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Calcium channel regulation in vascular smooth muscle cells: Synergistic effects of statins and calcium channel blockers

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Abstract

In the Anglo-Scandinavian Cardiac Outcomes Trial–Lipid Lowering Arm (ASCOT-LLA) we have reported a positive interaction between atorvastatin and amlodipine-based antihypertensive strategy in terms of the prevention of coronary events. In cellular and molecular studies on human vascular smooth muscle cells (VSMC) we have reported that transformation from a differentiated to a synthetic or dedifferentiated phenotype is associated with loss of function of L-type calcium channels and hence loss of potential responsiveness to calcium channel blockers (CCB). Statins directly inhibit cell cycle progression and dedifferentiation of VSMC due to their ability to inhibit the synthesis of isoprenoid cholesterol intermediates. We hypothesize that statins promote a more differentiated VSMC phenotype that results in upregulation of L-type calcium channels and restoration of a CCB-sensitive calcium influx pathway in VSMC, favourably affecting the balance that exists between VSMC proliferation, apoptosis and matrix metalloproteinase production with an associated increase in stability of atheromatous plaques.

Keywords

Synergy; Calcium channel blockers; Statins; Hypothesis

1. Introduction and background

The Anglo-Scandinavian Cardiac Outcomes Trial–Lipid Lowering Arm (ASCOT-LLA) recently demonstrated the large beneficial effects of adding a lipid lowering agent (atorvastatin) to blood pressure (BP) lowering therapy in hypertensive patients not deemed dyslipidaemic [1]. The study showed a 35% reduction in the primary endpoint of fatal coronary heart disease (CHD) and non-fatal myocardial infarction for the atorvastatin versus placebo treated groups. Two BP lowering regimens were used in ASCOT-LLA. Amlodipine, a calcium channel blocker (CCB) plus perindopril, an angiotensin converting enzyme inhibitor (ACEI) added as required, represented a newer combination (termed amlodipine-based). Atenolol, a beta blocker, plus bendro-flumethiazide-K, a thiazide diuretic added as required represented an older, standard combination (termed atenolol-based).

An analysis recently reported comparing the effect of lipid lowering between the two BP lowering combinations showed that, compared with placebo, atorvastatin reduced primary events by 53% in the amlodipine-based group and by 16% in the atenolol-based group, p interaction = 0.02 [2]. These data show a difference far greater than that found between the

two BP lowering combinations in the absence of static (the ASCOT-Blood Pressure Lowering Arm). Given the large relative reduction in events in patients receiving amlodipine-based therapy plus atorvastatin, in addition to the early manifestation of benefit within 3 months of randomization [2], we have sought potential biological explanations based on data from clinical trials and molecular and cellular studies from our own and other laboratories. In particular, we hypothesise a new protective mechanism based on synergistic actions of CCB and inhibitors of hydroxymethylglutaryl coenzyme A (statins) on vascular smooth muscle cells (VSMC).

Atheroma development is widely viewed within an inflammatory framework [3]. The process is believed to be initiated by endothelial dysfunction and permeation of low density lipoproteins (LDL) into the vessel intimal layer in early life [4]. Subsequent infiltration of monocytes/macrophages results in release of growth factors, cytokines and matrix metalloproteinases (MMP) causing modifications in the intimal microenvironment inducing phenotypic modulation of adjacent VSMC. As a consequence, differentiated VSMC (also termed, contractile, mature or quiescent) in the blood vessel wall lose contractile capability. The resultant dedifferentiated VSMCs (also termed, synthetic or immature) are better able to enter the cell cycle, secrete extracellular matrix proteins and undergo proliferation, chemotaxis and apoptosis [5]. Dedifferentiated VSMCs comprise an array of phenotypes that ultimately form the largest cellular component of the developed plaque. As plaque progresses, it develops a necrotic, thrombogenic lipid core. This is protected from contact with blood by a fibrous cap dominantly populated by VSMCs that secrete a collagen rich matrix. Structural integrity of the cap may be compromised by a local increase in inflammatory cells. Leukocyte-derived mediators inhibit VSMC proliferation and induce apoptosis [6] and MMP secreted by inflammatory cells degrade the collagen matrix. This results in depletion of VSMC, weakening of the fibrous matrix and increased cap vulnerability.

2. Actions of statins

Statins are the first line of treatment in the therapy of dyslipidaemia and have well documented favourable effects on the stability of established plaques [7]. These vasculoprotective actions are thought to be due to a number of mechanisms, some LDL-dependent and some LDL-independent. Many of the 'pleiotropic' actions of statins have been attributed to beneficial effects on endothelial function (reviewed in [8]), while possible actions on VSMC have been relatively neglected. Although the 'pleiotropic' effects of statins do not depend on lowering LDL-cholesterol, they are a consequence of inhibition of HMG CoA Reductase, the rate-limiting enzyme in cholesterol synthesis (Fig. 1). HMG CoA catalyses the conversion of HMG CoA to mevalonate which can then be further metabolized to cholesterol. However, mevalonate is also the precursor of the isoprenoids, farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (ggPP) which play a key role in the lipid modification of small G proteins, such as ras and rho [9]. Statins therefore not only block cholesterol synthesis but may also inhibit the ras and rho signalling pathways and inhibition of rho has been proposed to account for increased endothelial NO bioavailability [10]. In animal models of pulmonary hypertension, statins have been demonstrated to increase eNOS expression in endothelial cells [11] and to inhibit rho kinase expression and activity in the lung [12]. Our studies on human VSMCs *in vitro* have also shown that statins directly inhibit cell cycle progression and dedifferentiation of VSMC [13,14] and others have demonstrated similar effects using a variety of statins in human [15-17] and animal-derived VSMC [18]. These effects are due to effects on isoprenylation as they can be prevented by supplementation with isoprenoids [13,14] and probably involve inhibitory effects on small G proteins, such as rho. In rat aortic cells, RhoA and Rho kinase influence multiple SMC-specific promoters and regulate the organization of the actin-cytoskeleton

suggesting that RhoA may be particularly important in the regulation of VSMC phenotype [19].

3. Calcium channel blockers: Mode of action

Calcium channel blockers (CCB) are a chemically heterogeneous class of drugs consisting of dihydropyridines (DHPs), phenylalkylamines and benzothiazepines. All CCB share a common mechanism of action—the inhibition of Ca^{2+} influx through L-type calcium channels. This channel constitutes the dominant Ca^{2+} influx route in contractile VSMC and inhibition of L-type calcium channels accounts for the antihypertensive and vasodilator properties of CCB. Other Ca^{2+} influx routes exist in VSMC which are insensitive to CCBs at clinically used doses [20,21] but the contribution of these other pathways to total Ca^{2+} influx is generally minor in contractile cells. Several Ca^{2+} -independent effects have also been proposed to contribute to the actions of different CCB [22] but the therapeutic relevance of these remains uncertain. At the molecular level Ca^{2+} influx plays a key role in the regulation of phenotype, attachment, migration, growth and proliferation, and apoptosis *via* modulation of intracellular signalling pathways [23–26]. Inhibition of Ca^{2+} influx into VSMC by CCB might therefore be anticipated to have beneficial effects in atherosclerosis and myointimal hyperplasia over and above blood pressure lowering. However, evidence on this question is contradictory. A number of studies in animal dietary models of atherosclerosis have shown that CCB slow the formation of atherosclerotic lesions independent of blood pressure lowering and without significant effect on plasma lipids (reviewed in [27]). By way of contrast, more recent studies in transgenic mice models, such as the Apo E, have either reported no effect of a CCB on atheroma formation [28,29] or a reduction [30] that was not attributed to blockade of L-type calcium channels, but to the anti-oxidant action of the CCB used [30,31]. In animal models of vascular injury and myointimal hyperplasia (e.g. balloon or cuff injury) CCB have been shown to inhibit cell proliferation and reduce neointimal area [32–34]. The efficacy of CCB has been noted to be related to timing of administration and it has been suggested that CCB inhibit the early phase of response to injury [34].

4. Clinical trials involving calcium channel blockers

Observations in man are even more inconsistent than in animal models. In three studies using coronary angiography, the Montreal Heart Institute trial [35], the International Nifedipine Trial on Antiatherosclerotic Therapy (INTACT) [36] and The Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial (PREVENT) [37] treatment with CCB did not influence the overall rate of coronary lesion progression or minimal coronary artery diameter, although CCB-treated patients had less progression of minimal lesions in the Montreal Heart Institute trial [35] and a reduced rate of appearance of new coronary lesions in the INTACT study [36]. More recently, in the Cardiovascular Events in Patients With Coronary Disease and Normal Blood Pressure (CAMELOT) study [38], amlodipine (which was compared with placebo and enalapril) was reported to reduce cardiovascular events compared with placebo but had no significant effect on plaque atheroma volume measured by intravascular ultrasound. In the recent Coronary Calcification (CC) trial no effect of nifedipine was observed with respect to progression of coronary calcification assessed by multi-slice computerized tomography, although there was a trend suggesting a reduction in new lesions [39]. A number of early studies failed to show beneficial effects of CCB on restenosis rates following coronary angioplasty [40,41], although Hoberg et al. reported that verapamil reduced restenosis [42]. Two subsequent studies, Coronary AngioPlasty Amlodipine REStenosis Study (CAPARES) [43] and the Verapamil Slow-Release for Prevention of Cardiovascular Events After Angioplasty

(VESPA) trial [44] have not shown a significant effect of CCB on angiographic restenosis, although clinical endpoints were reduced.

In contrast to this conflicting data from the coronary circulation a number of trials comparing the effects of CCBs with other antihypertensive agents on carotid intima-media thickness (IMT) (an indicator of early atherosclerosis), have been more consistent in showing greater reduction in IMT in people treated with CCB compared with other antihypertensive agents despite comparable reductions in BP [37,45-48]. This has been emphasized by a recent meta-analysis [49], which confirms a statistically greater reduction in IMT progression by CCB compared with other agents even when blood pressure changes are accounted for. In summary the data indicate that CCB are effective in animal models of atherosclerosis or restenosis when there is no pre-existing disease. In man data is not wholly conclusive, but suggest that CCB may influence the progression of early atherosclerosis and new lesion formation, but have little or no effect on established disease.

5. Molecular mechanisms

Recently we proposed a molecular mechanism to account for these observations [50]. We showed that transition of human VSMC from a relatively differentiated (growth arrested or contractile phenotype) to a less differentiated state (synthetic phenotype) following serum stimulation was associated with loss of effect of CCB on both intracellular Ca^{2+} signalling and chemotaxis in response to platelet-derived growth factor (an important chemotactic agent and mitogen for VSMC). These observations could explain why CCB are effective in uninjured arteries and at the early stages of disease, but subsequently lose efficacy as the disease progresses and VSMC become progressively more de-differentiated. Our findings and this suggestion are consistent with the work of others showing increased expression of L-type calcium channels in an aortic cell line (A7r5) following growth arrest [51]; absence of a detectable L-type calcium channel currents in immature neonatal VSMC as compared with adult rat aortic VSMC [52] and loss of L-type calcium channel currents following vascular injury in the rat [53] and fowl [54]. Interestingly, recent work by Kumar et al. [55] has indicated that there is upregulation of TRP channels in VSMC following vascular injury and increased TRP channels in intimal venous VSMC that may compensate for the loss of L-type calcium channels. Most recently we have demonstrated that loss of functional responses mediated by L-type calcium channels induced by dedifferentiation of human VSMC is attributable to a reduced expression of mRNA and protein for the α_1 subunit of the L-type calcium channel ([56] and unpublished data).

6. The hypothesis

Together these observations suggest a hypothesis that incorporates the loss of efficacy of CCBs on advanced plaques and provides a basis for the co-operative action of CCBs with statins. As VSMCs dedifferentiate, CCB-sensitive L-type calcium channels are functionally downregulated. The effect is independent of context and may occur in response to vascular injury, development, remodelling, plaque formation, or in cell culture. This in turn imposes a ceiling on CCB efficacy such that efficacy will decline with plaque progression and increasing VSMC dedifferentiation. We propose that statins promote a more differentiated VSMC phenotype that results in upregulation of L-type calcium channel expression and restoration of a CCB-sensitive Ca^{2+} influx pathway in VSMC (Fig. 2). Under these conditions CCBs favourably affect the balance that exists between VSMC proliferation, apoptosis, and matrix metalloproteinase production. This model has several strengths. Firstly it contains a dynamic component of CCB-VSMC interaction. Based on VSMC phenotypic plasticity it predicts large early efficacy of CCBs that diminish with time, a feature that is consistent with observations in human and animal studies. Secondly it assumes only the

primary molecular mechanism of action of statins and CCBs (although it does not exclude the possibility of lipid independent and Ca^{2+} independent actions). Thirdly it provides a novel mode of action relevant only to the statin-CCB combination and targeted at VSMC. It therefore provides a biological mechanism that explains the observations of synergy between these agents seen in ASCOT-LLA and provides a rationale for the specific combination of these agents.

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The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [57].

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Fig. 1.

Schematic of pathway for cholesterol synthesis from 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) by HMG CoA reductase and its inhibition by statins.

Fig. 2.

As shown in this figure we propose that statins lead to adoption of a more differentiated VSMC phenotype with increased expression of L-type ($\text{Ca}_v1.2$) calcium channels and increased efficacy and benefit from calcium channel blockers (CCBs).