Atherosclerosis, calcium, and calcium antagonists

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ALTHOUGH it has been generally recognized that atherosclerosis is associated with a deposition of calcium in the arterial wall, the pathogenic significance of this calcification remains unclear. In recent years, a number of calcium-chelating agents, lanthanum chloride, and organic calcium antagonists have been shown to exert antiatherogenic effects in animals with dietary hypercholesterolemia. This brief review discusses possible interrelations between arterial calcification and atherogenesis, and summarizes some of the data in the literature on the effects of chelating and calcium antagonist agents.

Deposition of calcium salts in the walls of human arteries begins in childhood and progresses with age. Electron microscopic studies of human atherosclerotic arteries have shown that calcification in the media and intima is associated with extracellular membranous vesicles structurally resembling matrix vesicles in bone and other calcifying tissues. These vesicles contain noncrystalline particles and needle-shaped crystals that have the diffraction pattern of apatite. The membranous structures occur in the vicinity of degenerating cells that enclose similar vesicular formations. This suggests that calcifying vesicles represent the debris of mitochondria and other organelles from degenerating medial and intimal cells. The biochemical composition of the membranes has not yet been determined, but it appears likely that phospholipids constitute an important component. It is of interest that atheromatous lesions contain a vitamin K–dependent (γ-carboxyglutamic acid–containing) calcium-binding protein that appears to be functionally analogous but structurally dissimilar to osteocalcin. Like γ-carboxyglutamic acid–containing proteins of the blood clotting system, this protein may bind calcium and phospholipid and play a role in regulating the calcification of atherosclerotic plaques. Results of the studies cited above are based on analysis of material obtained at autopsy and prepared by conventional histologic techniques, a procedure that does not satisfactorily preserve the physical state and localization of electrolytes in soft tissues. Additional investigations in which rapidly frozen (frozen-hydrated) samples and electron-probe microanalysis are used are needed, since with these techniques it would be possible to determine if calcium in degenerating macrophages and smooth muscle cells accumulates in the mitochondria, as has been demonstrated in other syndromes associated with cell necrosis.

Blumenthal et al. were among the first to point out that in the human aorta medial calcification (or other processes altering vascular elastance, such as syphilitic aortitis) is a structural alteration always preceding the development of intimal plaques. A similar sequence of events is observed in rabbits and swine fed vitamin D in doses inducing little or no hypercalcemia. In these normolipidemic animals atherosclerotic lesions appear only months after fibromuscular changes, cell necrosis, and calcifications have developed. Recent findings with regard to the influence of mechanical factors on atherogenesis suggest that arterial stiffness may play a role in the localization and development of atheromas. The relationship between the physical properties of arteries, fluid mechanics, and atherogenesis will require further investigative attention. In addition, the possibility must be considered that crystalline deposits alter the deformability of atheromatous lesions. In coronary arteries, vessels subjected to continual bending movements, crystalline bodies could be traumatic to surrounding soft tissues and cause acute structural changes such as plaque hemorrhage or rupture. It has been recently reemphasized that the dense neovascularization of atheromas may render the artery susceptible to intramural hemorrhage, a possible mechanism of coronary thrombosis.

Recently, evidence has accumulated indicating that calcium antagonists may protect muscle and nonmuscle cells by a variety of pathophysiologic processes. Protective effects have been reported in ischemic, cytotoxic, and degenerative syndromes involving skeletal muscle, myocardium, brain, liver, kidney, intes-
tine, and the eye.\textsuperscript{7} The fact that calcium antagonists have a protective effect supports the notion that excessive calcium uptake, in particular mitochondrial calcium overload, plays a role in mediating cell death after various insults. An important question is whether there are membrane physiologic alterations in atherosclerosis that might favor an excessive uptake of calcium. It is well established that modest changes in membrane lipids may alter the functional characteristics of membrane proteins such as ion channels, ion transporters, and receptors. The importance of the abundance of membrane cholesterol in regulating the functional characteristics of membranes has been well demonstrated in red blood cells and platelets. Certain membrane enzymes, such as ATPase transporters, appear to be particularly sensitive to changes in membrane cholesterol.\textsuperscript{8, 9} Recently Locher et al.\textsuperscript{9} have demonstrated in human red blood cells that increases in membrane cholesterol augment the entry of calcium through a channel that can be blocked with a dihydropyridine calcium antagonist. Inspired by experiments relating changes in membrane lipids to calcium uptake we performed experiments with isolated canine coronary arteries incubated in a high-cholesterol environment.\textsuperscript{8} Arteries exposed to low concentrations of cholesterol in aqueous solution (10^{-12}M) or to human low-density lipoprotein underwent slow contractions and preincubation of arteries with cholesterol sensitized the arteries to the constrictor effects of calcium over a wide range of external calcium concentrations. The uptake of radioactive cholesterol associated with these changes in arterial reactivity was very low. The fact that the constrictor effects of cholesterol were blocked by calcium antagonists and that sterols structurally closely related to cholesterol were ineffective suggested to us that cholesterol did not act by a simple detergent effect. Our findings support the hypothesis that physiologic changes in membranes caused by a high-cholesterol environment may favor calcium uptake by arterial smooth muscle.

Interrelations between membrane lipids, calcium uptake, and cell necrosis may be relevant to atherogenesis. Necrosis of arterial cells may play a pivotal role in the initiation, progression, and regression of atherosclerotic lesions.\textsuperscript{10} Results of studies of coronary arteries of children and young adults support the view that cell death and accumulation of cellular debris in the intima are important features distinguishing early fibrotic plaques from the pure foam cell lesions (fatty streaks).\textsuperscript{11} Cell death also results in the release of cytotoxic substances such as polar lipids and lytic enzymes, a process that may amplify cell necrosis and contribute to the stimulation of a fibroproliferative response.

If cell necrosis is an important process in the formation of atheroma and if cell death is mediated in part by changes in membranes that facilitate the intracellular accumulation of calcium, drugs capable of reducing calcium uptake might exert antiatherogenic effects. Almost 20 years ago, Wartman et al.\textsuperscript{12} treated cholesterol-fed rabbits with subcutaneous injections of magnesium EDTA and concluded that this chelating agent suppressed the deposition of collagen and elastin in the arteries. Unfortunately, Wartman’s experiments have to my knowledge never been repeated, but other chelating agents were subsequently tested for their antiatherogenic effects. Kramsch et al.\textsuperscript{13} administered diphosphonic acid and tiophene derivatives to rabbits fed a high-cholesterol diet. The treatments did not influence serum cholesterol or triglycerides, but inhibited calcification, lipid accumulation, and plaque formation in arteries. In addition, these investigators demonstrated that LaCl\textsubscript{3} exerted antiatherogenic effects in rabbits and monkeys.\textsuperscript{13}

In view of our considerations concerning altered calcium uptake in atherosclerosis and the therapeutic results obtained with chelating agents, we evaluated the effects of nifedipine, a calcium antagonist, on atherogenesis in rabbits with diet-induced atherosclerosis.\textsuperscript{14} Rabbits were fed a 2% cholesterol diet for 8 weeks with or without treatment with oral nifedipine (40 mg/rabbit/day). High-dose oral nifedipine was well tolerated and produced modest transient hypotensive effects. Compared with rabbits receiving placebo, treated animals exhibited highly significant reductions in sudanophilic lesions, aortic total cholesterol, and aortic calcium. As with the chelating agents, the drug did not influence the hyperlipidemic response to the diet. Our findings were subsequently confirmed by Willis et al.,\textsuperscript{15} who demonstrated that high-dose nicardipine and nifedipine produced substantial reductions in aortic lesions, aortic total cholesterol, and aortic triglyceride in cholesterol-fed rabbits. In another study, low-dose nifedipine (2 mg/rabbit/day) was reported to exert antiatherogenic effects in rabbits, although the number of animals studied was small.\textsuperscript{16} Subsequently, calcium antagonists structurally unrelated to nifedipine, such as verapamil, diltiazem, and flunarizine, were demonstrated to exert antiatherogenic effects in cholesterol-fed rabbits.\textsuperscript{17–19}

Two groups of investigators have failed to demonstrate that calcium antagonists exert antiatherogenic effects in cholesterol-fed rabbits. In the first study,\textsuperscript{20} however, high-dose nifedipine was administered by
the sublingual route, which may evoke severe hypotensive episodes and trigger a sympathetic discharge. The reflex release of catecholamines, agents that stimulate the uptake of calcium, may have promoted the intracellular accumulation of calcium. Such an effect would oppose the therapeutic action of calcium antagonists. There is evidence that antiadrenergic drugs such as reserpine, guanethidine, and propranolol suppress atherogenesis in cholesterol-fed rabbits. In the second study, in which calcium antagonists were found ineffective in suppressing atherogenesis in cholesterol-fed rabbits given antiadrenergic drugs, plaques of the whole aorta were approximately one-tenth to one-fifth of those reported in the literature. Moreover, in two control groups receiving the same cholesterol diet, plaque areas expressed as a percent of the intimal area varied by more than 300% (4.4% vs 13.9%), a variation that would make the detection of significant effects in drug-treated groups difficult if not impossible.

The mechanism of action of chelating agents and calcium antagonists remains unclear. Calcium antagonists might exert antiatherogenic effects by reducing arterial pressure. In conscious normotensive rabbits, however, nifedipine administered by the oral route exerts only modest transient hypotensive effects. Blumlein et al. concluded that the antiatherogenic action of verapamil was unrelated to lowering of arterial pressure. Nakao et al. have reported that the calcium antagonist nicardipine inhibits the migration of cultured vascular smooth muscle from rat aorta, an effect that might play a role in inhibiting atherosclerosis. Recently Stein et al. demonstrated that high concentrations of verapamil enhanced the receptor-mediated uptake and disposal of low-density lipoprotein (LDL) by aortic cells in culture. They proposed that verapamil-induced stimulation of receptors might aid in the removal of LDL cholesteryl esters from the aortic interstitium. An action of calcium antagonists via LDL receptors might explain why the Watanabe rabbit, an animal that lacks LDL receptors, does not respond favorably to nifedipine. However, studies in Watanabe rabbits should be repeated in larger groups of animals receiving drug under controlled conditions. Recently, Etingin and Hajjar demonstrated that nifedipine produced a 50% loss of cholesterol and cholesteryl esters from lipid-laden rabbit aortic smooth muscle in culture. They concluded that nifedipine decreased cholesterol and cholesteryl ester accumulation in arterial smooth muscle by increasing cholesteryl ester hydrolysis.

In summary, current evidence indicates that calcium-chelating (anticalcifying) agents and calcium antagonists (calcium-channel blockers) have antiatherogenic effects in rabbits and monkeys. The drugs exert their effects without reducing dietary hypercholesterolemia. Therefore, the mechanism of action differs from that of hypolipidemic interventions.

**Clinical implications.** For many years it has been claimed by some that the chelating agent EDTA exerts beneficial effects in patients suffering from coronary and peripheral artery disease. Unfortunately, treatment with EDTA has never been subjected to a controlled trial and no statement can be made regarding the efficacy of this controversial therapy. On the other hand, several controlled studies with calcium blockers have recently been initiated in North America and Europe. To my knowledge, the ongoing trials are all based on the evaluation of sequential coronary arteriograms of patients undergoing catheterization for symptomatic coronary disease. For such trials to be successful, patients in placebo groups will have to be kept off calcium antagonists, and patient groups will have to be matched with respect to antiadrenergic manipulations (β-adrenergic blockade), which may influence the progression of atherosclerosis.

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