms of inflammatory bowel disease [10], rheumatoid arthritis [11] and chronic obstructive pulmonary disease (COPD) [12]. Furthermore, statins have been shown to have a beneficial effect on perioperative morbidity and mortality of cardiac, as well as non-cardiac surgery patients, by reducing major adverse cardiac and cerebrovascular events, but also by possibly reducing renal injury, respiratory complications and infections [13]. Statins may have also an effect on the bone favouring the proliferation and differentiation of osteoblast and reducing osteoclasts, opening an opportunity for their use in the management of osteoporosis. In a recent meta-analysis, it has been evidenced that statins significantly reduce the risk of fracture, improve bone mineral density and osteocalcin concentrations, especially in male patient subgroups [14].

Finally, a possible chemopreventive/chemotherapeutic role of statins in cancer has attracted a lot of interest in recent years [15]. Data collected so far are often contradictory and inconclusive, being based on epidemiological studies with highly heterogeneous cases in terms of cancer type and statin use. Nevertheless, a recent meta-analysis on statins and survival in colorectal cancer evidenced a weak improvement in survival associated with statin use [16], while another study in kidney cancer patients indicated that statin use is associated with a significantly reduced risk both of cancer-specific and all-cause related mortality [17].

Pleiotropic effects are regarded however by some authors as controversial and accounting. At least in part, for the side effects of statins. In fact, inhibiting the mevalonate pathway, statins not only inhibit cholesterol biosynthesis, but also that of many other products such as coenzyme Q, selenoproteins and heme A. Muscular adverse effects, ranging from fatigue to rhabdomyolysis, are the most common side effect of statins, and led in 2001 to the withdraw from the market of cerivastatin. Hepatotoxicity, neprotoxicity, erectile dysfunction along with increased risk of developing diabetes mellitus, peripheral neuropathy and autoimmune diseases are other relevant adverse effects to take into account during statins treatment, even if in all trials published benefits exceeded all potential risks [18].

Potential central nervous systems applications of statins

Some of the most interesting novel potential indications of statins are those related to brain diseases. Several hurdles, among which the crossing of the blood brain barrier (BBB), hamper the success of drug therapies having as a target the brain and the central nervous system (CNS) [19,20]. However, some of the statins, such as simvastatin, atorvastatin, lovastatin and fluvastatin, are lipophilic in nature and have, in principle, the potential to cross the BBB [21,22] opening the opportunity for their use for treatment of brain diseases. Their action at central level could be neuroprotective, a feature that could introduce statins as a new approach for a range of diseases in which the current therapy is considered unsatisfactory [23].

The actions of statins in the brain are both cholesterol-dependant and cholesterol-independent and could be beneficial to a range of CNS disease characterized by a combination of the following pathophysiological mechanisms: inflammatory reaction, chronic immune activation, increased production of reactive-oxygen species, cell oxidative damage, altered neurotransmission, neuronal dysfunction and death, reduced cognitive and memory performance.

In fact, in addition to the previously mentioned pleiotropic effects, it is suggested that statins might be able to lower cholesterol levels also in the brain, depleting cholesterol-rich microdomains in the cell membranes (lipid rafts) and having an impact on neurotransmission, synaptogenesis and various pathophysiological proteins processing. Statins appear to activate neuroprotective pathways, such as PKB/Akt, to induce the expression of neurotropic factors, such as brain-derived neurotrophic factor (BDNF) and even to recruit stem cells [24].

One of the most straightforward and studied statins application at central level is their neuroprotective and neurorestorative role in cerebrovascular diseases, such as ischemic and haemorrhagic stroke. In this sense, statins are considered a preventive treatment of stroke and of stroke recurrence [25]. In addition, they have a role in acute ischemic stroke therapy: pre- and post-stroke statin use might reduce stroke severity, functional disability and mortality, whereas statin withdrawal leads to worse outcomes. This has been attributed to improved cerebral flow, enhanced fibrinolysis and reduced infarct size related to statin use [26].

The other major interest in the use of statin in the CNS is the increasing epidemiological evidence of the role they have in the management of neurodegenerative diseases [27].

Parkinson's disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantia nigra, probably as a consequence of neuroinflammation, oxidative stress and mitochondrial dysfunction. Despite the discordant results present in literature, a relatively recent meta-analysis of epidemiological studies correlating statin use to development of the disease indicated a significant reduction in the risk of PD [28]. The highlighted benefits are possibly due to the cholesterol lowering action, the reduction of inflammation mediators such as TNF-α, nitric oxide and superoxide and the modulation of some brain receptors, such as dopamine D1/D2 receptors and NMDA receptors [27].

Multiple sclerosis (MS) is an immune-mediated disorder in which CNS resident and invading immune cells lead to inflammation, oxidative stress, excitotoxicity, demyelination, axonal degeneration and ultimately neuronal loss. Statins may have a role in the management of MS via their anti-inflammatory and immunomodulatory actions. In particular, statins action is mediated by the inhibition of myelin-antigen presentation, of the recruitment of leucocyte into the CNS and of the activation and differentiation of pro-inflammatory T cells, redirecting the immunomodulation towards the secretion of anti-inflammatory cytokines (IL-4, IL-5, IL-10, TGF-β) and the suppression of pro-inflammatory signalling (IL-2, IL-12, IFN-γ). Also in this case, clinical trials with oral statin mono-therapy were only partially effective in showing effects on MS and some studies have been carried out with combinations of statins and IFN-β with conflicting results [29]. However, in a recent study high-dose simvastatin treatment has evidenced a significant reduction in the annualised rate of whole-brain atrophy in patients affected by secondary progressive MS, for which no satisfactory treatment exists [30].

Recently, the use of statins as a disease-modifying strategy in Alzheimer's disease (AD) has elicited substantial interest. A series of epidemiological studies had suggested an association between high blood cholesterol and AD [31]. Subsequent retrospective studies suggested a decrease in the incidence of AD and dementia for patients who were treated orally with statins [32]. The effects of statins on AD could be related to several actions: reduction of cholesterol *de novo* synthesis in the brain, leading to reduced neurofibrillary tangles and amyloid β formation; improvement of brain perfusion, reduction of atherosclerosis and cerebrovascular risk; anti-inflammatory effects and up regulation of eNOS [33]. However, recent prospective clinical trials in which statins (atorvastatin, simvastatin) have been administered orally to AD patients were disappointing and failed to provide improvements in their cognitive status [34].

Furthermore, statins may have an anti-proliferative, proapoptotic and anti-invasive effect on brain tumours. Studies on human glioma cells for example are supporting such effects as a result of proapoptotic effects mediated by lipid raft modulation, Fas translocation and activation of the phosphatidylinositol 3-kinase (PI3K)/Akt/caspase-3 pathway [35]. This role has been further supported recently by an epidemiological study in Taiwan that once more suggests that statins (all statins were considered, i.e. lovastatin, pravastatin, rosuvastatin, fluvastatin, simvastatin, or atorvastatin) use may reduce the risk of brain cancer [36].

Nanomedicines for statins drug delivery to the brain

Despite the clear potential of statins for the treatment of CNS diseases, the clinical evidences are still limited and sometime even contradictory, with risks of side effects outweighing

benefits. Furthermore, beneficial effects have been generally associated with statin high dosing. These limitations appear as a direct consequence of the pharmacology of statin oral administration. Despite good absorption, statins undergo a heavy hepatic extraction to low bioavailability values, with the only exception of rosuvastatin [3]. If we look at lipophilic statins, considered capable of crossing the BBB, not only the metabolites are more hydrophilic and then less favoured for BBB crossing, but the unmodified drug shows a high protein binding. As a consequence that not all statins can cross the BBB, and those which can might do it at relatively low concentrations [24,37].

The use of novel and more efficient dosage form able to deliver statins to the brain appear necessary to advance the use of statins. In particular, nanomedicines are extremely appealing for this application providing drug encapsulation and protection from chemical and enzymatic degradation, control of drug release, improved delivery and bioavailability in particular of poorly-water soluble drugs and targeted distribution in the body [38]. In addition, the administration of nanoencapsulated statins may contribute to reduce or eliminate altogether some of the common adverse effects related to statin therapy.

While the literature is rich of nanotechnological approaches for improving statin oral absorption [39] or their delivery to other body compartments [38], the development of statin nanomedicines for the treatment of brain diseases is a quite new topic. In fact, the application of pharmaceutical nanotechnologies for brain delivery is itself an emerging subject in medicine and only few papers have been published on the delivery to the CNS of nanoencapsulated statins. The object of this review is to highlight the potential of this approach and to foster further research in this field.

However, the approaches for using these new formulations in clinical setting appears already quite well defined: direct local administration to the brain, targeting of the BBB after intravenous injection and nose-to-brain delivery appear to be the viable options [40].

Undoubtedly, the most fascinating option is the targeting of the nanocarrier across the BBB to preferentially distribute the statin into the CNS. In this approach, nanoparticles can be targeted towards the BBB and/or specific cell types in the CNS. Several proteins and receptors, such as the transferrin receptor, have been identified on the BBB as potential targets to improve enhanced nanoparticle uptake and targeting of CNS cells [41]. A specific delivery appears even more necessary if the therapeutic target are tumoral cells, due to the necessity of spare as much as possible the healthy tissues surrounding the malignancy [42]. Generally, nanoparticles have to be administered intravenously, but recently the group led by Silvia Guterres and Adriana Pohlmann showed nanoparticle accumulation in the CNS even after oral administration [43].
Additionally, Simsek and collaborators demonstrated the possibility to accumulate atorvastatin-loaded PLGA-PEG nanoparticles coated with polysorbate 80 in the rat brain. Nanoparticles produced showed particle size in the range of 30-172 nm and atorvastatin encapsulation efficiencies up to 50%. Nanoparticles brain accumulation was evaluated by fluorescent imaging of brain tissues up to 48 hours after IV administration of 15 mg/kg nanoparticle formulation loaded with the fluorescent marker Nile red. This targeting is based on the work carried out by Kreuter that attributed to the preferential adsorption in plasma of apolipoprotein E (ApoE) to the surface of nanoparticles stabilized with polysorbate 80 [44]. The specific adsorption of ApoE on the nanoparticle corona allows its interaction with the LDL receptors present on the endothelial cells of the BBB [45].

In some approaches however, the BBB can be eliminated as a barrier either by direct brain administration to the brain region affected by the pathology through either surgery or

stereotactic injection [46] or by exploiting pathological or deliberate alterations of the BBB permeability, for example via an osmotic shock or focused ultrasounds [47]. A very recent research reported the peri-neural injections of lovastatin-loaded PLGA nanoparticles (250-300 nm, drug loading up to 25% w/w on nanoparticle weight). These nanoparticles were used for assessing nanoencapsulated statin efficacy in an animal model of acute inflammatory demyelinating polyneuropathy. Nanoparticles loaded with lovastatin administered next to the sciatic nerve (250 µl single bolus of 20 mg of nanoparticles containing 5 mg of lovastatin) significantly attenuated the autoimmune neuritis, demonstrating, despite the peripheral nerve model, that local delivery of nanoparticles is an interesting option for treating autoimmune diseases possibly also at central level [48].

An alteration of the BBB can be a consequence of the disease, as in the case of ischemic stroke.

Campos-Martorell and co-workers evidenced how neutral and negatively charged liposomes, but not positively charged liposomes of the same size (around 150 nm) accumulated in the infarcted area of rats undergoing middle cerebral arterial occlusion. Simvastatin administered intravenously as 1 ml of 6 mg/ml simvastatin-loaded neutral liposomes not only was accumulated in the infarcted area of ischemic rats but unexpectedly it was delivered and retained in the brain of sham animals better than the free drug, suggesting a potential improvement when simvastatin is used as a preventive treatment [49].

Nasal delivery has been indicated as a possible and convenient way of delivering the drug to the CNS non-invasively [50,51]. The olfactory region placed in the uppermost part of the nasal cavity, represent the only part where the CNS is in direct contact with the environment and may represent a route to bypass the BBB to gain access to the brain. However, the administration of statins via the nasal route is challenging due to the poor water solubility of these drugs and the barrier effect provided by mucus. Nanoparticles could favour the nose-to-brain delivery by favouring mucus and/or adhesion, providing penetration enhancement through the nasal mucosa or via targeting of the olfactory neurons.

A recent study of our group proposed the use of lecithin/chitosan nanoparticles loaded with simvastatin able to be biodegraded by the enzymes present in the nasal secretions. In preliminary gamma scintigraphy studies in rats, technetium (Tc99m) labelled nanoparticles (200 nm, 1 mg/ml simvastatin), but not a suspension of the drug or the radiolabel alone, where able to increase the radioactivity detected in the brain of the animals after the nasal instillation of 10 µl of the nanoparticle formulation in each nostril of the animals [52].

Future Perspectives

Statins are emerging with a range of possible applications for the treatment of a number of brain and CNS diseases. It appears clear that the pharmacokinetics and pharmacodynamics of this class of drugs do not allow for a real role in these new indications to the traditional oral statins administration. Nanotechnology-enabled formulations, in this case more than in many others, could provide a clinical advantage over classic formulation. In fact, nanocarriers appear to be pivotal for the protection, the targeting and the delivery of statins to the brain through local, intravenous and nasal delivery. The relevance of each clinical approach using the nanomedicines will be closely related to the disease to be treated, to this regards acute diseases would probably require local delivery or intravenous administration, while chronic diseases should be addressed with targeted approaches or via nasal delivery, to limit the invasiveness and/or the side effects related to the treatment.

Executive Summary

Statins: a future beyond cholesterol lowering?

- Statins block liver cholesterol synthesis by inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase
- Through the same pathway however statins inhibit the product isoprenoids involved in the post-transcriptional modification of proteins
- Signalling GTPase proteins (G proteins), such as Rho, Ras, Rac, Rap, Ral are affected leading to "pleiotropic" effects
- Pleiotropic effects of statins include anti-inflammatory, immunomodulatory and anti-oxidant
- Statins may hinder leukocyte infiltration of inflamed tissue by blocking the β2 integrin leukocyte function antigen-1 (LFA-1)

Potential central nervous systems applications of statins

- Statins have the potential to be beneficial in brain diseases
- In stroke statins may improve cerebral flow, enhance fibrinolysis and reduce infarct size
- Statins may reduce Parkinson's disease risk via the cholesterol lowering action, the reduction of inflammation mediators and the modulation of some brain receptors
- In Multiple Sclerosis statins may have a role reducing myelin-antigen presentation, leucocytes recruitment and pro-inflammatory signalling
- Several effects appear to be beneficial in Alzheimer's disease, such as inhibition of neurofibrillary tangles and amyloid β formation, improvement of brain perfusion and anti-inflammatory effects
- Despite lipophilic statins may cross the BBB, first pass metabolism and low CNS concentrations hinder statins use in brain diseases

Nanomedicines for the drug delivery to the brain

- Pharmaceutical nanotechnologies may enable the use of statin in the brain
- Nanomedicines can improve drug solubility, to protect the active substance from chemical or enzymatic degradation, to provide controlled and/or targeted delivery, to favour the crossing of the biological barriers and ultimately improve brain availability
- Local invasive delivery, targeting and crossing of the BBB and nose-to brain delivery appear as the main route for using clinically nanoencapsulated statins

Future perspectives

- Not many studies have explored the use of nanoencapsulated studies for brain diseases
- The potential of these innovative formulations is high as traditional administration does not appear to be clinically effective for CNS indications
- The clinical use of nanoencapsulated statins for brain diseases has to be carefully tailored as a function of the characteristics of the disease to be treated

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