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REVIEW

Endothelial progenitor cells: Exploring the pleiotropic effects of statins

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Abstract

Statins have become a cornerstone of risk modification for ischaemic heart disease patients. A number of studies have shown that they are effective and safe. However studies have observed an early benefit in terms of a reduction in recurrent infarct and or death after a myocardial infarction, prior to any significant change in lipid profile. Therefore, pleiotropic mechanisms, other than lowering lipid profile alone, must account for this effect. One such proposed pleiotropic mechanism is the ability of statins to augment both number and function of endothelial progenitor cells. The ability to augment repair and maintenance of a functioning endothelium may have profound beneficial effect on vascular repair and potentially a positive impact on clinical outcomes in patients with cardiovascular disease. The following literature review will discuss issues surrounding endothelial progenitor cell (EPC) identification, role in vascular repair, factors affecting EPC numbers, the role of statins in current medical practice and their effects on EPC number.

Key words: Statins; Endothelial progenitor cells; Pleiotropic effects; Ischaemic heart disease; Pleiotropic mechanisms

Core tip: Statin therapy is a cornerstone of current management in coronary artery disease. Conventional thinking of stain therapy is for reduction of low-density lipoproteins. However a number of studies have observed an early benefit prior to any significant change in lipid profile. Therefore alternative pleiotropic mechanisms to account for this have been proposed. One such proposed mechanism is the ability of statins to augment both number and function of endothelial progenitor cells (EPCs). The following literature review discusses issues surrounding EPC identification, role in vascular repair, the role of statins in current medical practice and their effects on EPCs.

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INTRODUCTION

The maintenance of endothelial integrity is essential for the preservation of a healthy vasculature[1]. This integrity results from a balance between on-going endothelial damage and the rate of vascular repair. Disruption of endothelial integrity or impairment of endothelial repair mechanisms is a central step in both the initiation and progression of atherosclerosis[2]. Endothelial repair is dependent on undifferentiated cells migrating to sites of vascular injury[3-5] then differentiating into mature endothelial cells[6-13]. These undifferentiated cells are called endothelial progenitor cells (EPCs) have a central role in vascular repair by virtue of their ability to proliferate, migrate to site of vascular injury and then differentiate into mature vascular endothelium[13,14]. EPCs perpetuate this cycle by secreting pro-angiogenic cytokines[15].

Statins form the corner stone of treatment of coronary artery disease. The safety and efficacy of statins in reducing cardiac events by decreasing serum LDL-cholesterol has been well described[16-18]. Recently however studies have shown the early beneficial effect of statins occurs before any significant change in lipid profile. This led to the hypothesis that cardiovascular benefits of statins may occur *via* alternative mechanisms other than reduction of LDL-cholesterol alone[19,20]. One such proposed mechanism is the ability of statins to augment both number and function of EPCs.

The following literature review discusses issues surrounding EPC identification, role in vascular repair, factors affecting EPC numbers, the role of statins in current medical practice and their effects on EPC number.

RESEARCH AND LITERATURE

We performed a review of various studies within the literature available on endothelial progenitor cell and statins. The authors searched various databases (EMBASE, OVID, PubMed) using the keywords: "Endothelial progenitor cells", "statin", "pleiotropic effects". We studied the various publications that we obtained from the search results. Full text manuscripts were obtained. We only included papers in the English language.

EPCS

Cellular identification and staging of differentiation has been made possible by specific surface receptors called epitopes that allow immunophenotyping. This process allows identification of subset of cellular surface molecule termed cluster of differentiation (CD). Cellular subtypes may be defined by the presence or absence of a particular CD molecule. Therefore "CD" may be "+ "or "-" denoting either presence or absence of a particular CD, and is used to describe stem cells rather than fully differentiated cell types. Certain cell types may have variable CD marker expression during maturation for example, and therefore classed as bright (high), mid (mid) or dim (low) denoting intensity of expression[21,22].

Vascular repair had previously been thought to be due to migration and proliferation of fully differentiated endothelial cells, in a process called angiogenesis[23]. Asahara *et al*[24] identified putative cells with cell surface marker $CD34^+$, alternately named kinase insert domain receptor (KDR/VEGFR) markers capable of differentiating into endothelial cells both *in vitro* and *in vivo*[24-26]. Subsequent studies recognised that in fact undifferentiated cells subsequently termed EPCs migrated to sites of neovascularization and then differentiate into endothelial cells[24,26] in a process called vasculogenesis[27]. EPCs are derived from pluripotential stem cells within the bone marrow. These then evolve into mature endothelial cells[24] accounting for only 0.001%-0.0001% of peripheral blood cells in an unstressed state[28]. Circulating EPCs may be isolated from bone marrow or the circulation as mononuclear cells[24,29,30], expressing a variety of endothelial surface markers[31]. However, there currently remains a lack of consensus on phenotypic and functional definition of endothelial precursor cells[32,33].

EPCs are a diverse group of cells of different lineages that have angiogenic potential, but are not always necessarily able to differentiate into functional endothelial cells as would be suggested from their name[32]. EPCs are derived from $CD34^+$ hematopoietic progenitor cells[6,24,29,31], with the subset of EPCs characterized by co-expression of endothelial marker proteins[6,29,31]. Studies have identified 3 markers associated with early functional EPCs including CD133, CD34, and the vascular endothelial growth factor receptor-2 (VEGFR-2) also known as kinase insert domain receptor (KDR, Flk-1 or CD309)[7,31]. Therefore EPCs express markers of both hematopoietic stem cells (CD34 and CD133) and endothelial cells (CD146, vWF, and VEGFR2)[26,28,29,31,34-37]. The presence of certain cell surface markers are depend on the stage of maturations of the EPC. For example the cell surface marker CD133, a 120-kDa trans-membrane polypeptide, is expressed on bone marrow derived hematopoietic stem and progenitor cells in peripheral blood[37]. The expression of CD133 on EPCs declines during maturation within the peripheral circulation. Currently there remains some uncertainty as to when EPCs lose the CD133 surface marker, whether during transmigration from bone marrow to circulation or later whilst in the peripheral blood system[38]. Nevertheless the loss of CD133 represents the transformation into more mature EPCs that are endothelial-like cells[37].

Table 1 Table to show cell markers during development of endothelial progenitor cell

	Endothelial progenitor cells		
	Bone marrow	Circulation	
		Early EPCs	Mature EPCs
$CD133^+$	+	+/-	-
$CD34^+$	+	+	+
$VEGFR2^+$	+	++	+++
$CD31^+$	-	+	+
VE-cadherin	-	+	+
vWF	-	+	+

EPC: Endothelial progenitor cell; VEGFR: Vascular endothelial growth factor receptor.

Whereas the expression of CD34 a cell surface marker found on immature pluripotential stem cells[31] that acts as an adhesion molecule, although the precise function remains unclear, gradually increases as the CD133 decreases as the EPC matures[37]. During the course of maturity EPCs begin to have increased expression of other markers specific to endothelial cells such as VEGFR-2, VE-cadherin, and von Willebrand factor (vWF)[37].

The expression of $CD34^+$, $CD133^+$, and/or $VEGF2^+$ has been used as identifying markers of EPCs in a number of studies[28,32,39-41]. Whereas as other studies advocate the use of $CD133^+$ either alone or in combination with $CD34^+$/VEGFR-2^+ cells for identification of EPCs[31,42]. In contrast, other studies have suggested that $CD133^+$ are haematopoietic cell lines and have not been identified in and therefore unable to form endothelial phenotypic EPCs[7,40,43]. Ingram *et al*[28] proposed that $CD45^-$ cells incorporated "true" circulating EPCs and verified by other studies[28,39,40,43]. Interestingly, $CD34^+$/$VEGFR2^+$ and diminished (dim) CD45 ($CD45^{dim}$) cells have been found to have greater correlation with coronary heart disease severity and response to statin therapy[44,45].

In summary, the maturity of the EPC is marked by the gradual loss of CD133, gradual increased expression of $CD34^+$ and the appearance of CD31, VE-cadherin and vWF cell surface markers (Table 1).

EPCS AND CORONARY ARTERY DISEASE

Endothelial integrity is essential for healthy vasculature, and can be thought of as a balance between continued endothelial damage and the capacity to repair by a pool of EPCs[9,46]. It is now generally accepted that cardiovascular risk correlates with EPC numbers. Highlighting the integral relationship between endothelium and atherosclerosis[47-51], disruption of endothelial integrity by endothelial cell injury has been shown to be a stimulus for the development of atherosclerosis[2], but also as a stimulus for augmentation of EPC number and function[9,52,53]. Continued endothelial damage[54] may lead to an eventual reduction of the number of EPCs. Elevated EPC numbers have been shown to be associated with augmented formation of collaterals in coronary artery disease[55] and restoration of endothelial vasodilator function[9]. A reduction in EPC numbers may lead to deficient endothelial repair and progression of atherosclerosis, with further EPC depletion and perpetuation of atherosclerosis[9,56]. However, it is uncertain whether low numbers of circulating EPCs represents enhanced usage by vascular repair processes, or reduced production by bone marrow.

$CD34^+$ $VEGFR2^+$ EPCs cells have been shown to be reduced in patients with atherosclerotic coronary and peripheral disease[57]. Vasa *et al*[56] found not only reduced numbers, but also impaired function of EPCs in patients with coronary artery disease. Elevated numbers of EPC have been associated with freedom from myocardial infarction, hospitalization, revascularization and cardiovascular death in patients with coronary artery disease[56,58]. Furthermore the predictive value of EPC count has been shown to be independent of traditional cardiovascular risk factors[9,46,59]. In fact, the extent of the reduction in EPC numbers has been associated not only with coronary artery disease burden[60], but also the presence of symptoms[61,62].

Finally, elevated numbers of circulating $CD34^+$/$CD133^+$/VEGFR2 EPCs have been observed after an acute myocardial infarction[42,63]. This may be regarded as a consequence of cardiac ischaemia together with raised inflammatory and haematopoietic cytokines stimulating EPC mobilisation from the bone marrow[64-66]. A similar response is seen following coronary angioplasty[67], and interestingly, the combination of an acute coronary syndrome (ACS) treated by angioplasty provoked an enhanced EPC response[68]. Therefore, EPC may have a central role not only in repairing coronary vessels after plaque rupture, but also after any coronary intervention.

STATIN THERAPY

Statins act by competitively inhibiting 3 hydroxy-3-methylglutaryl Coenzyme A (HMG CoA) reductase, the rate limiting step in the mevalonate pathway producing isoprenoids including cholesterol. The competitive inhibition of HMG CoA reductase induces the expression of LDL receptors within the liver, thereby increasing the catabolism of plasma LDL, with a consequent decrease in LDL-cholesterol levels[69].

The safety and efficacy of statins in reducing cardiac events by decreasing serum LDL-cholesterol has been well described[16-18]. Statin therapy has been shown to reduce death and cardiovascular events in primary prevention of atherosclerosis[70], stable coronary artery disease[16,71-73], ACS[74,75] and secondary prevention[72]. Statins also appear to reduce development of atherosclerotic lesions[76,77] and decrease plaque burden[13,78].

The beneficial effect of intensive statin therapy was studied in a prospective meta-analysis of 90056 patients from 14 randomised trials and found greater

cholesterol reduction was associated with better patient outcomes[19]. The study found that the 5-year incidence of major adverse cardiac events, coronary revascularization and stroke was reduced by one fifth for every millimoles per liter reduction in LDL cholesterol, which was irrespective of the initial lipid profile[19].

Another meta-analysis found aggressive statin therapy was associated with reduced peri-procedural myocardial infarction and a 44% risk reduction in major adverse cardiovascular events at 30-d irrespective of clinical presentation[79]. Moreover, the ARMYDA-RECAPTURE study[80] found reloading of the high dose statin, atorvastatin 80 mg in 383 NSTEMI and stable angina patients on chronic therapy prior to percutaneous coronary intervention (PCI) had a 50% reduction in 30-d major adverse cardiac events in both group with a greater reduction in NSTEMI group[80].

These studies led to the universal adoption of statin therapy in patients with coronary artery disease irrespective of presentation from stable angina to ACSs[81,82].

THE EFFECT OF STATIN THERAPY ON EPCS

Several studies have shown the early beneficial effect of statins occurs before any significant change in lipid profile. This led to the hypothesis that cardiovascular benefits of statins may occur *via* alternative mechanisms other than reduction of LDL-cholesterol alone[19,20]. These potential beneficial effect(s) may represent a potential therapeutic target for ischemic heart disease patients, and therefore is of great interest. There have been a number of mechanisms proposed to account for pleiotropic effects of statin therapy. These include reduction in vascular inflammation[83], reduction of platelet aggregability and thrombus deposition[77,84-86], enhancement of fibrinolysis[87] and increased endothelium derived NO production[88-90]. However the mechanism that has evoked the most interest is the impact of statins on EPCs[70].

Statin therapy has been associated with greater numbers of circulating EPCs by enhancing mobilization, differentiation, increasing longevity, enhance homing to sites of vascular injury with augmentation of re-endothelisation by enhancing expression of adhesion molecules on EPC cell surface membrane[3,70,91-94].

However, the duration of the effect on EPC number by statin therapy continues to remain contentious.

In one study, atorvastatin therapy was shown to significantly increase circulating EPC as soon as 1 wk with plateauing after 3-4 wk with a 3-fold increase of EPCs from baseline in a stable angina population was also observed[70]. Whereas Deschaseaux *et al*[95] investigated whether EPCs could be firstly detected and secondly characterized in patients receiving long-term statin therapy defined as 4 wk. The group found a significantly greater number of $CD34^+$, $CD34^+/CD144^+$ circulating EPC in patients receiving statin therapy compared to statin naïve patients. Interestingly two types of EPCs were detected, early and late EPCs. The early EPCs were found to form elongated cells whereas the late EPC population gave rise to cobblestone-like colonies with strong proliferation capacities seen *in-vitro* cell culture. The numbers of early EPCs were significantly higher in patients not receiving statin therapy whereas late EPCs were significantly higher in patients receiving statin therapy. The study also observed that long term statin therapy specifically maintained late EPCs in circulation with a $CD34^+/CD144^+$ phenotype. Rodent studies have found rosuvastatin resulted in a greater than 3 fold increase in EPC numbers when compared with placebo as long as 10 wk after myocardial infarction[56]. Long-term atorvastatin 10 mg for 12 mo markedly increased EPC number with an associated decrease in oxidative DNA damage[35]. However to the contrary, Hristov *et al*[96] found reduced numbers of circulating EPCs in CHD patients on long-term statin treatment.

Statins appear to have a dose dependant effect on EPC count. A double blinded randomised pilot study found greater number of circulating $CD34^+$ $VEGFR\text{-}2^+$ EPCs after 12 wk of therapy with pravastatin 20 mg when compared to atorvastatin 10 mg[97]. Similarly, in ACS patients' intensive statin therapy with atorvastatin 80 mg after primary or rescue PCI was associated with greater EPC count at 4-mo follow up as compared to 20 mg atorvastatin. The authors found no beneficial effect in an improvement of LV function[98]. Furthermore statin reloading in patients on moderate statin therapy undergoing percutaneous coronary intervention has been shown to increase EPC count[99,100] this correlates with the beneficial effect of statin reloading of high dose statin in patients on chronic therapy[80] mentioned above.

PLEIOTROPIC EFFECTS OF STATIN THERAPY

Several proposed intracellular signaling mechanisms accounting for the pleiotropic effect of statin therapy have been put forward. Figure 1 below summarizes the positive and negative effects on EPC proliferation, mobilisation and longevity but also the effect of statin therapy.

Nitric oxide pathway

The first proposed intracellular signaling mechanisms involves nitric oxide pathway. The endothelium releases nitric oxide (NO), a primary mediator of smooth muscle tone that causes vasodilatation through the activity of endothelial-type nitric oxide synthase (eNOS)[101-104]. NO has an central role in vascular homeostasis with its bioavailability dependent on expression of endothelial eNOS[105], presence of eNOS substrate and or co-factors[106], phosphorylation of eNOS[107,108] or due to excessive depletion of NO such as seen with presence of

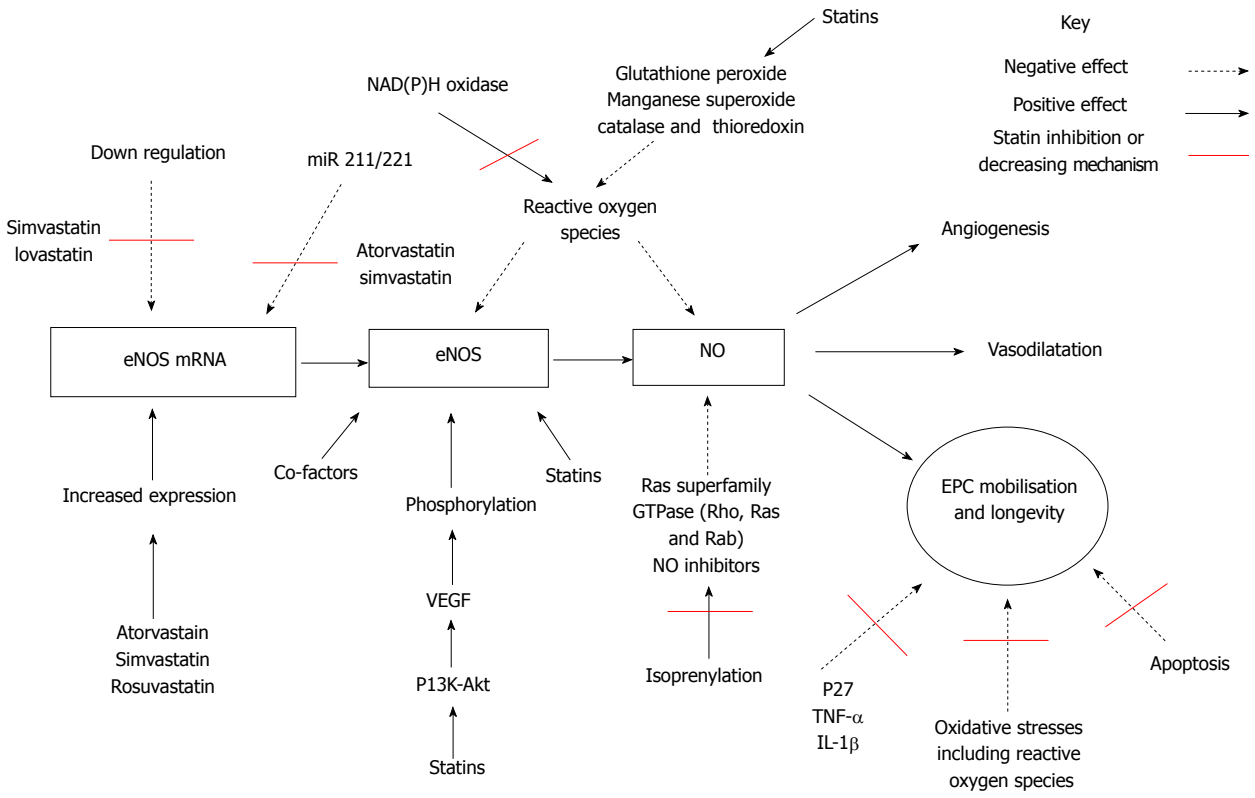

Figure 1 Simplified diagram illustrating the positive and negative effects on endothelial progenitor cell proliferation, mobilisation and longevity together with proposed mechanisms of action of statin therapy. EPC: Endothelial progenitor cell; NO: Nitric oxide; eNOS: Endothelial nitric oxide synthase; VEGF: Vascular endothelial growth factor; mRNA: Messenger ribonucleic acid; TNF: Tumor necrosis factor alpha; IL-1: Interleukin 1; P13k-AKT: Phosphoinositide 3-kinase - protein kinase B pathway; NAD(P)H oxidase: Nicotinamide adenine dinucleotide phosphate-oxidase; miR : Micro non-coding ribonucleic acid.

excessive reactive oxygen species[109]. However the main functions of NO is as a cellular signaling molecule[101], an angiogenic factor involved in stimulation, promotion, and stabilization of new blood vessels together with VEGFs, FGFs, Angiopoietins, PDGF, MCP-1, TGF, various integrins, VE-cadherin[110-113]. Statin therapy has been proposed to both enhance expression and activity of eNOS[114] a prerequisite stage for statin-mediated EPC mobilisation[115]. Statins are known to augment eNOS activity[116-118], increase eNOS expression and restoration of endothelial function[104,119-121]. Statins have also been associated with increased EPC longevity *via* several pathways including inhibition of p27[122], down regulating TNF-α or IL-1β expression[123] and prolonging eNOS expression[122] and finally by increasing eNOS mRNA half-life[124,125]. Kosmidou *et al*[126] found simvastatin and rosuvastatin prolonged expression by increasing 3' polyadenylation of eNOS mRNA. Laufs *et al*[88,124] firstly noted simvastatin and lovastatin reversed the down-regulation of eNOS expression caused by hypoxia and secondly simvastatin reversed down regulation of eNOS expression induced by oxidized LDL[88,124] a recognised cause of atherosclerosis.

miR 221 and miR 222 levels

A second observed pleotropic mechanism of statin therapy has been a decreased level of micro non-coding RNAs called miR 221 and miR 222. These negatively regulate protein expression at post-transcriptional stage[127]. This down regulating effect occurs by targeting 3' untranslated regions resulting in either degradation of target mRNA or impairing translation[128]. Furthermore miR-221 and miR-222 have been observed to regulate proliferation and differentiation of CD34-positive haematopoietic progenitor cells by reducing expression of *c-kit* receptor factor impairs haematopoietic progenitor cell proliferation[129]. Increased miR-221 and or miR-221 expression in EPC down regulates EPC differentiation and mobilisation *via* c-kit and or eNOS pathways in coronary artery disease patients[127]. Atorvastatin has been shown firstly to decrease miR 221 and miR 222, and secondly increase EPC numbers[127]. Cerda *et al*[130] found both atorvastatin and simvastatin increased NO levels and NOS3 mRNA expression, whereas ezetimibe did not. Atorvastatin, simvastatin and ezetimibe have all been shown to down-regulate the expression of miR-221, whereas miR-222 was reduced only after atorvastatin treatment. The magnitude of the reduction of miR-221 and miR-222 after treatment with statins correlated with an increment in NOS3 mRNA levels[130]. The eNOS and miR221/222 are thought likely to be components of the same pathway[131].

The PI3K/Akt/mTOR pathway

The third proposed pleiotrophic mechanism involves the phosphoinositide 3-kinase (PI3K)/protein kinase B

(Akt)/mammalian target of rapamycin (mTOR) signaling pathway plays. The PI3K/Akt/mTOR pathway plays a central role in multiple cellular processes, including cell proliferation, angiogenesis, metabolism, differentiation and longevity[132,133]. PI3K generates phosphatidylinositol 3,4,5-triphosphate (PIP3) an important lipid secondary messenger which in turn plays a central role in several signal transduction pathways[134,135] including activation of the serine/threonine kinases PDKl and Akt. Akt controls protein synthesis and cell growth *via* the phosphorylation of mTOR[136]. The PI3K/Akt pathway has been associated with angiogenesis through the regulation of the NO signaling pathway[137]. The PI3K pathway releases a group of angiogenic factors including VEGF. VEGFR2 has a central role in VEGF-induced angiogenesis[138]. VEGF is required for the migration of endothelial cells and *via* PI3K-Akt dependent manner allows formation of capillary like structures[139]. Studies have shown that NO production may be induced by VEGF and appears to be attenuated by the inhibition of PI3K[140]. This is thought to occur *via* phosphorylation of eNOS at the serine 1177 residue by Akt[107,141], required for the VEGF induced endothelial cell migration[142]. Factors that stimulate the PI3K/Akt protein kinase pathway, including statins, have been shown also to activate eNOS[87,141,143]. In turn, the expression of eNOS appears to be fundamental for mobilization of EPC and any impairment in PI3K/Akt/eNOS/NO signaling pathway may result in decreased EPC number[91,92].

The PI3K/Akt/mTOR intracellular pathway *via* inhibition of the Rho kinase has also been shown to preserves mitochondrial permeability transition pore preventing mitochondrial apoptosis, and therefore death, while conserving cardiomyocyte function[144,145].

These proposed mechanisms may account for difference in the effect of statin therapy in acute or chronic therapy. Statins given during acute ischaemic stress have been shown to firstly potentiate adenosine receptors[146,147] eventually leading to downstream regulation of eNOS and therefore increases NO production. Secondly statins augment activation of the reperfusion injury salvage kinase (RISK) pathway[148]. This results in enhanced activity of the PI3K/Akt/mTOR intracellular signal pathways[149], leading to preservation of mitochondrial function and cardio-protection. Short-term high dose statin therapy have shown an increase in both EPC mobilisation from bone marrow and augmented function[92,150-154].

Whereas chronic statin therapy has been linked to a phenomenon termed pre-ischaemic conditioning, protecting the myocardium against ischaemia[155]. This is believed to be secondary to statin induced NO availability by up regulation of eNOS and stabilisation of eNOS mRNA. Secondly, by increased production of NO and superoxide radicals improves vascular function and reducing vascular inflammation respectively[88,156]. Statins also inhibit isoprenylation of a number of Ras superfamily GTPase including Rho, Ras and Rab[157] NO inhibitors resulting in increased NO bioavailability. Thirdly, by preventing mitochondrial apoptosis and preservation of cardiomyocyte function *via* the up-regulation of the PI3K/Akt/mTOR intracellular signalling pathway by inhibition of Rho kinase[144,145]. However, the RISK pathway has been shown the down regulated with chronic statin therapy[158] and has been shown to become reactivated by statin re-loading[159]. The latter may account for the increase in EPC count in patients on chronic statin therapy reloaded with statin therapy[80,99,100].

Oxidative stresses

Finally, EPC mobilisation and or function may also be affected by oxidative stress[153,160]. Oxidative stresses occur secondary to generation of oxygen free radicals or reactive oxygen species (ROS). Oxidative stress has a central role in cardiovascular disease, and a pivotal role in atherosclerosis[161]. Cellular oxidative stress seen with oxidized low-density lipoprotein (ox-LDL) has a central role in the pathogenesis of atherosclerosis. LDL is oxidised by reactive oxygen species from both circulating cells and cells on vascular walls[162,163]. In essence, LDL oxidation is a result of a chain reaction of free radicals converting polyunsaturated fatty acids into lipid peroxides and as a consequence, formation of active aldehydes[164]. The biochemical reaction forming ox-LDL have been found to cause senescence of EPCs[165]. Whereas high density lipoprotein is regarded as atheroprotective due to some part of its antioxidant properties also has a positive effect on EPC number and function[166]. There are a number of endogenous antioxidants exerting protective effects by scavenging ROS. An indirect way ROS effects EPCs includes ROS reacting with NO forming a potent oxidant[167] with a consequent decrease in NO. Decrease in NO either by excessive oxidation or impaired production reduces EPC mobilisation and/or function[161,168]. Secondly, direct exposure to oxidative stresses or in disease conditions with high oxidative stress, for example diabetes, is associated with induced EPC apoptosis with significant reduction in EPC numbers[168,169], mobilisation, function[170] and reduced ability to migrate and or integrate into vasculature[161,171].

In an attempt to counteract the effects of oxidative stress EPCs produce superoxide dismutase[172]. Interestingly, cardiovascular risk factors have been found to alter and or reduce the EPC antioxidant ability. Healthy volunteers have found to express higher levels of antioxidative enzyme catalases including glutathione peroxidase and manganese superoxide dismutase when comparing patients with cardiovascular disease[173,174]. The underlying pathophysiological mechanism currently remains undetermined. The antioxidant pleiotropic effect of statins may include indirect mechanism increasing NO bioavailability accounting for antioxidant properties contributing to an increase in EPC mobilisation and or function[114,175]. Secondly statin therapy has also been shown to inhibit activation of NAD(P)H oxidase and ROS release[176] but also activate catalase and thioredoxin

ROS scavenging mechanisms[176,177]. Finally, statins appear to have a direct effect by significantly reducing peroxide induced apoptosis of EPCs[169] and decreasing the oxidative damage to DNA in EPCs[178].

G proteins and G protein-coupled receptors

G protein-coupled receptors (GPCR) are comprised of seven trans-membrane domain proteins and are a super family consisting of a large and diverse number of proteins encoded by approximately 5% of human genes[179]. There have been a number of classification systems proposed the most recent "GRAFS" (Glutamate, Rhodopsin, Adhesion, Frizzled/Taste2 and Secretin)[180]. In mammals there are five main families[181]. GPCRs have an integral role in transfer of extracellular stimuli to within the cell by conformational changes in trans-membrane domain structure[182-185]. They regulate physiological responses to a myriad of endogenous ligands including amines, glycoproteins, peptides and lipids. Therefore, not surprisingly that GPCRs have been implicated in regulation of cellular maintenance, differentiation, proliferation and migration of various stem cells[186-189].

GPCRs modulate activity of intracellular signaling *via* G proteins. There are currently four known G protein subfamilies each able to potentiate a number of drown stream effectors triggering a number of signaling pathways[182]. These include activation of Rho associated kinases[190,191], activation or inhibition of cyclic AMP production[192] and PI3Ks and therefore modulate the PI3K/Akt pathway[193,194]. The aforementioned have been implicated in EPC proliferation and function as described above. GPCRs have evoked great interest as a possible target for novel drug therapy[195] as an estimated 50% of all currently prescribed drugs target only a small proportion of GPCRs[196]. They are also becoming increasingly recognised as having a major role in stem cell signaling[197]. The role of GPCR in regulation and function of EPCs and the effect of statin therapy remains yet to be elucidated however current evidence suggests that they may have a pivotal role.

CONCLUSION

EPCs have a pivotal role in the maintenance of vascular integrity. However, factors that influence EPC number, migration and function are now becoming recognised and have potentially a significant role in management of ischaemic heart disease patients. Statins once thought to modify cardiovascular risk only by lowering LDL-cholesterol are now being acknowledged as having alternative mechanisms that appear to have beneficial pleiotropic effects. One such mechanism may be mediated by EPCs. A number of studies have shown positive pleiotropic effect of statins on EPCs, both function and number. There appears to be a complex interaction between statins and EPC that is only now becoming recognised. Despite great progress since Asahara's pioneering work, there remain gaps within our knowledge regarding the pleiotropic effect(s) of statins on EPCs. Further studies are required to elucidate and fully understand any pleiotropic effect and this may guide future beneficial therapeutic interventions.

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