SYNERGISTIC EFFECTS OF AMLODIPINE AND ATORVASTATIN

Cross Reference to Related Applications

This application claims priority from the following four United States provisional patent applications: U.S. Application No. 60/130,665, filed on April 23, 1999; U.S. Application No. 60/145,305, filed on July 23, 1999; U.S. Application No. 60/151,121, filed on August 27, 1999; and U.S. Applications No. 60/166,592, filed on November 19, 1999.

Field of Invention

The present invention relates to pharmaceutical compositions and combinations to treat arterial and related heart disease and related ailments.

Background of the Invention

Coronary artery disease (CAD) is the leading cause of mortality in the developed world, and is associated with substantial morbidity as well. Typically, the patient with CAD has several concomitant conditions, including hypertension, diabetes, and dyslipidemia, increasing overall risk for poor outcomes and complicating treatment.

Among antihypertensive therapies, the lipophilic dihydropyridine-type calcium channel blocker (CCB) amlodipine besylate (AML) is a very well-tolerated agent with an established record of safety and effectiveness for the treatment of hypertension and angina. A potential therapeutic role for AML in the treatment of patients with CAD was recently shown in the Prospective Randomized Evaluation of the Vascular Effects of Norvasc® (AML) Trial (PREVENT). This three-year trial evaluated the effects of AML compared to placebo on the development and progression of atherosclerotic lesions in coronary and carotid arteries among patients with documented CAD (Byington RP, Miller ME, Herrington D, et al. Rationale, design, and baseline characteristics of the Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial (PREVENT). *Am. J. Cardiol.* 1997; 80:1087-1090). The results of PREVENT showed impressive clinical benefits with AML therapy, including an overall 30% reduction in major documented events or procedures (Byington RP, Chen J, Furberg CD, Pitt B. Effect of amlodipine on cardiovascular events and procedures. *J. Am. Coll. Cardiol.* 1999; 33:314A). AML therapy was also associated with a significant slowing in the progression of carotid atherosclerosis, as measured by B-mode ultrasonographic assessments (Byingyton R, Riley W, Booth D, et al. Effect of amlodipine on progression of carotid atherosclerosis in patients with documented heart disease. *Am. J. Hypertens.* 1999; 12:42A-43A). The clinical benefit seen with AML in CAD has not been previously reported with other CCBs, including dihydropyridine-type agents that have been used to examine this question (Waters D, Lesperance J,

Francetich M, et al. A controlled clinical trial to assess the effect of a calcium channel blocker on the progression of coronary atherosclerosis. *Circulation* 1990; 82:1940-1953; Lichtlen PR, Hugenholtz PG, Rafflenbeul W, et al. Retardation of coronary artery disease in humans by the calcium-channel blocker nifedipine: Results of the INTACT study (International Nifedipine Trial on Antiatherosclerotic Therapy). *Cardiovasc. Drugs Ther.* 1990; 4:S1047-S1068; Borhani NO, Mercuri M, Borhani PA, et al. Final outcome results of the Multicenter Isradipine Diuretic Atherosclerosis Study (MIDAS). A randomized controlled trial. *JAMA* 1996; 276:785-791). This observation has led to interest in potential antiatherogenic properties of AML, including antioxidant effects that are independent of calcium channel modulation (Mason RP, Leeds PR, Jacob RF, et al. Inhibition of excessive neuronal apoptosis by the calcium antagonist amlodipine and antioxidants in cerebellar granule cells. *J. Neurochem.* 1999; 72:1448-1456; Tulenko TN, Laury-Kleintop L, Walter MF, Mason RP. Cholesterol, calcium and atherosclerosis: Is there a role for calcium channel blockers in atheroprotection? *Int. J. Cardiol.* 1997; 62 (2 Suppl):55S-66S; Kramsch DM, Sharma RC. Limits of lipid-lowering therapy: The benefits of amlodipine as an anti-atherosclerotic agent. *J. Hum. Hypertens.* 1995; 9 (Suppl 1):S3-S9).

Hypolipidemic therapy has also been demonstrated to be very useful in reducing morbidity and mortality associated with CAD. Among HMG-CoA reductase inhibitors, atorvastatin calcium (AT) has been shown to be very effective

as hypolipdemic therapy (Nawrocki JW, Weiss SR, Davidson MH, et al. Reduction of LDL cholesterol by 25% to 60% in patients with primary hypercholesterolemia by atorvastatin, a new HMG-CoA reductase inhibitor. *Arterioscler. Thromb. Vasc. Biol.* 1995; 15:678-682; Bakker-Arkema RG, Davidson MH, Goldstein RJ, et al. Efficacy and safety of a new HMG-CoA reductase inhibitor, atorvastatin, in patients with hypertriglyceridemia. *JAMA* 1996; 275:128-133). In a recent clinical study involving patients with stable and advanced CAD, aggressive lipid-lowering therapy with atorvastatin significantly delayed the time to a first ischemic event and reduced, by 36%, the overall incidence of cardiovascular events, as compared to angioplasty with usual medical care (Pitt B, Waters D, Brown WV, et al. Aggressive lipid-lowering therapy combined with angioplasty in stable coronary artery disease. *N. Engl. J. Med.* 1999; 341:70-76). In addition, the hydroxylated metabolites of AT have been shown to exhibit antioxidant effects in lipoprotein preparations (Aviram M, Rosenblat M, Bisgaier CL, Newton RS. Atorvastatin and gemfibrozil metabolites, but not the parent drugs, are potent antioxidants against lipoprotein oxidation. *Atherosclerosis* 1998; 138:271-280).

However, no pharmaceutical composition currently exists that treats both hypertension and hyperlipidemia. Such a pharmaceutical composition would have several benefits. For example, the multiple risk factors for arterial and related heart disease that are often present in an individual patient could be targeted simultaneously. Additionally, the ease of taking one combined dosage could

significantly enhance patient compliance with therapeutic regimens.

Therefore, it is an object of this invention to provide a combination therapy that will treat the multiple pathological processes involved in arterial and related heart disease. These include, but are not limited to, hypertension and hyperlipidemia. It is also an object of this invention to develop useful and convenient dosage levels and forms of such a combination therapeutic. Preferably, this pharmaceutical composition would have synergistic effects on these hallmarks of arterial and related heart disease, such that the individual effects of the components of this composition would be enhanced by their combination.

Thus, this invention encompasses a therapeutic goal for the treatment of CAD that entails the development of drugs that can simultaneously target multiple underlying disease processes that contribute to atherosclerosis, thereby altering the course of the disease. Therefore, using this invention, CAD therapy may have increased positive outcomes if the use of an antihypertensive agent and HMG-CoA reductase inhibitor was combined in a single delivery system.

Summary of the Invention

Unexpectedly, when AML and AT were combined, they had a synergistic effect in preventing lipid peroxidation in human low-density lipoproteins (LDL) and lipid membranes; this effect occurred in addition to their well-established

therapeutic effects on both dyslipidemia and hypertension. The activity of the combination is considered synergistic as the measured effect significantly exceeded any additive effects of the two drugs. Therefore, these agents have heretofore unrecognized synergistic antioxidant effects, a property that would enable these agents to increase the resistance of LDL and vascular cell membranes to oxidative modification during atherogenesis. Indeed, oxidative modification of lipids is a well-established cause of injury to the endothelium and underlying smooth muscle (Ross R. Atherosclerosis -- An inflammatory disease. *N. Engl. J. Med.* 1999; 340:115-126; Diaz MN, Frei B, Vita JA, Keaney JF. Antioxidants and atherosclerotic heart disease. *N. Engl. J. Med.* 1997; 337:408-416). Lipophilic agents that protect against lipid peroxidation have been shown to reduce lesion development in various models of atherosclerosis as well as clinical studies (Diaz MN, Frei B, Vita JA, Keaney JF. Antioxidants and atherosclerotic heart disease. *N. Engl. J. Med.* 1997; 337:408-416). Moreover, the benefit associated with hypolipidemic therapy is attributed to both its effect on plasma very low-density lipoproteins (VLDL), LDL, and high-density lipoproteins (HDL) levels and, as a consequence, to a reduction in the potential formation of atherogenic oxidized lipoproteins.

Scientific analyses that support the combined use of AML (Norvasc®) and AT (Lipitor®) in a single delivery system for the treatment of cardiovascular disease are described in this invention. Specifically, the synergistic antioxidant activities of

the calcium channel blocker, AML, and the HMG-CoA reductase inhibitor, AT, including its active hydroxylated metabolite (ATM), were evaluated in human LDL and lipid membranes enriched with polyunsaturated fatty acids (PUFA), the key target for oxy-radical damage in atherosclerosis. The synergistic effects of these agents were demonstrated in membranes prepared in the presence of cholesterol. The combination of AML with ATM effected a dramatic and sustained reduction in lipid oxy-radical damage at concentrations as low as 10.0 nM. The dose-dependent antioxidant activity associated with the combination of these drugs at therapeutic levels was highly synergistic and could not be effectively reproduced by the endogenous agent, vitamin E. Synergistic inhibition of lipid peroxidation was also observed with the combination of AML and AT. Antioxidant activity was not observed, however, when AML was combined with other HMG-CoA reductase inhibitors, including lovastatin and mevastatin. As determined by x-ray diffraction and chemical analyses, the distinct activity described for this drug combination can be attributed to strong physico-chemical interactions with the membrane bilayer that are independent of the well-characterized effects of these drugs on calcium transport and cholesterol metabolism. This synergistic antioxidant benefit constitutes a new pharmacologic mechanism of action for these compounds and a compelling rationale for the combined use of the active ingredients in Norvasc® and Lipitor® in the treatment of cardiovascular disease by reducing the levels of LDL in plasma and improving protection of LDL and cellular membranes against oxidation. This new property complements the established effects of these drugs on hypertension and

dyslipidemia.

Other objects, features, and advantages of the present invention will be apparent from the following Detailed Description of the Preferred Embodiments taken in conjunction with the accompanying drawings in which:

Brief Description of the Drawings

Figure 1 shows the synergistic effects of AML and ATM on lipid peroxidation at a low, therapeutic concentration of drug (100.0 nM) in membranes containing physiologic levels of cholesterol. x is percent inhibition of lipid peroxidation and y is treatment with amlodipine (AML), atorvastatin metabolite (ATM), and the combination of both (AML + ATM) at a level of 100 nM. Values are mean ± standard deviation for n = 6. ... indicates $p<0.001$ versus control and other treatments.

Figure 2 shows the dose-dependent antioxidant effect of the combination of AML and ATM over a broad range of concentrations (0.01 through 10.0 μM). x is percent inhibition of lipid peroxidation and y is treatment with the combination of AML and ATM at the micromolar concentrations indicated. Values are mean ± standard deviation for n = 6. ... indicates $p<0.001$ versus control and other treatments.

Figure 3 shows the superior antioxidant activity of the AML/ATM combination over vitamin E as a function of time at an identical concentration (10.0 μM). x is percent inhibition of lipid peroxidation and y is treatment with the combination of amlodipine and atorvastatin metabolite (AML + ATM), darkened panel, or vitamin E (E), cross-hatched panel, both at 10 μM. Values are mean ± standard deviation for n = 6 to 12. ... indicates $p<0.001$ versus control and other treatments.

Figure 4 shows the comparative antioxidant activity of AML/ATM versus AML/AT at two different concentrations. x is percent inhibition of lipid peroxidation and y is treatment with the combination of amlodipine and atorvastatin metabolite (AML + ATM), darkened panel, or amlodipine and atorvastatin (AML + AT), cross-hatched panel, at the micromolar concentrations indicated. Values are mean ± standard deviation for n = 5 to 6. ... indicates $p<0.001$ versus control and other treatments.

Figure 5 shows the sites of proton abstraction (S1, S2, and S3) for ATM that contribute to antioxidant activity in the 5A panel along with resonance stabilization calculations (Panels 5B through 5D).

Figure 6 shows the comparative effects of AML antioxidant activity when combined with different HMG-CoA reductase inhibitors at the same concentration.

x is percent inhibition of lipid peroxidation and y is treatment with the combination of amlodipine and atorvastatin metabolite (AML + ATM), darkened panel; amlodipine and atorvastatin (AML + AT), darkened panel; amlodipine and mevastatin (AML + M), cross-hatched panel; or amlodipine and lovastatin (AML + L), no lipid peroxidation inhibition; all at 1.0 μM. Values are mean ± standard deviation for n = 6 to 12. ... indicates $p<0.001$ versus control and other treatments.

Figure 7 shows the synergistic antioxidant effects of AML and ATM in human LDL samples, as compared to Trolox C (soluble vitamin E). In panel A, x is thiobarbituric reactive substances (TBARS) formation measured at an absorbance of 532 nm and y is time in hours. The control is indicated by filled circles with solid lines, Trolox C (soluble vitamin E) is indicated by filled, inverted triangles with small dashed lines, AML alone is indicated by open diamonds with long dashed lines, ATM alone is indicated by open circles with alternating long and short dashed lines, and AML and ATM is indicated by filled squares with dotted lines. In panel B, x is thiobarbituric reactive substances (TBARS) formation measured at an absorbance of 532 nm and y is treatment with the combination of amlodipine (AML), darkened panel; atorvastatin metabolite (ATM), cross-hatched panel; or amlodipine and atorvastatin metabolite (AML + ATM), darkened panel, at a concentration of 3.0 μM. Values are mean ± standard deviation. ... indicates $p<0.001$ versus control and other treatments.

Figure 8 shows the computed enthalpies for ATM, AML and their respective radical species.

Figure 9 shows x-ray diffraction determination of the separate versus combined lipid membrane interactions of AML and ATM. x is relative electron density and y is angstroms from the middle of the lipid bilayer. In all three panels the control is the solid line. In panel A, AML is the dotted line; in panel B, ATM is the dotted line; in panel C, the combination of AML and ATM is the dotted line. The darkened areas in each panel are the difference in electron densities between the control and treatment electron density profiles.

Figure 10 shows the separate versus combined effects of AML and ATM on membrane bilayer dimensions as determined by x-ray diffraction analysis. x is the change in membrane width in angstroms and y is the treatment with AML, darkened panel; ATM, cross-hatched panel; AT, darkened panel; and the combination of AML and ATM, hatched panel. Values are mean ± standard deviation. ... indicates $p<0.001$ versus control and other treatments.

Detailed Description of Embodiments

Synergistic Antioxidant Effects of Amlodipine with the Atorvastatin/Atorvastatin Metabolite in Lipid Membranes: The separate and combined dose-dependent antioxidant activities of AML, AT and ATM were tested

in membrane vesicles reconstituted from phospholipids enriched with cholesterol and the PUFA, dilinoleoyl phosphatidylcholine, at a 0.5:1 mole ratio. Membrane vesicles were used in these experiments for the following reasons: 1) this system is well-defined and highly reproducible; 2) linoleic acid represents the primary target for oxidative damage and is common in vascular cell membranes and lipoprotein particles; 3) this membrane system does not contain calcium channels or the HMG CoA reductase enzyme and; 4) lipid peroxidation in this system can be initiated spontaneously at 37°C in the absence of exogenous chemical initiators, such as high levels of iron and ascorbate. In these experiments, oxidation occurred in a gradual, time-dependent manner that was measured spectrophotometrically over a 72 h period.

In Figure 1, the synergistic antioxidant activity of AML and ATM was demonstrated in membrane vesicles composed of cholesterol and phospholipid at levels that reproduce physiologic-like conditions (Tulenko TN, Chen M, Mason PE, Mason RP. Physical effects of cholesterol on arterial smooth muscle membranes: Evidence of immiscible cholesterol domains and alterations in bilayer width during atherogenesis. *J. Lipid. Res.* 1998; 39:947-956). At 100.0 nM, only the ATM separately produced any significant inhibition (9% of control) of lipid peroxidation in this membrane preparation enriched with cholesterol. When the agents were combined, however, the extent of inhibition increased to 33%, an effect significantly ($p<0.01$) greater than that measured for the agents separately. The

antioxidant activity of the combination was very apparent: the drugs inhibited lipid peroxide formation (>5 x 10^2 μM) at a concentration of 100.0 nM (the control level of lipid peroxide formation was 1.6 mM). This drug combination produced an effect that was highly dose-dependent over a broad range of concentrations (Figure 2). Significant inhibition ($p<0.05$) was observed as low as 10.0 nM with an IC_{50} of 500.0 nM. Greater than 90% inhibition (>1 mM lipid peroxide formation) was observed at a concentration of 10.0 μM for the combination (Figure 2). The fact that inhibition was observed at sub-micromolar levels indicates that the benefit observed with the combination of AML and ATM is of therapeutic relevance.

The antioxidant effect of the combination persisted over time in a manner that could not be reproduced by vitamin E, even at an elevated concentration (10.0 μM) (Figure 3). This observation is consistent with the concept that vitamin E or α-tocopherol is gradually consumed during the lipid peroxidation process. By contrast, the activity of the AML/ATM combination was not affected by the length of the incubation period in which the total lipid peroxide level increased to 2.2 mM at the 72 h time point.

The combination of AML with ATM was significantly more effective in inhibiting lipid peroxide formation than the combination of AML and AT (Figure 4). At levels of 100.0 nM and 1.0 μM, the effect of the combination of AML with ATM was 45% and 86% greater, respectively, than that observed for the

combination of AML and AT. The increased polarity of ATM, mediated by its additional hydroxy group, may facilitate stronger interactions with the formally charged AML, leading to distinct interactions with phospholipid molecules, as evidenced by x-ray diffraction analysis. The additional hydroxy group associated with the ATM also provides an additional abstractable proton that can be donated to free radical molecules (Figure 5). Following the loss of the proton, the remaining unpaired free electron can be effectively stabilized in resonance structures of the metabolite, as shown in Figure 5. The distinct antioxidant activity of the combination of AML with ATM is indicated by the observation that a similar effect could not be reproduced when AML was combined with each of two other statins (mevastatin and lovastatin), as demonstrated in Figure 6.

The results of *in vivo* investigations have indicated an important role for lipophilic antioxidants in reducing cardiovascular morbidity and mortality, especially CAD. LDL particles that have greater resistance to oxidative damage exhibit reduced cytotoxicity, interfere less with endothelium-derived nitric oxide production, and do not contribute to foam cell formation (Diaz MN, Frei B, Vita JA, Keaney JF. Antioxidants and atherosclerotic heart disease. *N. Engl. J. Med.* 1997; 337:408-416). Supplementation with an antioxidant has been shown to increase LDL resistance to oxidative modification and reduce endothelial cell cytotoxicity (Belcher JD, Balla J, Balla G, et al. Vitamin E, LDL, and endothelium. Brief oral vitamin supplementation prevents oxidized LDL-mediated vascular injury in vitro.

Arterioscler. Thromb. 1993; 13:1779-1789). Probucol, a lipophilic antioxidant, attenuated the formation of atherosclerotic plaques in cholesterol-fed primates, an effect that correlated with increased resistance of LDL to oxidative damage (Sasahara M, Raines EW, Chait A, et al. Inhibition of hypercholesterolemia-induced atherosclerosis in the nonhuman primate by probucol. I. Is the extent of atherosclerosis related to resistance of LDL to oxidation? *J. Clin. Invest.* 1994; 94:155-164). This antioxidant inhibited the formation of lesions in Watanabe hereditary hyperlipidemic (WHHL) rabbits, a well-characterized animal model of atherosclerosis, independent of cholesterol-lowering effects (Carew TE, Schwenke DC, Steinberg D. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: Evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Proc. Natl. Acad. Sci. USA* 1987; 84:7725-7729). Beyond these animal studies, a placebo-controlled clinical study demonstrated that probucol reduced restenosis by 47% in patients with CAD following coronary-artery balloon angioplasty, presumably due to its antioxidant effects (Tardif JC, Cote G, Lesperance J, et al. Probucol and multivitamins in the prevention of restonosis after coronary angioplasty. Multivitamins and Probucol Study Group. *N. Engl. J. Med.* 1997; 337:365-372). In a separate study, it was demonstrated that probucol, unlike antioxidant vitamins, had a beneficial effect on vascular remodeling in patients that had underwent angioplasty, as determined by intravascular ultrasound techniques

(Cote G, Tardif J-C, Lesperance J, et al. Effects of probucol on vascular remodeling after coronary angioplasty. *Circulation* 1999; 99:30-35).

Thus, a review of the available data provides a mechanistic rationale for the use of lipophilic antioxidants to interfere with inflammatory processes associated with CAD. By increasing the resistance of LDL and vascular cell membranes to oxidative damage, agents with antioxidant activity may effectively interfere with pathologic alterations in the vessel wall during atherogenesis. These processes include, but are not limited to, foam cell formation, endothelial dysfunction and toxicity, leukocyte and platelet adhesion, and arterial vasospasm, secondary to a loss of normal nitric oxide production. These cellular observations support epidemiologic analyses that indicate an inverse association between the serum levels of certain antioxidants and adverse outcomes associated with coronary disease (Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC. Vitamin E consumption and the risk of coronary disease in women. *N. Engl. J. Med.* 1993; 328:1450-1456; Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. *N. Engl. J. Med.* 1993; 328:1450-1456; Enstrom JE, Kanim LE, Klein MA. Vitamin C intake and mortality among a sample of the United States population. *Epidemiology* 1992; 3:194-202; Riemersma RA, Wood DA, Macintyre CC, Elton R, Gey KF, Oliver MF. Low plasma vitamins E and C. Increased risk of angina in Scottish men. *Ann. N. Y. Acad. Sci.* 1989; 570:291-295; Ramirez J,

Flowers NC. Leukocyte ascorbic acid and its relationship to coronary artery disease in man. *Am. J. Clin. Nutr.* 1980; 33:2079-2087; Hennekens CH, Buring JE, Manson JE, et al. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N. Engl. J. Med.* 1996; 334:1145-1149; Losonczy KG, Harris TB, Havlik RJ. Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: The Established Populations for Epidemiologic Studies of the Elderly. *Am. J. Clin. Nutr.* 1996; 64:190-196). Several of these epidemiologic studies showed benefit with vitamin E, an antioxidant with limited capacity to interfere with oxidative modification. The results of this study would predict that the combination of AML and ATM would be significantly more effective than vitamin E in reducing vascular injury associated with CAD.

Synergistic Antioxidant Effects of Amlodipine with the Hydroxy Atorvastatin Metabolite in Human LDL: The separate and combined antioxidant effects of AML and ATM were also evaluated in human LDL preparations. The ability of these agents to inhibit LDL peroxidation was assessed *in vitro* following addition of copper (10.0 μM) by measuring the levels of thiobarbituric reactive substances (TBARS), a marker of lipid peroxidation. Figure 7 shows that the rate of LDL oxidation was characterized by sigmoidal curve kinetics with an initial lag phase followed by a sharp propagation and final plateau phase (Esterbauer H, Gebicki J, Puhl H, Jurgens G. The role of lipid peroxidation and antioxidants in oxidative

modification of LDL. *Free Radic. Biol. Med.* 1992; 13:341-390). After a 4 h incubation period, the differential effects of these compounds could be clearly observed at a concentration of 3.0 μM. As compared to control lipid peroxidation (100% TBARS formation), TBARS formation for AML and ATM were 93.3 ±6% and 65.6 ±7%, respectively. When combined, however, TBARS formation was only 29.3 ±6%, levels that were significantly ($p<0.01$) lower than that observed for either drug alone. The synergistic antioxidant effect of the combination persisted at the 6 h time point. These findings provide further evidence for synergistic activity consistent with that observed in membrane vesicles, as the drug combination produced a level of inhibition that substantially exceeded their expected additive effect. The antioxidant activity of vitamin E was similar to that observed for ATM alone in these experiments.

Chemical Mechanisms for the Synergistic Activity of Amlodipine and Atorvastatin Metabolite: The synergistic antioxidant activity observed for this drug combination in LDL and reconstituted lipid membranes suggests that these compounds interact directly with each other to scavenge lipid radicals. Based on thermodynamic considerations (Figure 8), it is proposed that ATM reacts more quickly with lipid radicals (equation 1) than does AML, as described in equation 2. If these are the only two pathways available for these drugs when added together to the system, then their combined effect would only be additive. However, the combination of both compounds provides the possibility for a third pathway

(equation 3), an alternative that is supported by the results of the peroxidation experiments in both LDL and lipid membranes. The combination of pathways 1 and 3 would produce a synergistic effect as it occurs more rapidly than pathway 2. Indeed, semi-empirical calculations suggest that reaction 3 is a favorable, exothermic process (ΔH_f = -40.7 kJ/mol). Thus, the presence of the fast inhibitor (ATM) enables the slower inhibitor (AML) to remove free radicals more rapidly than if reacting with the lipid radicals on its own. The three pathways describing these interactions are as follows, in which LOO• represents a lipid radical:

(1) LOO• + ATM → LOOH + ATM• *FAST*

(2) LOO• + AML → LOOH + AML• *SLOW*

(3) ATM• + AML → ATM + AML• *FAST*

As a result of this synergy between AML and ATM, the recycled metabolite is now available for additional scavenging of lipid radicals. Overall, differences in the rates of inhibition between AML and ATM are based, in part, on the calculated enthalpies for these compounds and their respective radicals (Figure 8). The smaller (i.e., more negative) the ΔH_f value, the more favorable is hydrogen abstraction associated with radical formation. Once formed, the unpaired radical associated with radical species can be stabilized in resonance structures.

Molecular Membrane Interactions of Amlodipine and Atorvastatin/Atorvastatin Metabolite: Small-angle x-ray diffraction approaches

were used to examine the molecular membrane interactions of the AML and ATM combination. This highly quantitative technique provides direct information on the structure of the membrane lipid bilayer in the absence and presence of the drugs. It has been previously reported that AML has high affinity for membrane lipids (K_p> 10^3) under atherosclerotic conditions, as compared to other CCBs (Mason RP, Moisey DM, Shajenko L. Cholesterol alters the binding of Ca^{2+} channel blockers to the membrane lipid bilayer. *Mol. Pharmacol.* 1992; 41:315-321). The distinct lipophilicity of AML is attributed to its amphiphilic chemical structure that directs the molecule to an advantageous location in the membrane where it can then interfere with the propagation of free radicals by both biophysical and biochemical mechanisms, as previously described in detail by my laboratory (Mason RP, Leeds PR, Jacob RF, et al. Inhibition of excessive neuronal apoptosis by the calcium antagonist amlodipine and antioxidants in cerebellar granule cells. *J. Neurochem.* 1999; 72:1448-1456; Mason RP, Moisey DM, Shajenko L. Cholesterol alters the binding of Ca^{2+} channel blockers to the membrane lipid bilayer. *Mol. Pharmacol.* 1992; 41:315-321; Mason RP, Campbell SF, Wang SD, Herbette LG. Comparison of location and binding for the positively charged 1,4-dihydropyridine calcium channel antagonist amlodipine with uncharged drugs of this class in cardiac membranes. *Mol. Pharmacol.* 1989; 36:634-640; Mason RP, Walter MF, Trumbore MW, Olmstead Jr. EG, Mason PE. Membrane antioxidant effects of the charged dihydropyridine calcium antagonist amlodipine. *J. Mol. Cell. Cardiol.* 1999; 31:275-281) In membranes that are not enriched with cholesterol, amlodipine

inhibited lipid peroxidation in a manner that could not be reproduced by other CCBs or the angiotensin converting enzyme (ACE)-inhibitor, captopril (Mason RP, Walter MF, Trumbore MW, Olmstead Jr. EG, Mason PE. Membrane antioxidant effects of the charged dihydropyridine calcium antagonist amlodipine. *J. Mol. Cell. Cardiol.* 1999; 31:275-281). In the same way, the chemical structure of the atorvastatin metabolite has amphiphilic properties that would enable the drug to interact strongly with the membrane lipid bilayer, as recently reported by my laboratory (Mason RP. Inhibition of oxidative damage to low density lipoproteins and isolated membranes by atorvastatin and its active metabolite. *J. Am. Coll. Cardiol.* 2000; 35:317A).

For these studies, the combination of AML and ATM were added to membrane vesicles reconstituted from cholesterol and phospholipid at a 0.5:1 mole ratio (Figure 9). X-ray diffraction analysis of the membrane samples produced strong and reproducible diffraction patterns for representative control and drug-containing samples. In the absence of drug, the overall membrane bilayer width, including surface hydration, was 55.5 Å with an intrabilayer headgroup separation of 44 Å. The addition of the two drugs together at a ratio of drug to phospholipid of 1:15 produced distinct changes in the structure and organization of the phospholipid bilayer, as compared to the drugs added separately (Figure 9). In the presence of the drug combination, the overall membrane bilayer width, including surface hydration, was reduced to 53.5 Å with an intrabilayer headgroup separation of 41 Å. Separately, the membrane bilayer widths of membranes containing AML and ATM

alone were 54.8 Å and 58.0 Å, respectively (Figure 10). These structural findings provide direct evidence that the combination of these agents differentially modulate the structure of lipid molecules, as compared to their separate effects.

Direct subtraction of the membrane electron density profiles (Å versus electrons/$Å^3$) demonstrated large differences in lipid structure that could be attributed to the presence of the drugs (Figure 9). Specifically, the addition of the drug combination produced a broad increase in electron density associated with the upper hydrocarbon core/hydrated headgroup region of the membrane bilayer, ± 11-21 Å from the center of the bilayer. This large increase in electron density distributed over 10 Å is attributed to the equilibrium location of the drugs in the membrane. Concomitant with this change was an observed disordering effect associated with the central hydrocarbon core region of the membrane, ± 0-11 Å. This decrease in electron density is due to an increase in molecular volume resulting from the insertion of the drug molecules into a region of high molecular density near the membrane hydrocarbon core/water interface. Thus, it can be concluded from these data that the insertion of the drug combination into the membrane bilayer alters the intermolecular packing constraints of the phospholipid molecules in a manner similar to that observed with either reducing cholesterol content or increasing sample temperature (Tulenko TN, Chen M, Mason PE, Mason RP. Physical effects of cholesterol on arterial smooth muscle membranes: Evidence of immiscible cholesterol domains and alterations in bilayer width during

atherogenesis. *J. Lipid. Res.* 1998; 39:947-956; Chang HM, Reitstetter R, Mason RP, Gruener R. Attenuation of channel kinetics and conductance by cholesterol: An interpretation using structural stress as a unifying concept. *J. Membr. Biol.* 1995; 143:51-63). Such changes in biophysical properties have been shown to interfere with the propagation of free radicals though the lipid bilayer matrix (McLean LR, Hagaman KA. Effect of lipid physical state on the rate of peroxidation of liposomes. *Free Radic. Biol. Med.* 1992; 12:113-119).

Separately, AML and ATM effected distinct changes in membrane structure, as compared to the drug combination, due to specific interactions with constituent phospholipid molecules (Figures 9 and 10). While the drug combination effected a 2 Å or 4% decrease ($p<0.01$) in overall membrane width, the ATM separately produced a 5% increase ($p<0.01$) in width (2.5 Å) while AML alone did not significantly alter membrane dimensions, including the intrabilayer headgroup separation. As compared to AML alone, ATM produced a larger reduction in hydrocarbon core electron density. This effect on membrane structure may contribute to its greater antioxidant potency, as compared to AML. The combination of AML and ATM effected a new site of interaction with the membrane lipid bilayer, in addition to their separate locations in the membrane (Figure 9).

Therefore, this invention is drawn to a pharmaceutical composition

comprising amlodipine and an atorvastatin compound. These individual pharmaceutical agents can be formulated in combination, or separately, in salts, forms, and dosages that produce maximal therapeutic responses. This combination therapy is designed to treat the various pathophysiological manifestations of arterial and related heart disease, including, but not limited to, hypertension, hyperlipidemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris. Specifically, this combination therapy will be designed to lower blood pressure and systemic lipid concentrations as well as the related pathophysiological results of the lack of their regulation, including, but not limited to, arterial weakening and plaque deposition. The effects of these individual agents on these various processes and events related to arterial and related heart disease, when used in combination can be additive and/or synergistic.

It will now be apparent to those skilled in the art that other embodiments, improvements, details, and uses can be made that are consistent with the letter and spirit of the foregoing disclosure and within the scope of this patent and the appended claims.

Experimental Methods

Dilinoleoyl phosphatidylcholine (DLPC), 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) and unesterified cholesterol were obtained from

Avanti Polar Lipids Inc. (Alabaster, AL) and stored at -80°C. Aliquots of LDL (L-2139) from human plasma were obtained from Sigma Chemical Co. (St. Louis, MO). Sephadex G-25 M (PD-10) columns were purchased from Pharmacia Biotech Inc. (Piscataway, NJ). Amlodipine besylate was obtained from Pfizer Central Research (Groton, CT) while atorvastatin calcium and its hydroxy metabolite were provided by Parke Davis (Ann Arbor, MI). Vitamin E, Trolox (a vitamin E analog), lovastatin, and mevastatin were purchased from Sigma Chemical Co. (St. Louis, MO).

Membrane Lipid Peroxidation Analysis: The separate and combined dose-dependent antioxidant activities of these agents were examined in membranes enriched with PUFA prepared at a 0.5 cholesterol to phospholipid mole ratio (Mason RP, Walter MF, Trumbore MW, Olmstead Jr. EG, Mason PE. Membrane antioxidant effects of the charged dihydropyridine calcium antagonist amlodipine. *J. Mol. Cell. Cardiol.* 1999; 31:275-281). The lipid (dilinoleoyl phosphatidylcholine and cholesterol) used for these samples was dissolved in HPLC-grade chloroform (25.0 mg/ml). An aliquot of the lipids (1.0 mg) was added to individual glass 13 x 100-mm test tubes and chloroform was removed by shell-drying under a steady stream of N_2 gas. The lipids were dried down in the absence or presence of drug(s) dissolved in ethanol. Residual solvent was removed under vacuum while the samples were shielded from light. Membrane vesicles were produced by rapidly mixing the dried lipids at room temperature following addition of 1.0 ml HEPES

buffered saline (0.5 mM HEPES and 154.0 mM NaCl, pH, 7.2). The final phospholipid concentration was 1.0 mg/ml buffer and the final concentration of drug ranged from 10.0 nM through 10.0 μM.

Membrane lipid peroxidation was carried out at 37°C in a shaking water bath without the addition of exogenous stimulants, as previously described in detail (Mason RP, Walter MF, Trumbore MW, Olmstead Jr. EG, Mason PE. Membrane antioxidant effects of the charged dihydropyridine calcium antagonist amlodipine. *J. Mol. Cell. Cardiol.* 1999; 31:275-281). At various time intervals (24, 48, 65, 72h), an aliquot of lipid sample (10 to 100 μl) was removed before 25 μl of 5.0 mM ethylenediaminetetracetic acid (EDTA) and 20 μl of 35.0 mM butylated hydroxytoluene (BHT) was immediately added to the sample to stop the peroxidation reaction.

The extent of membrane lipid peroxidation was measured by the CHOD-Iodide assay as previously described in detail (El-Saadani M, Esterbauer H, el-Sayed M, Goher M, Nassar AY, Jurgens G. A spectrophotometric assay for lipid peroxides in serum lipoproteins using a commercially available reagent. *J. Lipid. Res.* 1989; 30:627-630). The quantity of I_3^- was measured from the following reaction in which *L* represents a phospholipid molecule:

$$LOOH + 2H^+ + 2I^- \text{-------}> LOH + H_2O + I_2$$

$$I_2 + I^- \text{-------}> I_3^-$$

An aliquot of the membrane sample was removed at various time points and then added to 1.0 ml of CHOD color reagent (E.M. Science, Gibbstown, NJ) that includes 20.0 μM BHT, 24.0 μM EDTA, and 0.2% Triton-X. The sample was then covered with foil and allowed to incubate for 2 h in the absence of light before measuring the absorbance of the sample at 365 nm ($\varepsilon = 2.4 \times 10^4 M^{-1}cm^{-1}$). The background sample was run along the test samples in triplicate and contains 76.7 μl of 0.652 mM HEPES, 20 μl of 5.0 mM EDTA and 3.3 μl of DDI water. The extent of lipid peroxidation was measured in triplicate for each drug concentration and compared to control samples that did not contain drug. The statistical significance of these experiments was assessed by the non-paired *t*-test. Significance was accepted at $p<0.05$.

LDL Oxidation Determination: In addition to lipid membranes, the antioxidant activity of AML and ATM was evaluated in human LDL. The EDTA content of the LDL samples obtained from human plasma was removed by gel filtration with PD-10 Sephadex G25-M filtration columns; PBS (nitrogen purged) was used as the eluent. The LDL samples (50 μg of protein/mL) were then preincubated with or without drug (3.0 μM) for 30 min at 37°C. Oxidation of LDL was then induced by the addition of 10.0 μM $CuSO_4$. The time course of LDL oxidation, measured by TBARS formation, was followed for 6 h at 37°C (Mak IT, Kramer JH, Weglicki WB. Potentiation of free radical-induced lipid peroxidative injury to sarcolemmal membranes by lipid amphiphiles. *J. Biol. Chem.* 1986;

261:1153-1157). LDL oxidation, as determined by the TBARS methods, followed sigmoidal curve kinetics with an initial lag phase followed by a sharp propagation and final plateau phase (Esterbauer H, Gebicki J, Puhl H, Jurgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic. Biol. Med.* 1992; 13:341-390). The protein content of the LDL was determined using the Coomassie Protein Plus assay kit from Pierce Chemical (Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976; 72:248-254).

Small angle X-ray diffraction analysis: Small-angle X-ray diffraction analyses were used to directly examine the molecular membrane interactions of AML, AT, and ATM. The lipids (1-palmitoyl-2-oleoyl phosphatidylcholine and cholesterol) used for these samples were dissolved in HPLC-grade chloroform (10.0 mg/ml). Membrane vesicles were produced from these lipids by the same method as described for the peroxidation experiments. The final phospholipid concentration was 5.0 mg/ml buffer and the mole ratio of drug to phospholipid was 1:15. Membrane samples were oriented for diffraction analysis by subjecting them to centrifugation as previously described (Chester DW, Herbette LG, Mason RP, Joslyn AF, Triggle DJ, Koppel DE. Diffusion of dihydropyridine calcium channel antagonists in cardiac sarcolemmal lipid multibilayers. *Biophys. J.* 1987; 52:1021-1030). Briefly, vesicles were placed in sedimentation cells that contained an aluminum foil substrate. The vesicles were sedimented in an SW-28 rotor

(Beckman Instruments, Fullerton, CA) at 35,000 x g for 1.5 h at 5°C. Following centrifugation, the supernatant was removed from the pellets and the samples were then mounted on to curved glass supports. Samples were placed in sealed canisters to control relative humidity and temperature during the diffraction experiments, as previously described in detail (Chester DW, Herbette LG, Mason RP, Joslyn AF, Triggle DJ, Koppel DE. Diffusion of dihydropyridine calcium channel antagonists in cardiac sarcolemmal lipid multibilayers. *Biophys. J.* 1987; 52:1021-1030).

X-ray diffraction experiments were conducted by aligning the samples at grazing incidence with respect to a collimated, nickel-filtered monochromatic X-ray source (CuK_α = 1.54 Å) produced by a high-brilliance rotating anode microfocus generator (Rigaku Rotaflex RU-200, Danvers, MA). The fixed geometry beam line consisted of a single, nickel-coated Franks mirror to define a line source where $K_{\alpha 1}$ and $K_{\alpha 2}$ are unresolved. The diffraction data were collected on a one-dimensional, position-sensitive electronic detector (Innovative Technologies, Newburyport, MA) placed at a distance of 150 mm from the sample. Each meridional diffraction peak was Lorentz- and background-corrected, as previously described (Mason RP, Gonye GE, Chester DW, Herbette LG. Partitioning and location of Bay K 8644, 1,4-dihydropyridine calcium channel agonist, in model and biological membranes. *Biophys. J.* 1989; 55:769-778). The phases for the four-order data were determined by swelling analysis (Moody MF. X-ray diffraction pattern of nerve myelin: A method for determining the phases. *Science* 1963; 142:1173-117). Fourier

transformations of the data were generated from the diffraction data with Origin software (Microcal Software, Northampton, MA).

What is claimed is:

1. A pharmaceutical composition comprising:

(a) a therapeutically effective amount of amlodipine;

(b) a therapeutically effective amount of an atorvastatin compound selected from the group consisting of atorvastatin and hydroxylated atorvastatin metabolite; and

(c) pharmaceutically acceptable formulation agents.

2. The pharmaceutical composition of claim 1 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

3. The pharmaceutical composition of claim 2 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

4. The pharmaceutical composition of claim 1 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

5. The pharmaceutical composition of claim 4 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

6. The pharmaceutical composition of claim 1 wherein said pharmaceutical composition reduces the risk of arterial and related heart disease.

7. The pharmaceutical composition of claim 6 wherein said arterial and related heart disease is selected from the group consisting of hypertension, hyperlipdemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

8. The pharmaceutical composition of claim 1 wherein said pharmaceutical composition lowers blood pressure.

9. The pharmaceutical composition of claim 8 wherein said pharmaceutical composition lowers blood pressure to a level consistent with a reduced risk of arterial and related heart disease.

10. The pharmaceutical composition of claim 8 wherein said pharmaceutical composition lowers blood pressure to a level statistically equivalent to normal.

11. The pharmaceutical composition of claim 1 wherein said pharmaceutical composition lowers systemic lipid concentrations.

12. The pharmaceutical composition of claim 11 wherein said pharmaceutical composition lowers systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

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13. The pharmaceutical composition of claim 11 wherein said pharmaceutical composition lowers systemic lipid concentrations to a level statistically equivalent to normal.

14. The pharmaceutical composition of claim 1 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations.

15. The pharmaceutical composition of claim 14 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

16. The pharmaceutical composition of claim 14 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

17. The pharmaceutical composition of claim 1 wherein said pharmaceutical composition concomitantly lowers blood pressure and systemic lipid concentrations.

18. The pharmaceutical composition of claim 17 wherein said pharmaceutical composition concomitantly lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

19. The pharmaceutical composition of claim 17 wherein said pharmaceutical composition concomitantly lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

20. The pharmaceutical composition of claim 1 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations.

21. The pharmaceutical composition of claim 20 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

22. The pharmaceutical composition of claim 20 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

23. The pharmaceutical composition of any of claims 17, 18, and 19 wherein the concomitant lowering of blood pressure and systemic lipid concentrations results at least partially from reduced lipid oxidation.

24. The pharmaceutical composition of any of claims 20, 21, and 22 wherein the synergistic lowering of blood pressure and systemic lipid concentrations results at

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least partially from reduced lipid oxidation.

25. A pharmaceutical composition comprising:

(a) a therapeutically effective amount of amlodipine;

(b) a therapeutically effective amount of an atorvastatin compound selected from the group consisting of atorvastatin and hydroxylated atorvastatin metabolite; and

(c) pharmaceutically acceptable formulation agents wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations.

26. The pharmaceutical composition of claim 25 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

27. The pharmaceutical composition of claim 26 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

28. The pharmaceutical composition of claim 25 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

29. The pharmaceutical composition of claim 28 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

30. The pharmaceutical composition of claim 24 wherein said pharmaceutical composition reduces the risk of arterial and related heart disease.

31. The pharmaceutical composition of claim 30 wherein said arterial and related heart disease is selected from the group consisting of hypertension, hyperlipdemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

32. The pharmaceutical composition of claim 25 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

33. The pharmaceutical composition of claim 25 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

34. The pharmaceutical composition of any of claims 25 through 33 wherein the lowering of blood pressure and systemic lipid concentrations results at least partially from reduced lipid oxidation.

35. A pharmaceutical composition comprising:

(a) a therapeutically effective amount of amlodipine;

(b) a therapeutically effective amount of an atorvastatin compound selected from the group consisting of atorvastatin and hydroxylated atorvastatin metabolite; and

(c) pharmaceutically acceptable formulation agents wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations at least partially as a result of reduced lipid oxidation.

36. The pharmaceutical composition of claim 35 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

37. The pharmaceutical composition of claim 36 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

38. The pharmaceutical composition of claim 35 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

39. The pharmaceutical composition of claim 38 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

40. The pharmaceutical composition of claim 35 wherein said pharmaceutical composition reduces the risk of arterial and related heart disease.

41. The pharmaceutical composition of claim 40 wherein said arterial and related heart disease is selected from the group consisting of hypertension, hyperlipdemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

42. The pharmaceutical composition of claim 35 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

43. The pharmaceutical composition of claim 35 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

44. A pharmaceutical composition comprising:

(a) a therapeutically effective amount of amlodipine;

(b) a therapeutically effective amount of an atorvastatin compound selected from the group consisting of atorvastatin and hydroxylated atorvastatin metabolite; and

(c) pharmaceutically acceptable formulation agents wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations.

45. The pharmaceutical composition of claim 44 wherein amlodipine comprises a

therapeutically effective derivative of amlodipine.

46. The pharmaceutical composition of claim 45 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

47. The pharmaceutical composition of claim 44 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

48. The pharmaceutical composition of claim 47 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

49. The pharmaceutical composition of claim 44 wherein said pharmaceutical composition reduces the risk of arterial and related heart disease.

50. The pharmaceutical composition of claim 49 wherein said arterial and related heart disease is selected from the group consisting of hypertension, hyperlipdemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

51. The pharmaceutical composition of claim 44 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

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52. The pharmaceutical composition of claim 44 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

53. The pharmaceutical composition of any of claims 44 through 52 wherein the synergistic lowering of blood pressure and systemic lipid concentrations results at least partially from reduced lipid oxidation.

54. A pharmaceutical composition comprising:

(a) a therapeutically effective amount of amlodipine;

(b) a therapeutically effective amount of an atorvastatin compound selected from the group consisting of atorvastatin and hydroxylated atorvastatin metabolite; and

(c) pharmaceutically acceptable formulation agents wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations at least partially as a result of reduced lipid oxidation.

55. The pharmaceutical composition of claim 54 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

56. The pharmaceutical composition of claim 55 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

57. The pharmaceutical composition of claim 54 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

58. The pharmaceutical composition of claim 57 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

59. The pharmaceutical composition of claim 54 wherein said pharmaceutical composition reduces the risk of arterial and related heart disease.

60. The pharmaceutical composition of claim 59 wherein said arterial and related heart disease is selected from the group consisting of hypertension, hyperlipdemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

61. The pharmaceutical composition of claim 54 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

62. The pharmaceutical composition of claim 54 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

63. A pharmaceutical composition comprising:

(a) a therapeutically effective amount of a combination of amlodipine and an atorvastatin compound selected from the group consisting of atorvastatin and hydroxylated atorvastatin metabolite; and

(c) pharmaceutically acceptable formulation agents.

64. The pharmaceutical composition of claim 63 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

65. The pharmaceutical composition of claim 64 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

66. The pharmaceutical composition of claim 63 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

67. The pharmaceutical composition of claim 66 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

68. The pharmaceutical composition of claim 63 wherein said pharmaceutical composition reduces the risk of arterial and related heart disease.

69. The pharmaceutical composition of claim 68 wherein said arterial and related

heart disease is selected from the group consisting of hypertension, hyperlipdemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

70. The pharmaceutical composition of claim 63 wherein said pharmaceutical composition lowers blood pressure.

71. The pharmaceutical composition of claim 70 wherein said pharmaceutical composition lowers blood pressure to a level consistent with a reduced risk of arterial and related heart disease.

72. The pharmaceutical composition of claim 70 wherein said pharmaceutical composition lowers blood pressure to a level statistically equivalent to normal.

73. The pharmaceutical composition of claim 63 wherein said pharmaceutical composition lowers systemic lipid concentrations.

74. The pharmaceutical composition of claim 73 wherein said pharmaceutical composition lowers systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

75. The pharmaceutical composition of claim 73 wherein said pharmaceutical

composition lowers systemic lipid concentrations to a level statistically equivalent to normal.

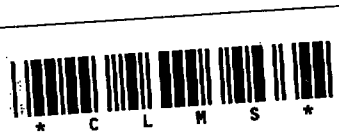
76. The pharmaceutical composition of claim 63 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations.

77. The pharmaceutical composition of claim 76 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

78. The pharmaceutical composition of claim 76 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

79. The pharmaceutical composition of claim 63 wherein said pharmaceutical composition concomitantly lowers blood pressure and systemic lipid concentrations.

80. The pharmaceutical composition of claim 79 wherein said pharmaceutical composition concomitantly lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.



81. The pharmaceutical composition of claim 79 wherein said pharmaceutical composition concomitantly lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

82. The pharmaceutical composition of claim 63 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations.

83. The pharmaceutical composition of claim 82 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

84. The pharmaceutical composition of claim 82 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

85. The pharmaceutical composition of any of claims 79, 80, and 81 wherein the concomitant lowering of blood pressure and systemic lipid concentrations results at least partially from reduced lipid oxidation.

86. The pharmaceutical composition of any of claims 82, 83, and 84 wherein the synergistic lowering of blood pressure and systemic lipid concentrations results at

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least partially from reduced lipid oxidation.

87. A pharmaceutical composition comprising:

(a) a therapeutically effective amount of a combination of amlodipine and an atorvastatin compound selected from the group consisting of atorvastatin and hydroxylated atorvastatin metabolite; and

(b) pharmaceutically acceptable formulation agents wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations.

88. The pharmaceutical composition of claim 87 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

89. The pharmaceutical composition of claim 88 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

90. The pharmaceutical composition of claim 87 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

91. The pharmaceutical composition of claim 90 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

92. The pharmaceutical composition of claim 87 wherein said pharmaceutical



composition reduces the risk of arterial and related heart disease.

93. The pharmaceutical composition of claim 92 wherein said arterial and related heart disease is selected from the group consisting of hypertension, hyperlipdemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

94. The pharmaceutical composition of claim 87 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

95. The pharmaceutical composition of claim 94 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

96. The pharmaceutical composition of any of claims 87 through 95 wherein the lowering of blood pressure and systemic lipid concentrations results at least partially from reduced lipid oxidation.

97. A pharmaceutical composition comprising:

(a) a therapeutically effective amount of a combination of amlodipine and an atorvastatin compound selected from the group consisting of atorvastatin and

hydroxylated atorvastatin metabolite; and

(b) pharmaceutically acceptable formulation agents wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations at least partially as a result of reduced lipid oxidation.

98. The pharmaceutical composition of claim 97 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

99. The pharmaceutical composition of claim 98 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

100. The pharmaceutical composition of claim 97 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

101. The pharmaceutical composition of claim 100 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

102. The pharmaceutical composition of claim 97 wherein said pharmaceutical composition reduces the risk of arterial and related heart disease.

103. The pharmaceutical composition of claim 102 wherein said arterial and related



heart disease is selected from the group consisting of hypertension, hyperlipdemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

104. The pharmaceutical composition of claim 97 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

105. The pharmaceutical composition of claim 97 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

106. A pharmaceutical composition comprising:

(a) a therapeutically effective amount of a combination of amlodipine and an atorvastatin compound selected from the group consisting of atorvastatin and hydroxylated atorvastatin metabolite; and

(b) pharmaceutically acceptable formulation agents wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations.

107. The pharmaceutical composition of claim 106 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

108. The pharmaceutical composition of claim 107 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

109. The pharmaceutical composition of claim 106 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

110. The pharmaceutical composition of claim 109 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

111. The pharmaceutical composition of claim 106 wherein said pharmaceutical composition reduces the risk of arterial and related heart disease.

112. The pharmaceutical composition of claim 111 wherein said arterial and related heart disease is selected from the group consisting of hypertension, hyperlipdemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

113. The pharmaceutical composition of claim 106 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

114. The pharmaceutical composition of claim 113 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

115. The pharmaceutical composition of any of claims 106 through 114 wherein the synergistic lowering of blood pressure and systemic lipid concentrations results at least partially from reduced lipid oxidation.

116. A pharmaceutical composition comprising:

(a) a therapeutically effective amount of a combination of amlodipine and an atorvastatin compound selected from the group consisting of atorvastatin and hydroxylated atorvastatin metabolite; and

(b) pharmaceutically acceptable formulation agents wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations at least partially as a result of reduced lipid oxidation.

117. The pharmaceutical composition of claim 116 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

118. The pharmaceutical composition of claim 117 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

119. The pharmaceutical composition of claim 116 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

120. The pharmaceutical composition of claim 119 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

121. The pharmaceutical composition of claim 116 wherein said pharmaceutical composition reduces the risk of arterial and related heart disease.

122. The pharmaceutical composition of claim 121 wherein said arterial and related heart disease is selected from the group consisting of hypertension, hyperlipdemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

123. The pharmaceutical composition of claim 116 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

124. The pharmaceutical composition of claim 116 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

125. A method of treating arterial and related heart disease comprising administering a therapeutically effective amount of a combination of amlodipine and an atorvastatin compound selected from the group consisting of atovastatin and hydroxylated atorvastatin metabolite.

126. The method of claim 125 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

127. The method of claim 126 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

128. The method of claim 127 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

129. The method of claim 128 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

130. The method of claim 125 wherein said arterial and related heart disease is selected from the group consisting of hypertension, hyperlipdemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

131. The method of claim 125 wherein amlodipine and the atorvastatin compound are administered in the same therapeutic.

132. The method of claim 125 wherein amlodipine and the atorvastatin compound are administered as separate therapeutics.

133. The method of claim 125 wherein amlodipine and the atorvastatin compound are administered at the same time.

134. The method of claim 125 wherein amlodipine and the atorvastatin compound are administered at different times.

135. A method of lowering blood pressure and systemic lipid concentrations comprising administering a therapeutically effective amount of a combination of amlodipine and an atorvastatin compound selected from the group consisting of atorvastatin and hydroxylated atorvastatin metabolite.

136. The method of claim 135 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

137. The method of claim 136 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

138. The method of claim 135 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

139. The method of claim 138 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

140. The method of claim 135 wherein amlodipine and the atorvastatin compound are administered in the same therapeutic.

141. The method of claim 135 wherein amlodipine and the atorvastatin compound are administered as separate therapeutics.

142. The method of claim 135 wherein amlodipine and the atorvastatin compound are administered at the same time.

143. The method of claim 135 wherein amlodipine and the atorvastatin compound are administered at different times.

144. The method of claim 135 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

145. The method of claim 144 wherein said arterial and related heart disease is selected from the group consisting of hypertension, hyperlipidemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

146. The method of claim 135 wherein said pharmaceutical composition lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

147. The method of any of claims 135 through 146 wherein the lowering of blood pressure and systemic lipid concentrations results at least partially from reduced lipid oxidation.

148. A method of synergistically lowering blood pressure and systemic lipid concentrations comprising administering a therapeutically effective amount of a combination of amlodipine and an atorvastatin compound selected from the group consisting of atorvastatin and hydroxylated atorvastatin metabolite.

149. The method of claim 148 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

150. The method of claim 149 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

151. The method of claim 148 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

152. The method of claim 151 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

153. The method of claim 148 wherein amlodipine and the atorvastatin compound are administered in the same therapeutic.

154. The method of claim 148 wherein amlodipine and the atorvastatin compound are administered as separate therapeutics.

155. The method of claim 148 wherein amlodipine and the atorvastatin compound are administered at the same time.

156. The method of claim 148 wherein amlodipine and the atorvastatin compound are administered at different times.

157. The method of claim 148 wherein said pharmaceutical composition

synergistically lowers blood pressure and systemic lipid concentrations to a level consistent with a reduced risk of arterial and related heart disease.

158. The method of claim 157 wherein said arterial and related heart disease is selected from the group consisting of hypertension, hyperlipdemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

159. The method of claim 148 wherein said pharmaceutical composition synergistically lowers blood pressure and systemic lipid concentrations to a level statistically equivalent to normal.

160. The method of any of claims 148 through 159 wherein the synergistic lowering of blood pressure and systemic lipid concentrations results at least partially from reduced lipid oxidation.

161. A method of inhibiting lipid oxidation comprising administering a therapeutically effective amount of a combination of amlodipine and an atorvastatin compound selected from the group consisting of atorvastatin and hydroxylated atorvastatin metabolite.



162. The method of claim 161 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

163. The method of claim 162 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

164. The method of claim 161 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

165. The method of claim 164 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

166. The method of claim 161 wherein amlodipine and the atorvastatin compound are administered in the same therapeutic.

167. The method of claim 161 wherein amlodipine and the atorvastatin compound are administered as separate therapeutics.

168. The method of claim 161 wherein amlodipine and the atorvastatin compound are administered at the same time.

169. The method of claim 161 wherein amlodipine and the atorvastatin compound are administered at different times.

170. The method of claim 161 wherein said pharmaceutical composition inhibits lipid oxidation to an extent consistent with a reduced risk of arterial and related heart disease.

171. The method of claim 170 wherein said arterial and related heart disease is selected from the group consisting of hypertension, hyperlipidemia, atheroclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.

172. A method of synergistically inhibiting lipid oxidation comprising administering a therapeutically effective amount of a combination of amlodipine and an atorvastatin compound selected from the group consisting of atorvastatin and hydroxylated atorvastatin metabolite.

173. The method of claim 172 wherein amlodipine comprises a therapeutically effective derivative of amlodipine.

174. The method of claim 173 wherein the therapeutically effective derivative of amlodipine comprises amlodipine besylate.

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175. The method of claim 172 wherein the atorvastatin compound comprises a therapeutically effective derivative of the atorvastatin compound.

176. The method of claim 175 wherein the therapeutically effective derivative of the atorvastatin compound is a hemicalcium salt.

177. The method of claim 172 wherein amlodipine and the atorvastatin compound are administered in the same therapeutic.

178. The method of claim 172 wherein amlodipine and the atorvastatin compound are administered as separate therapeutics.

179. The method of claim 172 wherein amlodipine and the atorvastatin compound are administered at the same time.

180. The method of claim 172 wherein amlodipine and the atorvastatin compound are administered at different times.

181. The method of claim 172 wherein said pharmaceutical composition synergistically inhibits lipid oxidation to an extent consistent with a reduced risk of arterial and related heart disease.

182. The method of claim 181 wherein said arterial and related heart disease is selected from the group consisting of hypertension, hyperlipdemia, atherosclerosis, arteriosclerosis, coronary artery disease, myocardial infarction, congestive heart failure, stroke, and angina pectoris.