MECHANISMS OF PLAQUE STABILIZATION FOR A CHARGED DIHYDROPYRIDINE CALCIUM CHANNEL BLOCKER: BEYOND HYPERTENSION MANAGEMENT

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Atherosclerosis is a systemic, vascular disorder that is the leading cause of death and
disability throughout much of the developed world. Certain cellular changes associated with the
evolving plaque are characterized by a loss of normal calcium regulation, including smooth
muscle cell migration and endothelial permeability. This observation has led to basic and
clinical investigations into potential antiatherogenic effects for calcium channel blockers
(CCBs).

Recently, the activity of the charged CCB amlodipine was tested in The Prospective
Randomized Evaluation of the Vascular Effects of Norvasc Trial (PREVENT). This 3-year trial
tested the effects of amlodipine on development and progression of atherosclerotic lesions in
coronary and carotid arteries among patients with documented CAD, as compared to placebo. The results of PREVENT revealed significant clinical benefits with this third-generation CCB in
a well-defined population consisting of either normotensive or controlled hypertensive patients. Patients assigned to amlodipine had a 33% reduction in hospitalization (p<0.01) and 46%
reduction in the need for revascularization (p<0.001), without a reduction in luminal loss.
Amlodipine therapy was also associated with significant slowing ($P = 0.007$) in the progression
of carotid atherosclerosis, an important surrogate marker for cardiovascular disease. The CCB
had no effect on the risk of all-cause mortality or major cardiovascular events, likely attributed to
a very low event incidence rate. When major events and procedures were combined, however,
there were significantly fewer adverse events in the amlodipine group (86 versus 116, HR 0.69
[0.52 to 0.92]) $^1$. The findings from PREVENT were consistent with another study known as the
Coronary Angioplasty Amlodipine Restenosis Study (CAPARES) $^2$. In a similar patient
population, the CCB significantly reduced the incidence of repeat percutaneous transluminal
coronary angioplasty (PTCA) and clinical events after PTCA $^2$. 
I. Antiatherosclerotic Mechanisms of Action for Third-Generation CCBs

A review of the scientific literature provides insights into direct cellular mechanisms that may contribute to the observed clinical benefit with amlodipine in CAD\textsuperscript{3-8}. These cellular actions could contribute to mechanisms of plaque stabilization among patients with CAD, resulting in reduced cardiovascular events. Many of these pharmacologic actions are attributed to the interactions of amlodipine with membrane lipid constituents under atherosclerotic-like conditions\textsuperscript{9,10}, mediated by its positive chemical charge\textsuperscript{9,11-13}.

A. Increased resistance of lipids to oxidative modification: Both \textit{in vitro} and \textit{in vivo} studies have shown that amlodipine inhibits oxidative damage to lipids associated with cellular membranes and lipoprotein particles\textsuperscript{3,14,15}. Under controlled experimental conditions, amlodipine inhibited lipid peroxide formation (>10\textsuperscript{2} µM) at concentrations as pharmacologic levels, independent of calcium channel modulation\textsuperscript{3,14,15}. This antioxidant activity of amlodipine is attributed to both its high lipophilicity in atherosclerotic membranes and a chemical structure that facilitates proton-donating and resonance-stabilization mechanisms that quench the free radical reaction\textsuperscript{3}. By inserting to a location in the membrane near polyunsaturated fatty acids at relatively high concentrations, amlodipine is capable of donating protons to lipid peroxide molecules, thereby blocking the peroxidation process. The remaining unpaired free electron associated with the amlodipine molecule can be stabilized in well-defined resonance structures associated with the dihydropyridine ring, as previously described in detail\textsuperscript{3}. The antioxidant activity of amlodipine was also observed \textit{in vivo} using various animal models, including nonhuman primates, thus representing a distinct antiatherogenic mechanism of action for this compound\textsuperscript{14,15}. 
B. Inhibition of smooth muscle cell migration: Smooth muscle cell migration from the media into the intima is an early hallmark feature of atheroma development. Amlodipine was shown to reduce vascular smooth muscle cell migration at subnanomolar concentrations following stimulation by a potent mitogen, platelet-derived growth factor \(^{16}\). This effect of amlodipine could not be reproduced by other CCB analogs in parallel experiments \(^{16}\) and support the hypothesis that amlodipine may interact with novel intracellular sites of action to effect changes in cell behavior, independent of calcium channel interactions.

C. Inhibition of cytokine-induced endothelial apoptosis: Endothelial cell dysfunction and toxicity are important early events in atherosclerosis. TNF-\(\alpha\) is a cytokine that is elevated in atherosclerosis and mediates damage to the vessel wall during the inflammatory process. Cytokines such as TNF-\(\alpha\) can activate resident macrophages that interfere with the structural integrity of the fibrous cap \(^{17}\). Disruption of the fibrous plaque reveals the underlying, highly thrombotic lipid core, leading to acute coronary syndromes in CAD. In addition to its effect on plaque destabilization, TNF-\(\alpha\) also interferes with endothelial function, leading to the expression of proteins that mediate programmed cell death or apoptosis. Amlodipine was shown to inhibit TNF-\(\alpha\)-induced endothelial apoptosis in a dose-dependent manner, starting at low nanomolar levels \(^{4}\). The ability of amlodipine to inhibit excessive cell loss by apoptosis was also observed in cultured cerebellar granule cells and its cytoprotective effects were observed at concentrations significantly lower than that observed for either nifedipine or the antioxidant vitamin E \(^{18}\).

D. Enhancement of nitric oxide bioavailability: Amlodipine was also shown to improve the enzymatic formation of nitric oxide (NO) in canine coronary microvessels by regulating nitric oxide synthase \(^{8}\). The authors of this study demonstrated that amlodipine enhanced endothelial NO production through a bradykinin-dependent pathway. This ability of
amlodipine to stimulate NO synthesis was dose-dependent and could not be reproduced by other CCBs (diltiazem, nifedipine) but by the ACE-inhibitor, enalapril. Amlodipine specifically increased the intracellular concentrations of endothelial nitric oxide synthase (eNOS) protein, leading to an enhanced rate of NO production.

E. Modulation of vascular cell gene expression, extracellular matrix formation, and LDL-proteoglycan binding: CCBs may exhibit additional antiatherosclerotic effects in patients with CAD by interfering with key molecular factors that contribute to the phenotypic conversion of vascular SMC from a contractile to synthetic state, leading to atheroma development. In both fibroblasts and vascular SMC, CCBs have been shown to inhibit the expression of procollagens I, III, and IV at nanomolar concentrations. Specifically, amlodipine was shown to increase the proteolytic activity of the 72-kDa type IV collagenase as measured by gelatin zymography while inhibiting the transcription of tissue inhibitor of metalloproteinases-2. It was observed that amlodipine, but not nifedipine, inhibited IL-1beta-induced metalloproteinase-1 expression in human endothelial cells. Finally, a recent study showed that cultured smooth muscle cells treated with amlodipine had decreased levels of de novo proteoglycan synthesis and markedly reduced LDL binding affinity for proteoglycan molecules, as compared to control. The latter effect may be attributed to electrostatic interactions of amlodipine with the negatively-charged proteoglycan molecules, thereby reducing LDL affinity.
CONCLUSION

As changes in calcium transport mechanisms contribute to cellular changes in
erogenesis, it has been proposed that CCBs may be effective in slowing the progression of
CAD. Recent clinical studies demonstrated significant clinical benefits with the long-acting
CCB amlodipine, including a marked reduction in cardiovascular morbidity, associated with
significant slowing in the progression of carotid atherosclerosis. The beneficial effects of
amlodipine in CAD may be attributed to antiatherosclerotic effects related to its physico-
chemical properties and distinct membrane interactions. These data support a potentially new
therapeutic role for certain third-generation dihydropyridine CCBs in the treatment of
atherosclerosis. These findings also suggest that agents such as amlodipine may work in a
synergistic fashion with other established treatments, including HMG-CoA reductase inhibitors,
a hypothesis that is currently being tested.
## TABLE I

MECHANISMS OF PLAQUE STABILIZATION FOR THE CALCIUM CHANNEL BLOCKER AMLODIPINE

<table>
<thead>
<tr>
<th>MECHANISMS OF ACTION</th>
<th>REFERENCE</th>
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<tr>
<td>High binding affinity to atherosclerotic cellular membrane</td>
<td>9</td>
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<td>LDL and Cell Membrane Antioxidant Activity</td>
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