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ST. MICHAEL'S HOSPITAL, UNIVERSITY OF TORONTO, ONTARIO

Scientific Update™

Low-Density Lipoprotein Oxidation Biomarkers Predict Cardiovascular Events in the PREVENT Study

Originally presented by: R. Preston Mason, MD

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Reported and discussed by:
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Oxidation of low-density lipoprotein (LDL) contributes to the development of endothelial dysfunction, a key initiating process in the development of atherosclerosis and coronary artery disease (CAD). The hypothesis that the oxidation of LDL predicts coronary events was recently tested by measuring circulating biomarkers of lipid oxidation in a substudy of the Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial (PREVENT), a 3-year prospective evaluation of the effect of amlodipine versus placebo on 825 patients with documented CAD. This *Cardiology Scientific Update* reviews the preliminary results of the PREVENT substudy and the prognostic role of LDL oxidation in CAD.

In humans, vascular walls are subjected to chronic insults and a progressive development of atherosclerosis from an early age.[1] In the past, most investigators considered atherosclerosis a type of lipid storage disease caused by excessive cholesterol accumulating in blood vessels in bland pools of extracellular lipid. However, recent work has recognized atherosclerosis as a systemic inflammatory process.[2,3] The ability of selected systemic markers of inflammation such as C-reactive protein (CRP) to predict ischemic events has now been clearly demonstrated in patients with stable and unstable CAD as well as in apparently healthy male and female subjects.[4-9]

The first steps in atherosclerosis recapitulate the classic host response-to-injury paradigm, with lipid playing an important role as a trigger. Lipoproteins enter the arterial intima, where they can undergo modification by oxidation, forming oxidized forms of lipid that can incite local and systemic inflammation.[10,11] Although LDL oxidation is a crucial process in the atherosclerotic process, whether this process can predict coronary events in patients with CAD remains unclear. To properly address this issue, one would need to measure appropriate circulating biomarkers of LDL oxidation and protein modification. Until recently, oxidized LDL had been considered absent in the circulation. However, with improved assay methods, a host of products of LDL oxidation can now be measured from the plasma.[12,13] Two examples of such biomarkers are lipid hydroperoxide (LOOH) and malondialdehyde (MDA)-modified LDL (MDA-LDL); their biochemical pathways are shown in Figure 1. LOOH can be considered an "early" oxidation product representing the oxidation of LDL, whereas MDA-LDL can be considered a "late" product representing protein modification to a pro-atherogenic peptide (Figure 2).[13]

The Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial (PREVENT) evaluated the effects of the dihydropyridine calcium channel blocker (CCB) amlodipine 10 mg versus placebo on the development and progression of atherosclerotic lesions in coronary arteries of 825 patients with angiographically documented CAD. The rationale and study design,[14] as well as the principal results of PREVENT have been published previously.[15] In brief, amlodipine had no impact on the progression of angiographic coronary stenosis at 36 months, the primary endpoint of PREVENT. However, amlodipine reduced the increase in carotid intimal-medial thickness. Patients treated with amlodipine had fewer hospitalizations for

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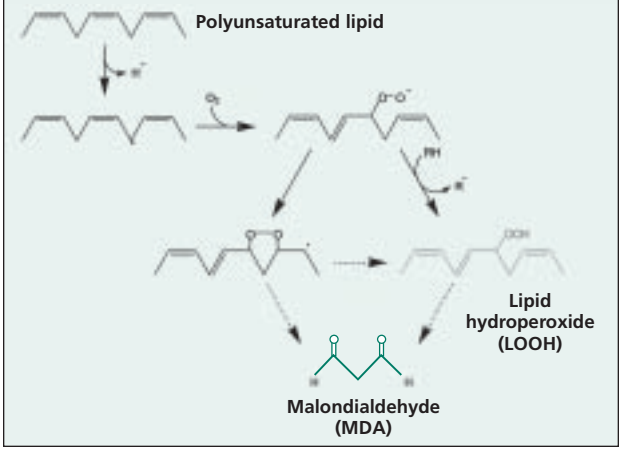
Figure 1: Biochemical pathways of markers of LDL oxidation



unstable angina and revascularizations. To study the role of LDL oxidation, a substudy of PREVENT was conducted to test the following hypotheses:

- Serum LDL oxidation markers (LOOH, MDA-LDL) predict cardiovascular (CV) events
- Amlodipine treatment is associated with a beneficial effect on oxidative mechanisms

2975 serum samples were collected at baseline and at 12, 24, and 36 months after randomization. LOOH was analyzed by the ferrous oxidation of xylenol orange using a spectrophotometric assay. MDA-LDL was measured by high-performance liquid chromatography and UV-VIS spectroscopy.

Results

The baseline characteristics of the patient population in the substudy are shown in Table 1. Over the course of the study, there were 51 major vascular events defined as myocardial infarction (MI)/stroke, 149 hospitalizations for nonfatal CV events (mainly unstable angina) and 139 patients underwent a major vascular procedure such as percutaneous coronary intervention (PCI)/ coronary artery bypass graft surgery (CABG). The relative risk of patients who were at the highest (4th) quartile of

Figure 2: The concept of "early" and "late" markers of LDL oxidation

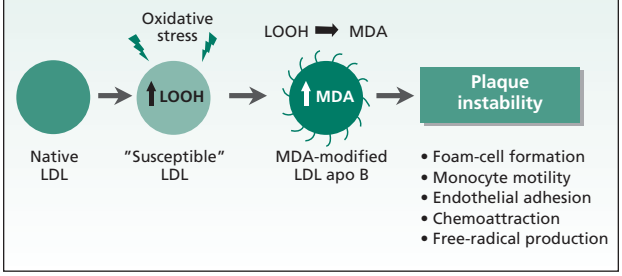


Table 1: Patient baseline characteristics

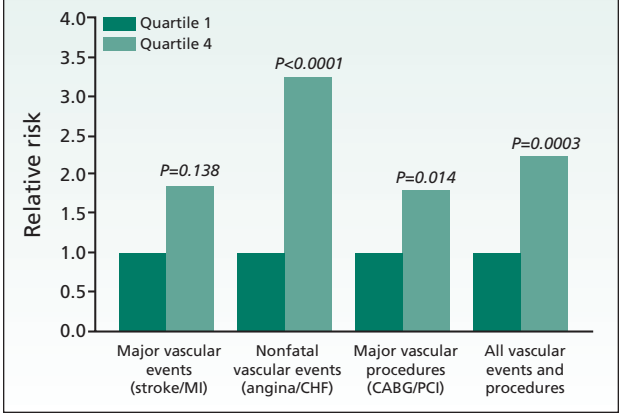
Variable	Mean ± SD
Age (years)	57.2 ± 9.6
Male (%)	80.9
Total cholesterol (mg/dL)	217.9 ± 39.1
LDL cholesterol (mg/dL)	132.4 ± 36.1
Triglycerides (mg/dL)	203.7 ± 131.7
Systolic blood pressure (mm Hg)	129.0 ± 17.4
Body mass index (kg/m^2)	27.9 ± 4.6
History of prior myocardial infarction (%)	47.3
History of prior angina (%)	51.6

serum LOOH level at baseline was compared with that of patients at the lowest quartile, with the 1st quartile patient set at unity (Figure 3). Compared to patients with the lowest quartile of serum LOOH, patients with the highest quartile of LOOH had significantly higher risk of experiencing non-fatal vascular events and undergoing revascularization procedures, with a trend for an increased risk of major vascular events such as MI and stroke. The prediction of LOOH for vascular events was independent of age, gender, body mass index, smoking index, systolic and diastolic blood pressure, as well as total cholesterol, LDL, high-density lipoprotein (HDL), triglyceride, and high-sensitivity CRP (hsCRP) levels. A similar analysis was conducted for baseline serum levels of MDA-LDL and the results are shown in Figure 4. Compared to patients with the lowest quartile of baseline MDA-LDL, patients with the highest quartile of MDA-LDL had significantly higher risk for experiencing major vascular events, non-fatal vascular events, as well as undergoing revascularization procedures. In addition, time-dependent analysis demonstrated that the changes of MDA-LDL over time were also highly predictive of vascular events (Figure 5). Importantly, treatment with amlodipine was associated with a 23% decrease in the turnover of LOOH levels over the 3-year period (p=0.032).

Discussion

Data from this substudy of PREVENT for the first time provide evidence that circulating "early" and "late" markers of LDL oxidation predict adverse vascular events in patients with documented CAD. While these data have not yet been published and should therefore be considered preliminary, they are nevertheless entirely consistent with previous *in vitro* observations, thereby substantiating the crucial role of LDL oxidation in the pathogenesis of CAD. The preliminary observations of the beneficial effect of amlodipine on the changes in LOOH levels are particularly interesting and raise the possibility that amlodipine interfered with mechanisms of LDL peroxidation, which might

Figure 3: Quartile analysis of risk prediction for lipid hydroperoxide at baseline



MI = myocardial infarction; CHF = congestive heart failure

account for the benefit on clinical outcomes observed in PREVENT,[15] and in the Coronary Angioplasty Amlodipine Restenosis (CAPARES) trial in patients following PCI.[16] The pharmacological basis for a clinical benefit with amlodipine in CAD may reside in the agent's physiochemical properties, including membrane lipophilicity and its positive charge that may contribute in part to its strong interaction with phospholipids near the surface of the atherosclerotic cell membrane (Figure 6).[17-19] High lipophilicity and a chemical structure that facilitates proton-donating, and resonance-stabilization mechanisms that quench the free radical generation likely contribute to antioxidant effects. If amlodipine is inserted in the membrane near polyunsaturated fatty acids at high concentrations, it is capable of donating protons to LOOH molecules, thereby stopping the peroxidation process. Indeed, an action along the LDL peroxidation cascade, such as following the formation of LOOH, thereby preventing conversion of LOOH to its derivatives, has

Figure 4: Quartile analysis of risk prediction for MDA-modified LDL at baseline

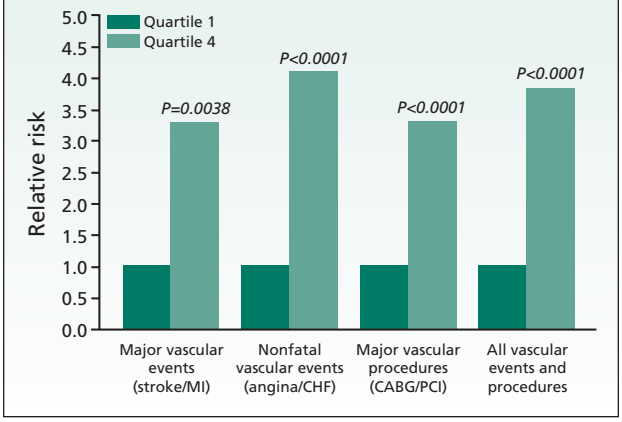


Figure 5: Predictive value of changes over time in MDA-modified LDL

CV Event	Number with event	RR	95% CI for RR	*P*-value
Major vascular events (fatal/ nonfatal MI, stroke)	51	3.07	1.53-6.16	0.0015
Hospitalizations for angina	149	4.17	3.11-5.58	0.0001
CABG	46	3.24	1.86-5.63	0.0001
PCI	93	3.70	2.58-5.32	0.0001
Vascular procedures (CABG/PTCA)	139	3.64	2.65-4.98	0.0001

been considered one of the most important mechanisms for the prevention of progression in CAD.[20]

In vitro experiments have demonstrated that amlodipine interfered with mechanisms of lipid peroxidation, independent of calcium channel modulation.[17,21] These effects have not been observed in other types of calcium channel blockers (Figure 7). The antioxidant effect of amlodipine and other dihydropyridine-type agents, has also been demonstrated *in vivo* in several experimental models.[19,21,22] Most of these studies demonstrated that amlodipine, when compared to controls, increased the resistance of lipoproteins to oxidative modification. Although the current substudy in PREVENT examined only LDL oxidation, other antiatherogenic mechanisms, including inhibition of smooth muscle cell proliferation and migration, inhibition of cytokine- and free radical-induced endothelial cell apoptosis, and modulation of vascular cell extracellular matrix,[18,19,23] have been described for amlodipine. These mechanisms were not examined in the current substudy of PREVENT, but they may also explain the clinical benefit of amlodipine in patients with CAD.

Figure 6: Membrane effects of amlodipine[18]

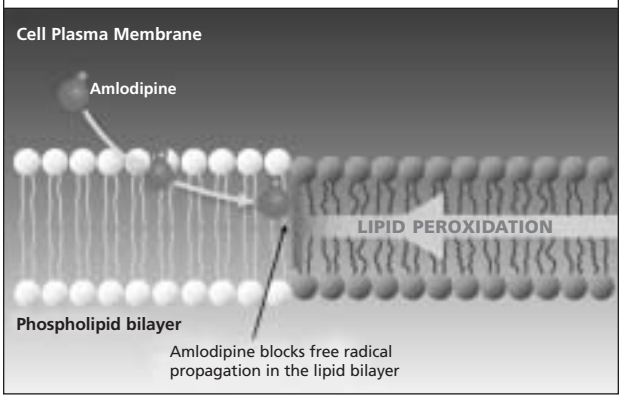
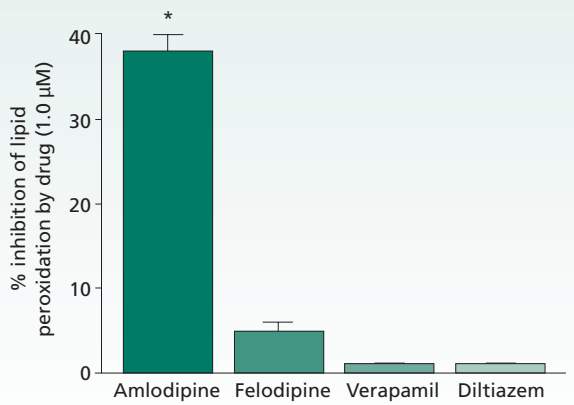


Figure 7: *In vitro* lipid peroxidation: Ca^{2+}-independent mechanism[17]



Summary

The new information from the PREVENT study has demonstrated the prognostic utility of circulating biomarkers of LDL peroxidation in patients with CAD. Furthermore, preliminary results of the effects of amlodipine on LOOH, which did not correlate with changes in blood pressure and lipids, suggest that one of the known pleiotropic and antiatherogenic mechanisms, namely the inhibitory effect on the important process of LDL oxidation, may account for the clinical benefit of amlodipine. The effect of amlodipine on clinical outcomes in patients with CAD will be addressed further in the ongoing Comparison of Amlodipine versus Enalapril to Limit Ischemic Occurrence of Thrombosis (CAMELOT) study.

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