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Expanding the Therapeutic Potential of Statins by Means of Nanotechnology Enabled Drug Delivery Systems

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Abstract: Statins are effective lipid lowering agents traditionally used for the primary and secondary prevention of cardiovascular disease. Statins also exert a range of pleiotropic effects that make them attractive candidates for use in a wide range of disorders, in particular inflammatory and immune mediated conditions. However, the exploitation of such pleiotropic effects has been greatly hindered by poor bioavailability and adverse effects on muscles and the liver at higher doses. Nanotechnology is often suggested as the solution to this problem, as it enables an increased bioavailability of statins. Moreover, colloidal carriers can offer targeted drug delivery approaches that enable localised biological effects of statins, further reducing their potential for unwanted toxicity and adverse effects. This article reviews the available evidences for the increased potential of statin therapy when administered in nano-formulations such as nanocrystals, nanoparticles, liposomes, micelles and various nano-enabled devices.

Keywords: Liposomes, micelles, nanocapsules, nanocrystals, nanoparticles, nanospheres, pleiotropic effects, statins.

1. INTRODUCTION

1.1. Statins as Cholesterol Lowering Agents

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or statins are ~~arguably~~ one of the biggest advances in cardiovascular care of the 20th century. Statins reduce cholesterol serum levels by reversibly inhibiting HMG-CoA reductase, an enzyme catalysing a rate-limiting step in cholesterol biosynthesis [1]. ~~Since statins were approved in 1987, their use has steadily increased worldwide, being the largest drug cost to the Australian Pharmaceutical Benefits Scheme (PBS) starting from 1997. Since 2001 atorvastatin has maintained the standing of prescription most dispensed each year in Australia [2].~~

The wide acceptance of statins as lipid lowering agents is justified after many years of thorough investigation into their effects. In 1994 the first significant cholesterol lowering trial, i.e., the Scandinavian Simvastatin Survival Study (4S trial), showed that simvastatin decreased blood levels of total cholesterol by 25%, LDL-cholesterol by 35% and raised HDL-cholesterol by 8% and more importantly, this trial showed simvastatin-dependent decreased mortality of patients, which sparked global interest [3]. Later studies showed the benefits of statins for primary prevention of cardiovascular disease [4-6]. Collectively, these extensive clinical trials have proven that long-term therapy with statins (for at least one year) reduces the risk of heart attack, stroke and all-cause mortality in patients with and without established coronary heart disease [7].

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Currently available statins include atorvastatin, fluvastatin, lovastatin, pravastatin, pitavastatin, rosuvastatin and simvastatin. From a chemical standpoint, statins have different structures, some being more lipophilic than others (Fig. **1**) [8].

These chemical differences among statins impact significantly on their pharmacokinetic profiles (Table **1**) [9-11]. Atorvastatin, fluvastatin, lovastatin, pitavastatin and simvastatin are lipophilic in nature, thus easily diffuse across enterocytes cell membranes and are active not only in the liver, but also in non-hepatic tissues. Rosuvastatin and pravastatin have a more hydrophilic character, which decreases their permeability across cell membranes, but are actively transported into the hepatocytes by organic anion transporters (OATP). Apart from pravastatin, which is transformed enzymatically in the liver cytosol, all statins undergo extensive metabolism by the cytochrome P450 (CYP) isoenzyme systems expressed in liver microsomes and in the gut wall [9]. Lipophilic statins generally exhibit poor bioavailability varying from 5% (simvastatin) to 24% (fluvastatin) upon oral administration [10]. The low bioavailability of these statins is due to a combination of poor aqueous solubility, low dissolution rate and extensive first pass metabolism. ~~The low systemic bioavailability of statins has previously been indicated as a desirable feature, since statins exert their main pharmacological effect by lowering cholesterol synthesis in hepatic cells.~~ However, statins are increasingly being investigated for other pharmacological effects in which higher systemic bioavailability is desired.

1.2. Beyond the Cholesterol Lowering Effect of Statins

Along with their lipid lowering effects, statins have been related to a range of other therapeutic outcomes or pleiotropic effects. These pleiotropic effects were first evidenced

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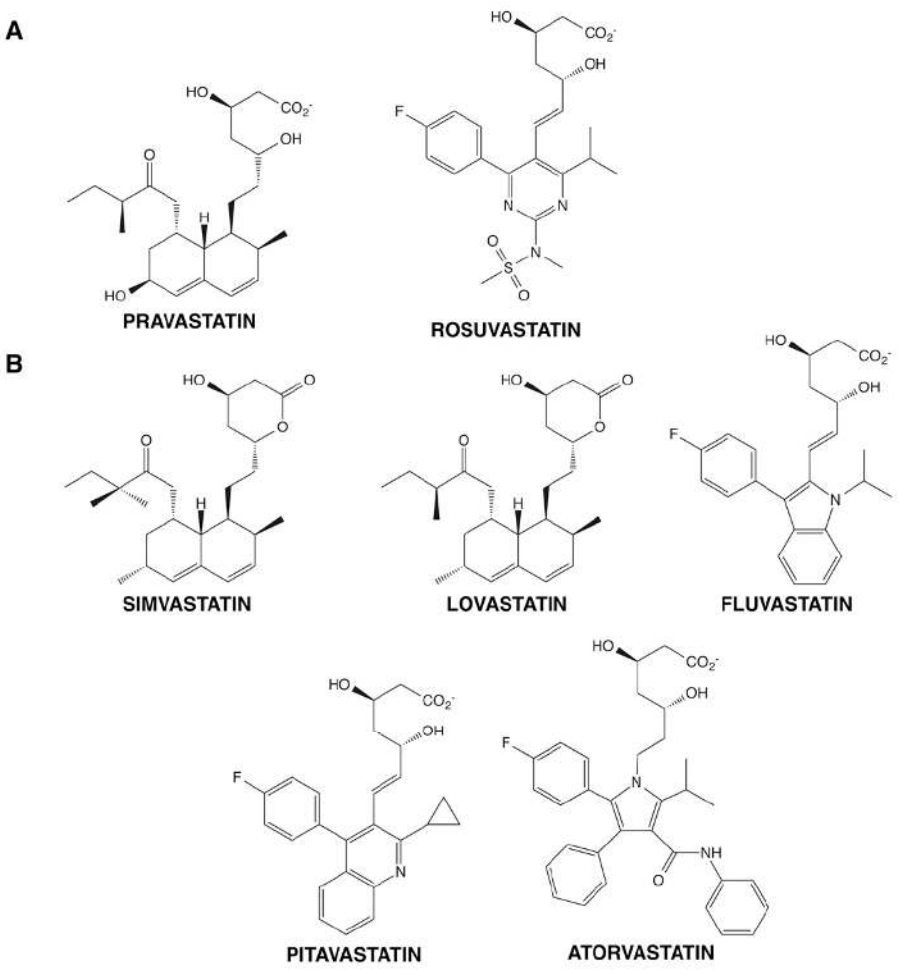

Fig. (1). Molecular structures of hydrophilic (**A**) and lipophilic (**B**) statins.

Table 1. Clinical pharmacokinetics of HMG-CoA reductase inhibitors (modified from [9-11]).

Parameter	Atorvastatin	Fluvastatin	Lovastatin	Pitavastatin	Pravastatin	Rosuvastatin	Simvastatin
Prodrug	No	No	Yes	No	No	No	Yes
Lipophilicity (Log P)	4.1	3.2	4.3	1.49	- 0.2	- 0.3	4.7
t_{max} (h)	2 – 4	0.5 – 1.5	2 – 4	0.5 – 0.8	0.9 – 1.6	3	1.3 – 2.4
C_{max} (ng/ml)	27 – 66	448	10 – 20	26	45 – 55	37	10 – 34
Absorption (%)	30	98	31	80	35	50	60 – 85
Hepatic extraction (% of absorbed dose)	>70	68	>70	NA	66	63	78 – 87
Bioavailability (%)	12	10 – 35	<5	>60	15 – 17	20	<5
$t_{1/2}$ (h)	15-30	0.5 – 2.3	2.9	11	1.3 – 2.8	20.8	2 – 3
Protein binding (%)	>98	>99	95	>95%	48	90	95
Metabolism	CYP3A4	CYP2C9	CYP3A4	CYP2C9 (minor)	Sulfation	CYP2C9 (minor)	CYP3A4
Lipid-Lowering Metabolites	Yes, Active	Yes, Mainly Inactive	Yes, Active	No	Yes, Mainly Inactive	Yes, Limited Effect	Yes, Active
Renal excretion (%)	2	6	30	<2	60	10	13

NA, not available

with the results of the large West of Scotland Coronary Prevention Study (WOSCOPS). The WOSCOPS study concluded that the lipid lowering effect of pravastatin could not exclusively account for the large benefits evidenced during the therapy with the drug, suggesting that other molecular mechanisms were associated to statin action [12]. After deep investigation, several additional beneficial properties have been attributed to statins, such as anti-inflammatory, antioxidant, immunomodulatory, antithrombotic, atherosclerotic plaque stabilising and also inhibitory of vascular smooth muscle proliferation inhibitory effects [1, 13-16]. ~~Due to these so-called pleiotropic effects~~, it is now considered that statins might have a future role in other pathological conditions including: rheumatoid arthritis [17], chronic obstructive pulmonary disease [18-20], cancer [21, 22], atrial fibrillation [24, 25], Parkinson's disease [26], Alzheimer's disease [27-29], sickle cell disease [30], sepsis [16, 31] and osteoporosis [32] (see Table **2**).

However, the extent to which these ~~'effects'~~ occur and their clinical relevance is largely debated and many still argue that statin effects have to be associated only to their cholesterol lowering ability [15, 33]. The mechanisms ~~by which these pleiotropic outcomes occur~~ are diverse and have not been fully elucidated. Statin-induced hypolipidemic effect is mainly related to the inhibition of the HMG-CoA reductase responsible for the production of mevalonate, a key intermediate in cholesterol biosynthesis. Reduction of hepatic intracellular cholesterol activates sterol regulatory element binding proteins (SREBPs), causing an increase in the transcription of the gene coding for the hepatic low density lipoproteins (LDL) receptor. Elevated numbers of LDL receptors lead to an increase in the clearance of circulating LDLs, the major mechanism by which statins reduce plasma LDL cholesterol. In addition, reduced availability of hepatic free cholesterol and/or cholesteryl esters for incorporation into very low density lipoproteins (VLDLs) may reduce the production rate of these lipoproteins. Efficacy on triglyceride reduction parallels LDL cholesterol lowering, as statins inhibit hepatic synthesis of apolipoprotein B-100, leading to a reduction of the synthesis and secretion of triglyceride rich lipoproteins [34].

On the other hand, inhibition of mevalonate synthesis also causes a decrease in the formation of other important isoprenoid intermediates in the cholesterol synthesis pathway, such as farnesyl phosphate (FPP), geranyl phosphate (GPP) and geranylgeranylphosphate (GGPP). These intermediates are involved in a post-translational modification of proteins, termed isoprenylation. Isoprenylation affects a number of G-proteins: Heme-a, nuclear laminins, small GTP binding proteins (GTPases) such as Ras, and Ras-like proteins Rho and Rac. GGPP and FPP are important lipids that, when added to intracellular proteins such as Ras, Rho and Rac, allow for their attachment to cell membranes. These signalling molecules are essential to membrane trafficking, transcriptional regulation, cell growth and development and switching on and off cellular signalling processes (Fig. **2**) [34]. The reduction of protein isoprenylation is considered the main molecular mechanism underlying most lipid-lowering-independent effects of statins, collectively termed pleiotropic effects [14, 35].

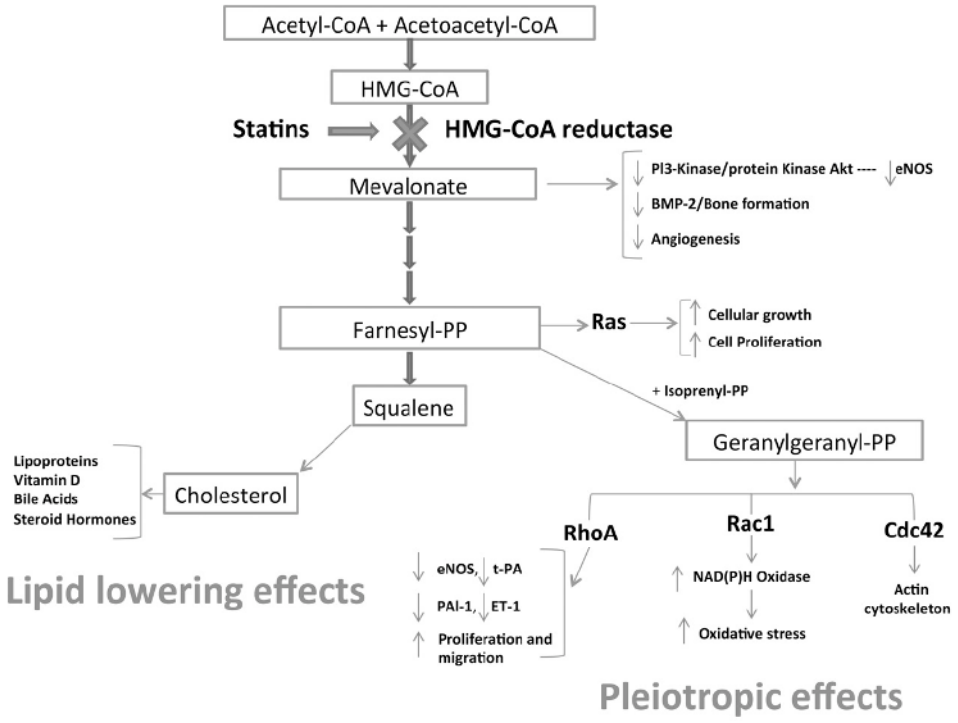

Fig. (2). Mechanism of action of statins through the inhibition of HMG-CoA reductase. Decrease in isoprenylation of signaling molecules, such as Ras, Rho, and Rac, leads to modulation of various signaling pathways. BMP-2: bone morphogenetic protein-2; eNOS: endothelial nitric oxide synthase; t-PA: tissue-type plasminogen activator; ET-1: endothelin-1; PAI-1: plasminogen activator inhibitor-1 (modified from [14]).

In particular, the increased nitric oxide (NO) bioavailability is considered to be a significant factor leading to some of the statin pleiotropic actions. Statins increase NO via three pathways. statins have been found to activate the protein kinase Akt, a signalling molecule, which can directly increase NO production. Statins also inhibit Rho pathway, which causes the increase of the expression and activity of endothelial NO synthase (eNOS) [36]. Lastly, restoration of eNOS activity can also be achieved via statin inhibition of Rac and the following reduction in in expression of caveolin-1 that acts as an eNOS inhibitor by forming a complex with the enzyme [37]. When NO is formed, it can diffuse into adjacent cells and have antithrombotic, anti-inflammatory and anti-proliferative effects on endothelial cells leading to statin-induced beneficial properties. Furthermore, Rho is important in the regulation of pro-inflammatory transcription factor in nuclear factor kappa B (NF-KB). NF-KB plays a key role in the transcription of variety of genes involved in immune and inflammatory responses. Statins may prevent the activation of NF-KB, inhibiting the synthesis of inflammatory cytokines. Collectively, these mechanisms may account largely for the anti-inflammatory, anti-atherosclerotic, pro-apoptotic and immunomodulatory effects exhibited by statins (Table **2**).

However, these effects are concentration-dependent and often are only observed at doses higher than the conventional cholesterol-lowering ones. At high doses, the risk of adverse effects such as myopathy and rhabdomyolysis is significantly increased [14]. This has largely limited statins pleiotropic actions being further investigated. Concurrently, statins poor bioavailability and toxicity at high concentrations mean that new and more efficient dosage forms are needed to allow for statin safe administration and to harness their new promising therapeutic actions.

2. BENEFITS OF NANOTECHNOLOGY IN THE DRUG DELIVERY OF STATINS

Numerous nano-sized drug delivery systems have been developed to facilitate and optimize the delivery of drugs with challenging biopharmaceutical properies [39]. Examples of such DDSs are nanocrystals, polymer nanoparticles (i.e. nanocapsules and nanospheres), solid lipid nanoparticles, liposomes and micelles, which are known as nano-sized drug delivery systems or nanocarriers (Fig. **3**).

All these formulation approaches differ in their favourable characteristics, applicability and drawbacks. However, a

Table 2. Proposed therapeutic non-cholesterol lowering role of statins in different disease states.

Disease State	Drug	Oral Dose	Effect of Statin	Reference
Alzheimer's Disease	Pravastatin	40 mg/day	Lowering cholesterol, antithrombotic, anti-1nflammatory effects	[28, 29]
Atrial fibrillation (AF)	Atorvastatin Pravastatin	10 mg/day 40 mg/day	Anti-inflammatory effect, Reduction in serum C-reative protein level	[24, 25]
Cardiac Transplant	Pravastatin	20 mg/day	Reduction in coronary vasculopathy and acute rejection. Direct inhibition of the induction of MHC-II expression and thus T cell activation.	[23]
Chronic Obstructive Pulmonary Disease (COPD)	Statin group	NA	Anti-inflammatory effects reducing exacerbations and decline of l lung function.	[18-20]
Colorectal cancer	Pravastatin Atorvastatin Simvastatin Rosuvastatin	10-40 mg/day	Anti-proliferative, pro-apoptotic and anti-invasive effects in cancer cells. Impair angiogenesis. Decrease the ability to metastasize	[22]
Inflammatory Bowel Disease	Atorvastatin Simvastatin Pravastatin	80 mg/day NA NA	Reduction in chemokines. Attenuate inflammatory response. Decrease the risk of progression to colorectal cancer	[21]
Osteoporosis	Simvastatin Pravastatin Atorvastatin Fluvastatin	20-40 mg/day	Reduction of risk of fractures. Induction of bone formation locally through expression of growth factors that promote angiogenesis, bone cell differentiation and osteogenesis.	[32]
Parkinson's Disease	Statin group	NA	Reduction of risk of Parkinson's Disease. Anti-inflammatory, immunomodulating and neuroprotecting effect.	[26]
Rheumatoid arthritis	Atorvastatin	40 mg/day	Anti-inflammatory effect. Reduction of vascular risk and synovial inflammation	[17]
Sepsis Syndrome	Simvastatin Pravastatin	10-40 mg/day	Protective effect against sepsis through anti-inflammatory and immunomodulating effect.	[31]
Sickle cell disease	Simvastatin	20-40 mg/day	Reduction in coagulation activation, homolysis, inflammation and thrombin generation in the vascular pathology of sickle cell disease.	[30]

NA, Not Available

comprehensive comparison of nano-sized drug delivery systems is beyond the scope of this review. This article will instead focus on nanotechnology-based formulations that have been proposed to improve established or enable innovative therapeutic applications of statins. In fact, the reformulation of statins with nanocarriers can contribute to ameliorate traditional clinical application of statins for cholesterol lowering by enhancing oral bioavailability, reduce frequency of administration and limiting drug toxicity. More interestingly, statin-containing nanoformulations allow the administration of this class of drugs via alternative administration routes for clinical applications yet to be explored [44].

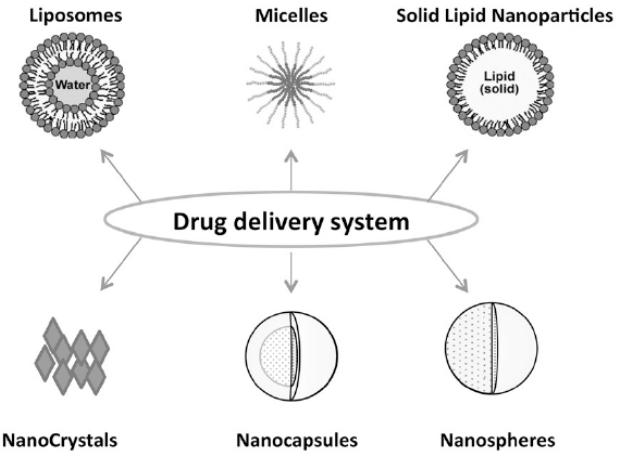

Fig. (3). Schematic representation of different nanotechnology based drug delivery systems widely used for statins.

2.1. Nanocrystals

Particle size reduction is one of the most widespread techniques to improve the absorption characteristics of drugs. Nanocrystals are the first approach that achieved drug size reduction to the nanoscale. Nanocrystals can be defined as crystals with a size ranging from 1 to 1000 nm. They differ from other nanosystems as they are composed of 100% drug, without polymeric or lipid excipients [45]. Nanocrystals increase the dissolution of drugs by three main mechanisms: increase of specific surface area, enhancement of solubility and production of high-energy solid state particles, most of them being in an amorphous state. Nanocrystals of poorly soluble drugs can be produced either by a ~~building up~~ approach (synthesis, self-assembly or precipitation) or by top-down technologies based on drug-fragmentation processes (high pressure homogenization, milling). Critical for the generation of stable nanocrystals able to avoid aggregation and Ostwald ripening is the addition to the nanoparticle dispersion of excipients able to provide steric and/or ionic stabilization to control particle size ~~and size~~ distribution [~~46,~~ 47].

Statins have been employed in the form of nanocrystals to improve their solubility and dissolution properties. Athul and co-workers prepared a promising simvastatin nanosuspension by high-pressure homogenization technique having good stability, enhanced dissolution rate, solubility and permeability compared to crude drug. These properties increased the drug oral bioavailability and its *in vivo* absorption rate. *In vitro* drug release data showed a maximum cumulative percentage of dissolved drug of 98.7% within 1 hour, whereas 45.9% only was reached by the crude drug [48].

Another group has developed nanocrystals of atorvastatin with the same approach, showing complete drug dissolution within 30 minutes from crystals with a d_{v50} of 0.241 μm [49]. Simvastatin nanocrystals (300 nm) obtained by nanoprecipitation in presence of surface stabilizers such as polyvinylpyrrolidone K-30 and sodium laurylsulphate, showed a similar increase in dissolution rates *in vitro* in comparison with commercial crystalline drug [50]. In a recent study, simvastatin nanocrystals prepared by sonoprecipitation method in presence of Pluronic F68 (360 nm) showed increased dissolution rate (4 fold), faster absorption (t_{max} reduced to 1.99 h from 2.88 h) and enhanced bioavailability (1.5 times increase) in comparison to crude simvastatin after oral administration to rats [51].

Amorphous atorvastatin calcium nanosystems were prepared using a supercritical antisolvent (SAS) process (Fig. **4**). Mean particle size of the spherical particles obtained ranged from 153 and 863 nm and was strongly influenced by drug concentration and CO_2/drug solution feeding rate. Intrinsic dissolution rate, solubility and oral bioavailability in rats were significantly enhanced when compared to the unprocessed crystalline raw material [52].

Anwar and colleagues developed an unconventional nanocrystal system of atorvastatin-chitosan conjugate [53]. The nanocrystal drug delivery system was obtained from the lyophilised drug-polymer conjugate, which after high pressure homogenization formed a colloidal suspension with an average size of 215 nm. When administered orally to rats, this nano-sized drug-chitosan conjugate was shown to improve the pharmacokinetic properties of atorvastatin, increasing its plasma half-life, C_{max} and AUC (5 fold) in comparison with unprocessed atorvastatin. The results were attributed to the conjugate increased solubility (100 times) and faster dissolution rate, while the mucoadhesive properties of chitosan allowed increased retention time in the gut. The enhanced bioavailability was attributed not only to particle nanosize, but also to a decreased metabolism of the drug by cytochrome P450 enzyme [53]. Such ~~a formulation~~ could be used to lower traditional dosing of statins and reduce the frequency of side effects. Additionally, ~~this nano-formulation~~ may ~~also~~ be promising for administration by other routes, where the mucoadhesive and permeation enhancing properties of chitosan are deemed favourable.

2.2. Nanoparticles

Nanoparticles in pharmaceutics are defined as solid colloidal particles up to 1000 nm in the form of nanospheres or nanocapsules. Nanospheres have a matrix type structure, in which the drug may be homogeneously dispersed throughout the core or adsorbed on the particle's surface. In contrast, nanocapsules are typical reservoir systems in which a core confining the drug is coated by an external shell controlling drug release [~~54-56~~]. ~~Nanoparticle formulations appear to offer unique characteristics for the future drug delivery of statins.~~ Nanoparticles ~~are able to~~ protect drugs from degrada-

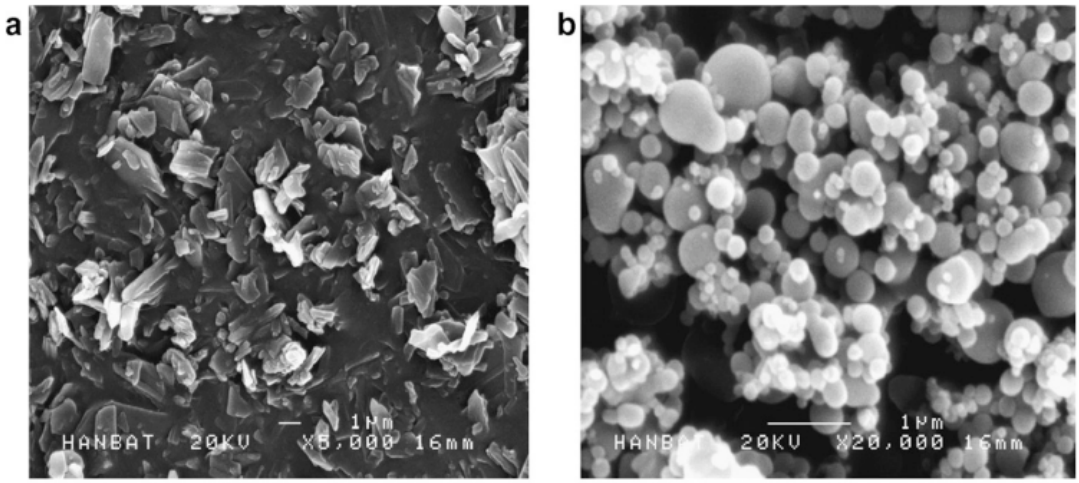

Fig. (4). SEM images of unprocessed atorvastatin calcium (**a**) and processed atorvastatin calcium precipitated by the SAS process (**b**) (modified from [52]).

tion and provide tailored release properties. Nanoparticle behaviour in the biological environment, stability and biodistribution will depend on their composition, surface properties, morphology and size. In particular, particles above one hundred nanometers provide high drug payload, but are more susceptible to interaction with biological matrices, such as mucus or blood proteins, and to scavenging by macrophages compared to smaller particles (<50 nm) [57].

As discussed, the short half-life and low bioavailability are the major noticeable drawbacks of the therapy with statins. To overcome these drawbacks researchers suggested the development of nanoparticulate formulations. Many methods have been employed to prepare/formulate nanoparticles with optimal particle size for the specific administration, reduced uptake by cells of the reticuloendothelial system (RES) when administered parenterally and/or preferential distribution to specific cells or tissues in order to obtain targeted drug delivery [44]. In particular, poly(lactic-co-glycolic acid) (PLGA) copolymers have been extensively used in developing statin-loaded nanoparticles. For example, Soni and co-authors have focused on improving the absorption of statins in the gastrointestinal tract using simvastatin PLGA nanoparticles [58]. These nanoparticles, administered *per os* as a suspension, showed a significant reduction in cholesterol serum lipid levels (as opposed to non encapsulated simvastatin), producing a 5 fold decrease in total cholesterol and 2.8 fold decrease in triglyceride levels. This was mainly attributed to the increased apparent solubility in aqueous media of simvastatin in consequence of its being loaded into nanoparticles. Interestingly, it was shown that PLGA nanoparticles had a biphasic release pattern. *In vitro* drug release from the nanoparticles showed an initial burst release of 40% in the first 4 hours, which was considered beneficial to improve drug absorption, followed by a phase of slow release of the drug [58]. In another approach, the water soluble statin fluvastatin was encapsulated into a novel fast degrading polyester, poly(propylene succinate) (PPSu), in view of a once-a-day formulation. Fluvastantin loaded nanoparticles were produced by w/o/w emulsification method using polymers various molecular weight and showed particles size between 150 and 1800 nm. After a burst release in the first hour, extended release profiles could be obtained only with high molecular weight PPSu. Such a drug delivery system appears promising to lower fluvastatin oral daily dosing and reduce adverse drug reactions associated with high dose statin regimens [59].

Cubic nanoparticles obtained from glyceryl monooleate and poloxamer 407 have been proposed as a potential drug delivery system to enhance bioavailability of simvastatin. Cubic nanoparticles obtained by fragmentation of the bulk cubic phase gel formed by the components showed particle sizes in the 100-150 nm range and a simvastation loading up to 5%. Despite simvastatin was not released significantly by the nanoparticles neither in simulated gastric fluid nor in fasted-state simulated intestinal fluid, pharmacokinetics studies carried out in dogs showed an increase in oral bioavailability of over 2.4-fold when simvastatin was administered as cubic nanoparticles compared to micronized crystal powder, as a result of a facilitated and prolonged absorption of the drug from the nanoformulation [60].

Lovastatin has been formulated in PLGA nanoparticles with the aim of enhancing bone repair when administered locally. Nanoparticles prepared by emulsification/solvent evaporation method showed particle size ranging from 200 to 400 nm increasing on lovastatin encapsulation. Release of lovastatin was found to be almost complete (97%) over 7 days. *In vivo* studies evidenced that 1 mg lovastatin-PLGA nanoparticles were the most efficient treatment in stimulating faster bone regeneration when locally administered to rats with surgically caused bony defects in comparison to control animals [61].

Pitavastatin PLGA nanoparticles have been proposed as an effective treatment for pulmonary artery hypertension. Pitavastatin was found to be the most potent among statins in mitigating endothelial injury, inhibiting vascular remodelling and inflammation. In a rat model of monocrotaline-induced pulmonary artery hypertension, a pitavastatin nanoparticles suspension administered via intratracheal instillation induced reduction of the hypertension and improved survival rate compared to pitavastatin alone administered in the same way or systemically. The beneficial effects of pitavastatin-

nanoparticles were attributed to their accumulation in alveolar macrophages and small pulmonary arteries up to 14 days after intratracheal instillation [62].

The use of statins has also been suggested for the treatment of many neurological disorders. Statins can provide neuroprotective effects via various mechanisms such as cholesterol lowering, reduction in reactive oxygen species, enhancement of cerebral blood flow and suppression of β-amyloid production and serum apolipoprotein E levels. Moreover, statin neuroprotective effects extend to antithrombotic effects, anti-inflammatory responses, modification of cognition related receptors, reduction of matrix metalloproteases and augmentation of endothelial nitric oxide synthase (eNOS). Therefore, it is expected that statins could provide benefit in diseases such as Alzheimer's disease, Parkinson's disease, cerebral ischemic stoke, vascular dementia and other cerebrovascular diseases which have been linked to high cholesterol as well as to prothrombotic and inflammatory states [63]. However, the passage of drugs across the blood brain barrier (BBB) has always represented a challenging physiological hurdle to the distribution of drugs to the central nervous system, the rate-limiting step being the presence of endothelial cells provided with tight junctions and efflux proteins, hindering the crossing to most drug molecules. Recent studies have shown how polysorbate 80-coated nanoparticles may constitute an interesting approach for improving BBB permeation. The mechanism by which this occurs is still not fully understood. However, it is known that coating with polysorbate 80 allows nanoparticles to accumulate at the brain micro-vessel endothelial cell surface [64, 65]. Simsek and collaborators explored this formulation approach to load atorvastatin in PLGA nanoparticles coated with poly(ethylene glycol) (PEG) and polysorbate 80 and to compare them to nanoparticles without polysorbate 80 on their surface. Both nanoparticles were labelled with Nile red fluorescent marker and tested on *in vitro* vascular endothelial cell cultures and *in vivo* in rat model. Interestingly, *in vitro* results indicated no significant effect of polysorbate 80 coating on nanoparticle uptake. However, *in vivo* results found that, although the imaging techniques were only qualitative, polysorbate 80 nanoparticles reached a higher fluorescence in the brain at 1 hour after administration over control nanoparticles, which may indicate an increase in polysorbate 80 nanoparticle CNS penetration [66]. This new formulation approach may be promising for a number of disorders such as Alzheimer's disease, stroke, Parkinson's disease and multiple sclerosis, for which a the beneficial effect of statins has been postulated, but not demonstrated via conventional statin administration.

More recently nanoparticles have been designed to target specific cells, organs and tissues. Nanoparticles are able to target cells through conjugation to a number of ligands able to bind to receptors and other membrane proteins typically expressed by a specific organ or cell population [55]. By localising the effect of statins to a particular area, some of the their pleiotropic effects may be amplified. For example, it has been postulated that a role exists for statins as potential new therapeutic agents able to promote neovascularisation in patients with ischemia and coronary artery disease. The mechanism by which this occurs has not been fully elucidated yet, but it has been suggested that statins increase endothelial progenitor cells (EPC) and promote their differentiation into mature endothelial cells by activating AKT protein kinase [67]. These effects have been observed in several studies at high doses of statins, but significant concerns were raised regarding potential adverse reactions [68]. Kubo and co-workers have tried to overcome this issue by developing biodegradable PLGA nanoparticles able to target the vascular endothelium [69]. Intramuscular injection of biodegradable pitavastatin loaded PLGA nanoparticles resulted in cell-selective delivery into capillary and arteriolar endothelial cells. Pitavastatin-loaded PLGA nanoparticles were shown to increase angiogenic activity and promote neovascularisation at doses that were 100 times as lower than those obtained by cumulative oral dosing. In addition, when nanoparticles containing pitavastatin were delivered to vascular endothelial cells, they induced increased neovascularization and improved tissue perfusion in a murine model of hind limb ischemia, indicating that this novel cell-selective delivery system is feasible for therapeutic neovascularization. It was noticed that nanoparticles were localised in the vascular endothelium and none detected in other organs such as the liver, kidney, or heart at any time point up to 14 days. This selectivity was attributed to an increased endocytosis of nanoparticles by the vascular endothelium cells. Furthermore, pitavastatin formulated in nanoparticles showed no effect on serum lipid levels in rat ischemic models. The absence of effects on lipid levels strongly suggests a different mechanism by which statins act, not related to the classic cholesterol-lowering effects. Although the authors of this study did not see an increase in EPC as they hypothesised, a rise in phosphorylated endothelial nitric oxide synthase (eNOS) and protein kinase AKT was observed which might account for the observed therapeutic effects [69].

A targeting approach has also been proposed to increase the potential for statins to be used in the stabilisation of atherosclerotic plaques. It has been hypothesised that statins role in plaque stabilisation may occur for a number of reasons including the up regulation of eNOS, a pathophysiological marker which is down regulated in a prothrombic or proatherogenic state. Moreover, statins have the ability to reduce matrix metalloproteinase secretion in macrophages, physiological proteins which result in plaque matrix degradation and consequent plaque vulnerability. Statins have also been shown to inhibit macrophage activity and proliferation adding to their effects on plaque stabilisation [70]. Broz and colleagues have tried to exploit the beneficial effect of statins by targeting activated macrophages present in atherosclerotic plaques. By creating a selective targeted system, the hope is to avoid local tissue damage and serious side effects such as rhabdomyolysis**.** Pravastatin-loaded targeted nanoparticles were obtained by functionalising the polymer, i.e. poly (dimethylsiloxane) (PDMS) with two poly(2-methyloxazoline) (PMOXA) side chains, with an oligonucleotide (polyG) through a biotin/streptavidin functionalization approach [71]. It was shown on cell cultures that functionalised nanoparticles enabled the pravastatin to penetrate into macrophages with controlled intracellular release only into desired cell lines with no toxic effect on smooth muscle cells viability. Interestingly, it was shown that pravastatin alone had no effect on macrophages being too hydrophilic to get into macrophages. However, owing to the targeted nanoparticle formulation, no such limitation was evident,

allowing pravastatin intracellular delivery [71]. Further investigation with a targeted nanoparticle therapy *in vivo* is needed to establish any potential therapeutic benefit of statins. Nanoparticles may provide a large benefit to patients at high risk of plaque rupture allowing statins to reach and exert inhibitory effects on macrophages safely for patients even at high concentrations.

2.3. Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) provide a promising approach for the delivery of poorly water soluble compounds such as statins. These are typically spherical solid nanoparticles with a drug-containing solid lipid core stabilised with surfactants or stabilisers, with sufficient physical stability for long time storage. The preparation process generally involves emulsification by homogenisation at high temperature of the molten solid lipid containing the drug. SLNs provide controlled release of drugs and have been shown to improve oral bioavailability of lipophilic drugs. Alternatively to polymeric nanoparticles, solid lipid nanoparticles are also considered to have appealing characteristics as nanocarriers. In fact, solid lipid nanocapsules and nanostructured lipid carriers offer additional advantages such as enhanced biocompatibility, lower toxicity, excellent biodegradability, increased drug encapsulation and stability [72]. Hence, SLNs are a promising sustained-release and drug-targeting system for statins. Furthermore, solid lipid nanoparticles administered orally may be able to bypass first pass metabolism by taking advantage of intestinal lymphatic transport [44]. A complete optimisation study of simvastatin loaded lipid nanoparticles has been conducted by Shah and Pathak [73]. It was found that second generation lipid carriers, called nanostructured lipid carriers (NCLs), composed of mixtures of solid and liquid lipids, were superior in improving bioavailability than conventional SLNs produced using only solid lipids. SLNs presented two main disadvantages in comparison with NLCs. The first was a reduced drug loading capacity and the second a progressive drug leakage during storage [73]. The pharmacokinetic parameters after oral administration in mice revealed a 4.8 fold increase in bioavailability as compared to simvastatin suspension and a 2.3 fold increase as compared to SLNs. A biodistribution study revealed preferential accumulation of NLCs in the liver and this was considered advantageous as the liver is the target organ for simvastatin [74]. In a similar approach, the bioavailability of lovastatin could be improved by administering it orally into solid lipid nanoparticles. Lovastatin loaded SLNs were developed using triglycerides by hot homogenization followed by ultrasonication, obtaining particles with a mean size range from 60 to 119 nm and a negative zeta potential. In addition, the report showed enhanced oral bioavailability in comparison with orally dosed lovastatin suspension. After intra-duodenal administration in rats relative bioavailability of lovastatin and lovastatin hydroxyacid of SLN formulation were increased by 173% and 324%, respectively, compared with the reference lovastatin suspension [75]. Therefore, formulating statins into NLCs may offer substantial benefit to increase their oral bioavailability.

However, this is not the only proposed use for statins formulated in lipid nanocarriers. Several studies have become available demonstrating statin ability to suppress growth and/or induce apoptosis of cancer cells. It has therefore been suggested that combining statins with other antiproliferative agents and/or formulating them into nanoparticles could enhance this effect. Nanoparticles are particularly beneficial in targeting tumours. Tumour vasculature, unlike the one in normal tissues, presents gaps as large as 600-800 nm between endothelial cells. As a consequence of tumour leaky and defective vessels and poor lymphatic drainage, nanoparticles are able to passively accumulate in the tumours. However, this phenomenon called enhanced permeability and retention effect, can be exploited by nanoparticles having sufficiently small particle size (10-100 nm) to both avoid the capture of liver and spleen macrophages and take advantage of the defective architecture of tumour blood capillaries [76]. To investigate this concept, simvastatin and tocotrienols were combined in lipid nanocarriers. Tocotrienols are a vitamin E family compounds found in palm oil. Previous studies showed that low dose statins and tocotrienols had no effect on cancer cells individually, whereas their combination significantly inhibited tumour cell growth. Collectively, simvastatin-tocotrienol co-encapsulated in lipid nanoparticles were shown to act synergistically increasing the intrinsic apoptotic effect compared to the two drugs separately [77]. These studies highlight the possibility of using nanoparticles for formulating multiple drugs into one drug delivery system, demonstrating their potential in enhancing and facilitating complex therapies.

2.4. Liposomes

Since they were first developed by Alexander Bangham in the 1960 as an analytical tool to study bio-membrane dynamics [78], the potential of these closed phospholipid bilayer vescicles as a drug delivery carrier promptly appeared with the first *in vivo* experiments. Liposomes are attractive drug carriers due to their tunable physical characteristics, good encapsulation efficiency of both hydrophilic or hydrophobic drugs, ability to protect drugs from degradation and the possibility of being functionalised with moieties to create targeted nanosystems [79, 80]. Due to these favourable characteristics, along with the fact that they are produced with highly biocompatible lipids, liposomes have become the first approved nano-sized drug delivery systems [81]. Despite chemical and physical stability of liposomes upon long-term storage as aqueous dispersion may still be considered a critical issue, with aggregation, vesicle fusion, drug leakage and phospholipids degradation being their most relevant drawbacks, up to date liposomes remain the most successful nanocarriers in clinical use [82, 83]. Therefore, it seems reasonable to consider liposomes a formulation approach to improve and extend the therapeutic use of statins.

Currently developed statin liposomal formulations have mostly explored the anti-inflammatory effects of statins. As previously described, it is hypothesised that statins anti-inflammatory properties are due their inhibition of the mevalonate pathway. Similarly to how statins inhibit the production of squalene and thus cholesterol, statins also inhibit the prenylation of Ras, Rho and Rac. As a consequence, statins have been shown to have anti-inflammatory properties such as augmentation of eNOS activity and inhibition of

cytokine production including MPC-1, IL-8, IL-1B and TNF alpha [13]. This makes statins interesting as drug therapies for inflammatory conditions related to damaged myocardium, percutaneous coronary intervention and tumours.

Afergan has investigated the anti-inflammatory properties of statin loaded liposomes. It has been shown that inflammation associated with percutaneous coronary intervention is one of the most significant predictors of poor therapeutic outcome with this surgery. The damaging inflammatory processes involve monocyte adhesion, macrophage accumulation and smooth muscle cell proliferation. Simvastatin liposomes administered intravenously to rats were found to be 1.5-2 times more potent in suppressing monocytes and macrophages activity than free circulating drug [84]. It is hoped that use of simvastatin liposomes may act as anti-inflammatory agents in percutaneous coronary intervention, the final goal being to decrease restenosis rates.

In another study, the effect of pravastatin-loaded liposomes was studied on the growth rate of three different tumour-related cells types, i.e., tumour cells, endothelial cells and macrophages. To do this, pravastatin was formulated into long circulating liposomes hoping for reaching high local drug concentrations in the tumor. *In vitro* experiments showed that liposomal pravastatin exerted the most significant effect on macrophage viability. For *in vivo* experiments, melanoma cells bearing mice were given intravenous injections of pravastatin or liposomal pravastatin. Liposomal pravastatin was shown to significantly slow down tumour growth by 70%, whereas pravastatin therapy alone had no effect. Interestingly, liposomal pravastatin was shown to effectively inhibit the several proteins involved in inflammation and angiogenesis compared to free circulating drug. When compared 48 hours post administration, drug loaded liposomes were shown to be present in the blood and other tissues, while the free drug was only found in the liver. Serum cholesterol levels were unaffected by pravastatin-loaded liposome therapy (Fig. **5**) [85]. This demonstrates the importance of the nano-formulation in controlling the tissue distribution of statins, thus promoting pleiotropic effects.

Targeted liposomal formulations have been developed to selectively deliver pravastatin to damaged myocardium where the statin can best exert its anti-inflammatory properties. Pravastatin was encapsulated in liposomes conjugated to N-acetylglucosamine, which has been shown to bind to cell surface lectins on cardiomyocytes. The hydrophilic nature of pravastatin normally makes intracellular uptake of pravastatin into cardiomyocytes difficult, unlike other lipophilic statins. It was shown that by encapsulating pravastatin into a N-acetylglucosamine conjugated liposome, pravastatin was successfully endocytosed into cardiomyocytes in *in vitro* experiments. This resulted in increased nitric oxide synthase expression and thus NO production. Similar results were not seen in non-targeted pravastatin liposomes [86]. A targeted N-acetyl glucosamine conjugated liposomal system encapsulated with cardio-protective agents such as statins as well as other anti-inflammatory agents, appears as promising drug delivery system to specifically target damaged myocardial cells. Further investigation is now needed to determine the level of expression of lectin receptors in different body regions to determine how specific this targeting approach might be *in vivo*.

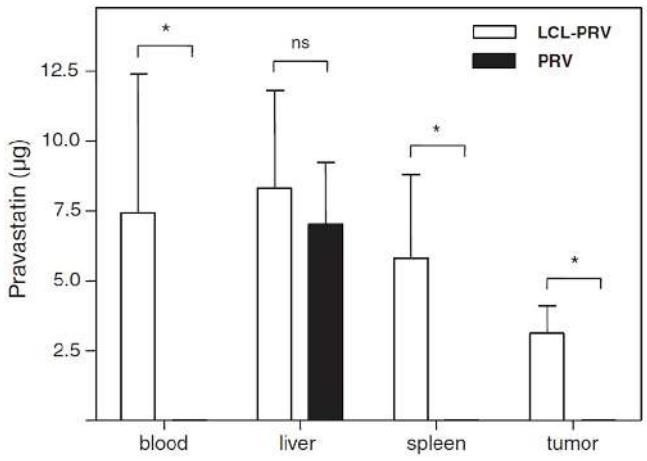

Fig. (5). Blood and tissue levels of pravastatin in melanoma-bearing mice receiving free (PRV) or liposomal pravastatin (LCL-PRV) (reproduced with permission from [85]).

2.5. Micelles

Micelles are successfully used as pharmaceutical carriers for poorly water soluble drugs for their small size, specific cell targeting capacity, minimized toxicity, stability in GI tract, good biocompatibility and solubilizing properties. Micelles are self-assembled nanosized colloidal particles (5 to 100 nm) with a hydrophobic core and a hydrophilic shell, which form spontaneously under certain concentrations and temperatures from amphiphilic or surface active agents. The hydrophobic core of micelles is capable of carrying poorly water-soluble drugs with high loading capacity (i.e., 5–25% weight), presently many of these drug-loaded micelles are at different stages of preclinical and clinical trials as useful tools to deliver drugs orally and enhance their bioavailability [87, 88]. Statins have been considered excellent candidates for encapsulation into micelles due to their mainly hydrophobic characteristics. As already discussed, statins show anticancer effects related to their anti-inflammatory and pro-apoptotic properties. These anticancer properties have been investigated using copolymers of stearyl chitosan (SC) and sulphated stearyl chitosan, which could self-assemble to form polymeric micelles containing atorvastatin. *In vitro* release studies showed that encapsulated atorvastatin micelles provide a sustained release profile, likely due to micelles forming a physical barrier around the drug. Cytotoxic studies were also conducted on human breast adenocarcinoma cell line (MCF 7) and human colon carcinoma cell line (HCT 116). Atorvastatin-loaded micelles showed enhanced cytotoxicity in both cell lines compared to free drug. It was suggested that chitosan facilitated adhesion to cell membranes allowing increased intracellular uptake of micelles. In particular, it was hypothesised that uptake occurred through the accumulation and endocytosis of micelles at few cationic sites present on the cell surface of tumours [89]. Simvastatin loaded poly(ethylene glycol)-poly (caprolactone) micelles were developed by Liu to optimise simvastatin delivery for bone regeneration. The *in vitro* release behaviour of simvastatin from the 80 nm micelles was prolonged. It was shown that simvastatin-loaded micelles caused an effective inhibition of the osteoblast early proliferation, stimulation of os-

teoblast differentiation and mineralization, and increased expression of the bone morphogenic protein 2 [90].

2.6. Nano-Enabled Drug Delivery Systems

Nanotechnology is further expanding, continually producing innovative drug delivery devices. Nano-enabled devices are drug delivery systems, generally novel implantable systems, which are macroscopic in size with some nanometric features crucial for their specific therapeutic action. These drug delivery devices combine the advantages of nanotechnology, such as increased drug availability and decreased side effects, with an extended release profile to aid compliance for patients and increase therapeutic efficacy. The extended release properties of these nano-enabled devices are particularity useful for chronic disease states [91]. A nanochannel drug delivery device incorporating atorvastatin and trans-resveratrol has been proposed to promote cardioprotection. Trans-resveratrol has has been proven effective in reducing oxidative stress and able to increase nitric oxide synthase expression in cardiomyocytes. Combining trans-resveratrol with atorvastatin creates a synergistic effect, which, when delivered locally to tissue, may help treat or prevent the progression of heart disease. The implantable drug delivery system is composed of a nano-fluidic membrane consisting of thousands of tightly packed nanochannels. The nanochannels achieve an extended constant release of drug through carefully geometrically designed slits. Testing of the drug delivery device was completed *in vitro* and showed zero-order release kinetics for more than one month. The implantable nano-enabled device displayed good endothelial biocompatibility [92]. In addition, the ability to finely tailor the number of nanochannels present offers a promising tool for personalised patient treatment.

Similarly, Tsukie and co-authors have investigated the delivery of statin drugs by means of innovative devices [93]. The authors incorporated pitavastatin nanoparticles into coronary artery stents in order to exploit statins vasoprotective properties. Current drug eluting stents incorporate sirolimus and paclitaxel. Concerns have been expressed over the safety of these devices as they are suspected to impair re-endorevascularisation and cause inflammation. Pitavastatin nanoparticle drug eluting stents were tested in pig coronary artery models to investigate their effectiveness in comparison to the current accepted therapy. Pitavastatin nanoparticles consisted of PLGA with surface modification of chitosan and were loaded onto stents by cationic electrodeposition coating technology. Pitavastatin nanoparticle eluting stents were shown to be as effective as sirolimus-eluting stents. In comparison, intracoronary administration of pitavastatin nanoparticles was shown ineffective, exhibiting the importance of the incorporation into an implantable local delivery device. Most significantly, pitavastatin therapy did not produce the delayed healing effects evidenced in sirolimus-eluting stents [93]. Local administration of pitavastatin nanoparticles may provide consistent and effective treatment of coronary artery disease, for patient at risk of in-stent thrombosis.

CONCLUSION

As Dr. Ruth Duncan, Chair of the European Science Foundation, defined it, nanomedicine is "the science and technology of diagnosing, treating and preventing disease and traumatic injury, of relieving pain, and of preserving and improving human health, using molecular tools and molecular knowledge of the human body" [94]. To achieve this goal, nanomedicines must be supplied, i.e. dosage forms, comprising an active drug plus excipients, substantially deriving from a dramatic size reduction of more conventional dosage forms.

Pharmaceutical nanotechnology offers a range of benefits to the therapeutic delivery of statins, the most significant being the increase in statin oral bioavailability. This allows statins to be administered at lower doses, reducing the risk of side effects such as rhabdomyolysis. The increased bioavailability of statins occurs mainly through two mechanisms including the increased dissolution of statins in the gastrointestinal tract and the bypass of first pass metabolism, a physiological barrier that significantly prevents statins from reaching significant systemic concentrations. Most importantly, the increased bioavailability achieved by statin-loaded nanocarriers allows statins pleiotropic effects to be exploited. Through nanocarriers, statins have the potential to exert anti-inflammatory, antioxidant, immunomodulatory and anti-thrombotic effects. In particular, nanotechnology targeted systems offer the highest potential for triggering these effects of statins, allowing them to be delivered locally to the site of action in significant concentrations. In an attempt to compare the nanotechnological approaches here reviewed, it is clear that the preference for one nanocarrier or another should be driven by the therapeutic need first and then also by technological considerations. The therapeutic need and goal determine the amount of drug to be administered via a certain route. Consideration should be paid to the fact that drug loading capacity changes among the available nanosytems. In the typical use of statins for lowering cholesterol blood levels, as oral administration is performed, improved dissolution is the goal to improve bioavailability and reduce the doses. Nanocrystals, micelles and lipid-based nanoparticles (SLNs, NLCs) should be considered as first choice for their positive effect on drug solubilisation in the gastrointestinal tract due to the reduced particle size of the pure drug or a "formulation effect" exerted by lipid excipients. Liposomes as well as polymeric nanoparticles should be considered for use via administration routes other than oral and, in particular, when the therapeutic goal requires sustained release, site-specific distribution and specific targeting properties of the carrier. Then, in front of the choice between liposomes and polymeric nanoparticles, stability and biocompatibility of the formulation should be taken into account as well as the drug release characteristics of each nanocarrier. As far as prolonged release of the drug at site of action is concerned, for instance after direct injection of the carrier in the tissue, polymeric nanoparticles may represent a golden standard to obtain a depot effect. Finally, as other potential applications of statins include the treatment of neurological disorders such as Alzheimer's disease, solid lipid nanoparticles so far appear as the most interesting nanocarrier to target the central nervous system.

As last reflection, when the actual nanoparticle-based drug delivery system has been manufactured, product development may not be over yet. Indeed, the nanocarrier eventually has to become a finished medicinal product to be used

safely and effectively in clinical practice. Beside any consideration on the large-scale production of these nanosystems, which could be not always straightforward, it cannot be excluded that for administration purposes, stability issues or patient convenience and compliance, the nanocarrier should be further transformed into a transitorily larger dosage form. Suitable technologies are needed to achieve this transformation that should only temporarily "mask" the nanoparticles within a bigger structure. For instance, an example of how this can be done was described by Cagnani *et al.* [95] who realised a powder for nasal delivery of drug-loaded nanoparticles: a spray drying process (in the presence of a filler) transformed the nanoparticles into microparticles, which were then agglomerated by tumbling to form coarse soft globules. After insufflation, these agglomerates broke down into smaller fragments of aggregated microparticles, which released intact, upon disintegration in physiological fluids, the original nanocarrier. In this way a solid finished product containing the nanocarrier can be proposed as components of classical dosage forms to suit different routes of administration, therapies and patients [96].

CONFLICT OF INTEREST

The authors report no financial, professional or intellectual conflicts of interest. The authors alone are responsible for the content and writing of this article.

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REFERENCES

[1] Bonetti, P.O.; Lerman, L.O.; Napoli, C.; Lerman, A. Statin effects beyond lipid lowering--are they clinically relevant?. *Eur. Heart J.*, **2003**, *24*(3), 225-248.

[2] Australian Department of health and ageing. PBS Information Management Section, Pharmaceutical Policy Branch. Expenditure and prescriptions twelve months to 30 June 2012. http://www.pbs.gov.au/statistics/2011-2012-files/expenditure-and-prescriptions-2011-2012.pdf (Last accessed April 20th, **2013**)

[3] Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet*, **1994**, *344*(8934), 1383–1389.

[4] Goldberg, R.B.; Mellies, M.J.; Sacks, F.M.; Moyé, L.A.; Howard, B.V.; Howard, W.J.; Davis, B.R.; Cole, T.G.; Pfeffer, M.A.; Braunwald, E. Cardiovascular events and their reduction with pravastatin in diabetic and glucose-intolerant myocardial infarction survivors with average cholesterol levels; Subgroup analyses in the Cholesterol and Recurrent Events (CARE) Trial. *Circulation*, **1998**, *98*(23), 2513-2519.

[5] Ong, H.T. The statin studies: from targeting hypercholesterolaemia to targeting the high-risk patient. *QJM*, **2005**, *98*(8), 599-614.

[6] Serruys, P.W.; de Feyter, P.; Macaya, C.; Kokott, N.; Puel, J.; Vrolix, M.; Branzi, A.; Bertolami, M.C.; Jackson, G.; Strauss, B.; Meier, B. Fluvastatin for Prevention of Cardiac Events Following Successful First Percutaneous Coronary Intervention. *JAMA*, **2002**, *287*(24), 3215-3222.

[8] Bełtowski, J; Jamroz-Wiśniewska, A. Modulation of H(2)S metabolism by statins: a new aspect of cardiovascular pharmacology. *Antioxid. Redox Signal.*, **2012**, *17*(1), 81-94.

[7] Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*, **2002**, *360*(9326), 7-22.

[8] Mekhail, G.M.; Kamel, A.O.; Awad, G.A.; Mortada, N.D. Anticancer effect of atorvastatin nanostructured polymeric micelles based on stearyl-grafted chitosan. *Int. J. Biol. Macromol.*, **2012**, *51*(4), 351-363.

[9] Bellosta, S.; Paoletti, R.; Corsini, A. Safety of statins: focus on clinical pharmacokinetics and drug interactions. *Circulation*, **2004**, *109*(23 Suppl 1), III50-57.

[9] Liu, X.; Li, X.; Zhou, L.; Li, S.; Sun, J.; Wang, Z.; Gao, Y.; Jiang, Y.; Lu, H.; Wang, Q.; Dai, J. Effects of simvastatin-loaded polymeric micelles on human osteoblast-like MG-63 cells. *Colloids Surf. B: Biointerfaces*, **2013**, *102*, 420-427.

[9] Grattoni, A.; Shen, H.; Fine, D.; Ziemys, A.; Gill, J.S.; Hudson, L.; Hosali, S.; Goodall, R.; Liu, X.; Ferrari, M. Nanochannel technology for constant delivery of chemotherapeutics: beyond metronomic administration. *Pharm. Res.*, **2011**, *28*(2), 292-300.

[9] Sih, J.; Bansal, S.S.; Filippini, S.; Ferrati, S.; Raghuwansi, K.; Zabre, E.; Nicolov, E.; Fine, D.; Ferrari, M.; Palapattu, G.; Grattoni, A. Characterization of nanochannel delivery membrane systems for the sustained release of resveratrol and atorvastatin: new perspectives on promoting heart health. *Anal. Bioanal. Chem.*, **2013**, *405*(5), 1547-1557.

[9] Tsukie, N.; Nakano, K.; Matoba, T.; Masuda, S.; Iwata, E.; Miyagawa, M.; Zhao, G.; Meng, W.; Kishimoto, J.; Sunagawa, K.; Egashira, K. Pitavastatin-Incorporated Nanoparticle-Eluting Stents Attenuate In-Stent Stenosis without Delayed Endothelial Healing Effects in a Porcine Coronary Artery Model. *J. Atheroscler. Thromb.*, **2013**, *20*(1), 32-45.

[9] Duncan, R.; Gaspar, R. Nanomedicine(s) under the microscope. *Mol. Pharm.*, **2011**, *8*(6), 2101-2141.

[9] Cagnani, A.; Sonvico, F.; Colombo, P.; Bettini, R.; Alonso, M.J.; Colombo, G.; Rossi, A. Chimerical agglomerates of lecithin/chitosan nanoparticles for powder nasal delivery. Poster presentation at the 2004 AAPS Annual Meeting and Exposition; November 7–11, 2004; Baltimore, MD (USA). Poster T3285.

[9] Buttini, F.; Colombo, G.; Rossi, A.; Sonvico, F.; Colombo, G. Particles and powders: tools of innovation for non-invasive drug administration. *J. Control. Release*, **2012**, *161*(2): 693-702.

[10] Igel, M.; Sudhop, T.; von Bergmann, K. Pharmacology of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins), including rosuvastatin and pitavastatin. *J. Clin. Pharmacol.*, **2002**, *42*(8), 835-845.

[11] Mukhtar, R.Y.,; Reid, J.; Reckless, J.P. Pitavastatin. *Int. J. Clin. Pract.*, **2005**, *59*(2), 239−252.

[12] West of Scotland Coronary Prevention Study Group. Influence of pravastatin and plasma lipids on clinical events in the West of Scotland Coronary Prevention Study (WOSCOPS). *Circulation*, **1998**, *97*(15), 1440-1445.

[13] Jain, M.K.; Ridker, P.M. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. *Nat. Rev. Drug Discov.*, **2005**, *4*(12), 977-987.

[14] Liao, J.K.; Laufs, U. Pleiotropic effects of statins. *Annu. Rev. Pharmacol. Toxicol.*, **2005**, *45*, 89-118.

[15] Palinski, W.; Napoli, C. Unraveling Pleiotropic Effects of Statins on Plaque Rupture. *Arterioscler. Thromb. Vasc. Biol.*, **2002**, *22*(11), 1745-1750.

[16] Blanco-Colio, L.M.; Tuñón, J.; Martín-Ventura, J.L.; Egido, J. Anti-inflammatory and immunomodulatory effects of statins. *Kidney Int.*, **2003**, *63*(1), 12-23.

[17] McCarey, D.W.; McInnes, I.B.; Madhok, R.; Hampson, R.; Scherbakov, O.; Ford, I.; Capell, H.A.; Sattar, N. Trial of Atorvastatin in Rheumatoid Arthritis (TARA): double-blind, randomised placebo-controlled trial. *Lancet*, **2004**, *363*(9426), 2015-2021.

[18] Marin, L.; Colombo, P.; Bebawy, M.; Young, P.M.; Traini, D. Chronic obstructive pulmonary disease: patho-physiology, current methods of treatment and the potential for simvastatin in disease management. *Expert Opin. Drug Deliv.*, **2011**, *8*(9), 1205-1220.

[19] Blamoun, A.I.; Batty, G.N.; DeBari, V.A.; Rashid, A.O.; Sheikh, M.; Khan, M.A. Statins may reduce episodes of exacerbation and the requirement for intubation in patients with COPD: evidence from a retrospective cohort study. *Int. J. Clin. Pract.*, **2008**, *62*(9), 1373-1378.

[20] Alexeeff, S.E.; Litonjua A.A.; Sparrow, D.; Vokonas, P.S.; Schwartz, J. Statin use reduces decline in lung function. Am. J. Respir. *Crit. Care Med.*, **2007**, *176*(8), 742-747.

[21] Samadder, N.J.; Mukherjee, B.; Huang, S.C.; Ahn, J.; Rennert, H.S.; Greenson, J.K.; Rennert, G.; Gruber, S.B. Risk of colorectal cancer in self-reported inflammatory bowel disease and modification of risk by statin and NSAID use. Cancer. **2011**, 117(8), 1640-1648.

[22] Bardou, M.; Barkun, A.; Martel, M. Effect of statin therapy on colorectal cancer. *Gut*, **2010**, *59*(11), 1572-1585.

[23] Kobashigawa, J.A.; Katznelson, S; Laks, H.; Johnson J.A.; Yeatman L.; Wang X.M.; Chia D.; Terasaki P.I.; Sabad, A.; Cogert, G.A.; Trosian, K.; Hamilton, M.A.; Moriguchi, J.D.; Kawata, N.; Hage, A.; Drinkwater, D.C.; Stevenson, L.W. Effect of pravastatin on outcomes after cardiac transplantation. N. Engl. J. Med. **1995**, 333(10), 621-627.

[24] Ozaydin, M.; Varol, E.; Aslan, S.M.; Kucuktepe, Z.; Dogan, A.; Ozturk, M.; Altinbas, A. Effect of atorvastatin on the recurrence rates of atrial fibrillation after electrical cardioversion. *Am. J. Cardiol.*, **2006**, *97*(10), 1490-1493.

[25] Tveit, A.; Grundtvig, M.; Gundersen, T.; Vanberg, P.; Semb, A.G.; Holt, E.; Gullestad, L. Analysis of pravastatin to prevent recurrence of atrial fibrillation after electrical cardioversion. *Am. J. Cardiol.*, **2004**, *93*(6), 780-782.

[26] Undela, K.; Gudala, K.; Malla, S.; Bansal, D., Statin use and risk of Parkinson's disease: a meta-analysis of observational studies. *J. Neurol.*, **2013**, *260*(1), 158-165.

[27] Kandiah, N.; Feldman, H.H. The therapeutic potential of statins in Alzheimer's disease. *J. Neurol. Sci.*, **2009**, *283*(1-2), 230-234.

[28] Sparks, D.L.; Kryscio, R.J.; Sabbagh, M.N.; Connor, D.J.; Sparks, L.M.; Liebsack, C. Reduced risk of incident AD with elective statin use in a clinical trial cohort. *Curr. Alzheimer Res.*, **2008**, *5*(4), 416–421.

[29] McGuinness, B.; Passmore, P. Can statins prevent or help treat Alzheimer's disease? *J. Alzheimers Dis.*, **2010**, *20*(3), 925-933.

[30] Adam, S.S.; Hoppe, C. Potential role for statins in sickle cell disease. *Pediatr. Blood Cancer,* **2013**, *60*(4), 550-557.

[31] Almog, Y.; Shefer, A.; Novack, V.; Maimon, N.; Barski, L.; Eizinger, M.; Friger, M.; Zeller, L.; Danon, A. Prior statin therapy is associated with a decreased rate of severe sepsis. Circulation **2004**, 110, 880-885.

[32] Jadhav, S.B.; Jain, G.K. Statins and osteoporosis: new role for old drugs. *J. Pharm. Pharmacol*., **2006**, *58*(1), 3-18.

[33] Robinson, J.G.; Smith, B.; Maheshwari, N.; Schrott, H. Pleiotropic effects of statins: benefit beyond cholesterol reduction? A meta-regression analysis. *J. Am. Coll. Cardiol.*, **2005**, *46*(10), 1855-1862.

[34] Stancu, C.; Sima, A. Statins: mechanism of action and effects. *J. Cell. Mol. Med.*, **2001**, *5*(4), 378-387.

[35] Alegret, M.; Silvestre, J.S., Pleiotropic effects of statins and related pharmacological experimental approaches. *Timely Top. Med. Cardiovasc. Dis.*, **2007**, *11*, E10.

[36] Wierzbicki, A.S.; Poston, R.; Ferro, A. Lipid and non-lipid effects of statins. *Pharmacol. Ther.,* **2003**, 99(1), 95-112.

[37] Plenz, G.A.; Hofnagel, O.; Robenek, H. Differential modulation of caveolin-1 expression in cells of the vasculature by statins. *Circulation*, **2004**, *109*(2), e7-e8.

[38] Irache, J.M.; Esparza, I.; Gamazo, C.; Agüeros, M.; Espuelas, S. Nanomedicine: novel approaches in human and veterinary therapeutics. *Vet. Parasitol.*, **2011**, *180*(1-2), 47-71.

[39] Sahoo, S.K.; Labhasetwar, V., Nanotech approaches to drug delivery and imaging. *Drug Discov. Today*, **2003**, *8*(24), 1112-1120.

[40] Farokhzad, O.C.; Langer, R. Impact of Nanotechnology on Drug Delivery. *ACS Nano*., **2009**, *3*(1), 16-20.

[41] Couvreur, P.; Vauthier, C. Nanotechnology: intelligent design to treat complex disease. *Pharm. Res.,* **2006**, 23(7), 1417-1450.

[42] Moghimi, S.M.; Peer, D.; Langer, R. Reshaping the future of nanopharmaceuticals: ad iudicium. *ACSNano.,* **2011**, 5(11), 8454-8458.

[45] Junghanns, J.U.; Mü ller, R.H. Nanocrystal technology, drug delivery and clinical applications. *Int. J. Nanomedicine*, **2008**, *3*(3), 295-309.

[48] Athul, P.V. Preparation and characterization of simvastatin nanosuspension by homogenization method. *Int. J. PharmTech Res.*, **2013**, *5*(1), 193-197.

[49] Arunkumar, N.; Deecaraman, M.; Rani, C.; Mohanraj, K.P.; Venkates Kumar, K. Preparation and solid state characterization of atorvastatin nanosuspensions for enhanced solubility and dissolution. *Int. J. PharmTech Res.*, **2009**, *1*(4), 1725-1730.

[50] Pandya, V.M.; Patel, J.K.; Patel, D.J. Formulation, Optimization and characterization of Simvastatin Nanosuspension prepared by nanoprecipitation technique. *Der Pharmacia Lettre,* **2011**, *3*(2), 129-140.

[51] Jiang, T.; Han, N.; Zhao, B.; Xie, Y.; Wang, S. Enhanced dissolution rate and oral bioavailability of simvastatin nanocrystal prepared by sonoprecipitation. *Drug Dev. Ind. Pharm.*, **2012**, *38*(10), 1230-1239.

[52] Kim, M.S.; Jin, S.J.; Kim, J.S.; Park, H.J.; Song, H.S.; Neubert, R.H.; Hwang, S.J. Preparation, characterization and *in vivo* evaluation of amorphous atorvastatin calcium nanoparticles using supercritical antisolvent (SAS) process. *Eur. J. Pharm. Biopharm.*, **2008**, *69*(2), 454-465.

[53] Anwar, M.; Warsi, M.H.; Mallick, N.; Akhter, S.; Gahoi, S.; Jain, G.K.; Talegaonkar, S.; Ahmad, F.J.; Khar, R.K. Enhanced bioavailability of nano-sized chitosan-atorvastatin conjugate after oral administration to rats. *Eur. J. Pharm. Sci.*, **2011**, *44*(3), 241-249.

[54] Mora-Huertas, C.E.; Fessi, H.; Elaissari, A. Polymer-based nanocapsules for drug delivery. *Int. J. Pharm.*, **2010**, *385*(1-2), 113-142.

[55] Muthu, M.S.; Singh, S. Targeted Nanomedicines: Effective Treatment Modalities for Cancer, AIDS and Brain Disorders. *Nanomedicine (Lond)*, **2009**, *4*(1), 105-118.

[56] Bei, D.; Meng, J.; Youan, B.B., Engineering Nanomedicines for Improved Melanoma Therapy: Progress and Promises. *Nanomedicine (Lond)*, **2010**, *5*(9), 1385-1399.

[57] Moghimi, S.M.; Hunter, A.C.; Murray, J.C. Nanomedicine: current status and future prospects. *FASEB J.,* **2005**, *19*(3), 311-330.

[58] Soni, A.; Gadad, A.; Dandagi, P.; Mastiholimath, V. Simvastatin-loaded PLGA nanoparticles for improved oral bioavailability and sustained release: Effect of formulation variables. *Asian J. Pharm.*, **2011**, *5*(2), 57-64.

[61] Ho, M.H.; Chiang, C.P.; Liu, Y.F.; Kuo, M.Y.; Lin, S.K.; Lai, J.Y.; Lee, B.S. Highly efficient release of lovastatin from poly(lactic-co-glycolic acid) nanoparticles enhances bone repair in rats. *J. Orthop. Res.*, **2011**, *29*(10), 1504-1510.

[62] Chen, L.; Nakano, K.; Kimura, S.; Matoba, T.; Iwata, E.; Miyagawa, M.; Tsujimoto, H.; Nagaoka, K.; Kishimoto, J.; Sunagawa, K.; Egashira, K. Nanoparticle-mediated delivery of pitavastatin into lungs ameliorates the development and induces regression of monocrotaline-induced pulmonary artery hypertension. *Hypertension*, **2011**, *57*(2), 343-350.

[63] Wang, Q.; Yan, J.; Chen, X.; Li, J.; Yang, Y.; Weng, J.; Deng, C.; Yenari, M.A. Statins: multiple neuroprotective mechanisms in neurodegenerative diseases. *Exp. Neurol.*, **2011**, *230*(1), 27-34.

[64] Sun, W.; Xie, C.; Wang, H.; Hu, Y. Specific role of polysorbate 80 coating on the targeting of nanoparticles to the brain. *Biomater.,* **2004**, *25*(15), 3065-3071.

[65] Kreuter, J.; Shamenkov, D.; Petrov, V.; Ramge, P.; Cychutek, K.; Koch-Brandt, C.; Alyautdin, R. Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood–brain barrier. *J. Drug Target.*, **2002**, *10*(4), 317-325.

[66] Simşek. S.; Eroğlu, H.; Kurum, B.; Ulubayram, K. Brain targeting of Atorvastatin loaded amphiphilic PLGA-b-PEG nanoparticles. *J. Microencapsul.*, **2013**, *30*(1), 10-20.

[67] Sata, M.; Nishimatsu, H.; Suzuki, E.; Sugiura, S.; Yoshizumi, M.; Ouchi, Y.; Hirata, Y.; Nagai, R. Endothelial nitric oxide synthase is essential for the HMG-CoA reductase inhibitor cerivastatin to promote collateral growth in response to ischemia. *FASEB J.*, **2001**, *15*(13), 2530-2532.

[68] Kureishi, Y.; Luo, Z.; Shiojima, I.; Bialik, A.; Fulton, D.; Lefer, D.J.; Sessa, W.C.; Walsh, K. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. *Nat. Med.*, **2000**, *6*(9), 1004-1010.

[69] Kubo, M.; Egashira, K.; Inoue, T.; Koga, J.; Oda, S.; Chen, L.; Nakano, K.; Matoba, T.; Kawashima, Y.; Hara, K.; Tsujimoto, H.; Sueishi, K.; Tominaga, R.; Sunagawa, K. Therapeutic neovascularization by manotechnology- mediated cell-selective delivery of pitavastatin into the vascular endothelium. *Arterioscler. Thromb. Vasc. Biol.*, **2009**, *29*(6), 796-801.

[70] Croons, V.; De Meyer, I.; Houten, S.M.; Martinet, W.; Bult, H.; Herman, A.G.; De Meyer, G.R. Effect of statins on the viability of macrophages and smooth muscle cells. *J. Cardiovasc. Pharmacol.*, **2010**, *55*(3), 269-275.

[71] Broz, P.; Ben-Haim, N.; Grzelakowski, M.; Marsch, S.; Meier, W.; Hunziker, P. Inhibition of macrophage phagocytotic activity by a receptor-targeted polymer vesicle-based drug delivery formulation of pravastatin. *J. Cardiovasc. Pharmacol.*, **2008**, *51*(3), 246-252.

[72] Wang, J.; Chen, J.; Ye, N.; Luo, Z.; Lai, W.; Cai, X.; Lin, Y. Absorption, pharmacokinetics and disposition properties of solid lipid nanoparticles (SLNs). *Curr. Drug Metab.*, **2012**, *13*(4), 447-456.

[73] Shah, M.; Pathak, K. Development and statistical optimization of solid lipid nanoparticles of simvastatin by using 2(3) full-factorial design. *AAPS PharmSciTech*, **2010**, *11*(2), 489-496.

[74] Tiwari, R.; Pathak, K. Nanostructured lipid carrier versus solid lipid nanoparticles of simvastatin: comparative analysis of characteristics, pharmacokinetics and tissue uptake. *Int. J. Pham.*, **2011**, *415*(1-2), 232-243.

[75] Suresh, G.; Manjunath, K.; Venkateswarlu, V.; Satyanarayana, V. Preparation, characterization, and *in vitro* and *in vivo* evaluation of lovastatin solid lipid nanoparticles. *AAPS PharmSciTech*, **2007**, *8*(1), E162–E170.

[76] Cho, K.; Wang, X.; Nie, S.; Chen, Z.G.; Shin, D.M. Therapeutic nanoparticles for drug delivery in cancer. *Clin. Cancer Res.,* **2008,** *14*(5), 1310-1316.

[77] Ali, H.; Shirode, A.B.; Sylvester, P.W.; Nazzal, S. Preparation, characterization, and anticancer effects of simvastatin-tocotrienol lipid nanoparticles. *Int. J. Pharm.*, **2010**, *389*(1-2), 223-231.

[78] Bangham, A.D.; Standish, M.M.; Watkins, J.C., Diffusion of univalent ions across the lamellae of swollen phospholipids. *J. Mol. Biol.*, **1965**, *13*(1), 238-252.

[80] Forssen, E.; Willis, M. Ligand-targeted liposomes. *Adv. Drug Deliv. Rev.*, **1998**, *29*(3), 249-271.

[81] Zhang, L.; Gu, F.X.; Chan, J.M.; Wang, A.Z.; Langer, R.S.; Farokhzad, O.C. Nanoparticles in medicine: therapeutic applications and developments. *Clin. Pharmacol. Ther.*, **2008**, *83*(5), 761-769.

[84] Afergan, E.; Ben David, M.; Epstein, H.; Koroukhov, N.; Gilhar, D.; Rohekar, K.; Danenberg, H.D.; Golomb, G. Liposomal simvastatin attenuates neointimal hyperplasia in rats. *AAPS J.*, **2010**, *12*(2), 181-187.

[85] Coimbra, M.; Banciu, M.; Fens, M.H.; de Smet, L.; Cabaj, M.; Metselaar, J.M.; Storm, G.; Schiffelers, R.M. Liposomal pravastatin inhibits tumor growth by targeting cancer-related inflammation. *J. Control. Rel.*, **2010**, *148*(3), 303-310.

[86] Aso, S.; Ise, H.; Takahashi, M.; Kobayashi, S.; Morimoto, H.; Izawa, A.; Goto, M.; Ikeda, U. Effective uptake of N-acetylglucosamine-conjugated liposomes by cardiomyocytes *in vitro*. *J. Control. Rel.*, **2007**, *122*(2), 189-198.

[88] Gaucher, G.; Satturwar, P.; Jones, M.C.; Furtos, A.; Leroux, J.C. Polymeric micelles for oral drug delivery. *Eur. J. Pharm. Biopharm.*, **2010**, *76*(2), 147-158.

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