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Oxidative stress and coronary heart disease

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Despite the fundamental biological role of oxygen as an efficient producer of energy, an altered form of oxygen – with modifications in key chemical bonds that may potentially result in alterations in cellular structure and function – contributes to disease progression. Reactive oxygen species (ROS) include both free radicals (that typically have an oxygen- or nitrogen-based unpaired electron in their outer orbitals) and other species (eg, hydrogen peroxide) that act as oxidants. The mitochondria and cellular membrane oxidases (eg, NADPH oxidase) are major sources of ROS (Table 1). The byproducts associated with metabolism of arachidonic acid by cyclooxygenase, lipoxygenase, and cytochrome p-450 mono-oxygenase also result in the production of ROS. The targets for damage by ROS are not only lipids, (eg, polyunsaturated fatty acids in cellular membranes and low density lipoproteins [LDL]), but also proteins and nucleic acids. Lipid peroxidation products react strongly with various biological substrates leading to cellular injury. Oxidative damage to the polyunsaturated fatty acids of membrane phospholipids leads to destabilization in their intermolecular packing characteristics and subsequent loss of membrane integrity. Lipid peroxides break down into smaller molecules that remain either covalently linked to the phospholipid glycerol backbone or are released into the cytosol. In addition, the oxidized LDL particle does not effectively bind to the high affinity LDL receptor, thus extending its circulation and enhancing its uptake into macrophages. Besides lipids, other constituents of the cell are vulnerable to ROS-induced damage, especially nucleic acids associated with nuclear and mitochondrial DNA, and oxidizable proteins. Damage to nucleic acids can be broadly categorized as either strand breaks or base modifications. In the case of strand breaks, nucleic acid modification leads to mutagenesis as a result of misincorporation of bases into the DNA template during repair or replication. Oxidizable amino acids associated with cellular proteins are also vulnerable to ROS-induced damage, leading to a loss in normal function. The effects of protein oxidation products (eg, residue-specific changes, fragmentation products, and cross-linked reaction products with other intracellular molecules) can be highly deleterious to the cell, for example disrupting proteins involved in transport or cell regulation. The effects of ROS and its modification on arteriosclerosis will be the focus of this issue of *Cardiology Rounds.*

Oxidative stress and atherosclerosis

Atherosclerosis is the leading cause of death worldwide. Coronary atherosclerosis accounted for 7.2 million deaths worldwide in 1996, representing one-third of total deaths in industrialized countries. During the same year, cerebrovascular atherosclerosis and disease accounted for an additional 4.6 million deaths. The direct and indirect costs of coronary artery disease (CAD) was 110 billion dollars in the U.S. last year. In addition, it has been projected that there will be a 28% increase in cardiovascular deaths over the next 5 years in developing countries. Based on global trends, the World Health Organization projects that, by 2020, approximately half of all deaths in developed countries and one-third in developing countries will be due to cardiovascular disease.

Atherosclerosis is now understood to be a chronic inflammatory disease characterized by excess accumulation of monocyte-derived macrophages within the arterial wall.[1] Compelling evidence points to oxidative stress as an important trigger in the complex chain of events leading to and promoting atherosclerosis[2] (Figure 1). The expression of chemotactic factors such as monocyte chemotactic protein-1 (MCP-1) is enhanced by oxidative stress and oxidized LDL.[3] Endothelial expression of vascular cell adhesion molecule-1 (VCAM-1), which is regulated through a redox-sensitive mechanism,[4] promotes the

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Table 1: Major sources of reactive oxygen species (ROS)

- Mitochondrial electron transport
- NADPH oxidase
- Cyclooxygenase and lipoxygenase
- Nitric oxide synthase
- Myeloperoxidase
- Xanthine oxidase
- Auto-oxidation of catecholamines

adhesion of monocytes to the endothelium. The release of macrophage colony-stimulating factor (M-CSF) is also stimulated by modified LDL.[5] Expression of these factors results in the attraction and adhesion of monocytes to the arterial wall and the promotion of their differentiation into tissue macrophages. Exposure to the superoxide ion, a ROS, activates the nuclear factor kappa-B (NF-kappa B) regulatory complex and triggers the transcription of several atherosclerosis-related genes (VCAM-1, MCP-1, tumor necrosis factor (TNF), matrix metalloproteinase (MMP)-9 and procoagulant tissue factor).[6] This series of events leads to the accumulation of macrophages in the arterial wall, which then avidly incorporate oxidized LDL to form foam cells. Oxidized LDL, in turn, stimulates the release of interleukin-1 from macrophages.[7] The activity of MMPs is also regulated by oxidative stress and appears to be closely linked to smooth muscle cell activation and migration.[8] MMPs have also been implicated in the physiopathology of plaque rupture. Furthermore, ROS can lead to platelet activation and thrombus formation.[9] Therefore, oxidative stress appears to be important in both the early and later stages of the atherosclerotic process.

There is also evidence that oxidative stress occurs early after angioplasty.[10,11] Damaged endothelium, activated platelets, and neutrophils at the angioplasty site can generate reactive intermediates. These oxidizing metabolites can induce chain reactions that result in endothelial dysfunction, macrophage activation, smooth muscle cell migration and proliferation, and matrix remodeling.[12,13] The accumulation of new extracellular matrix and smooth muscle cells results in the neointimal formation responsible for lumen narrowing after stent deployment and balloon angioplasty.

Lack of efficacy of vitamins and concerns associated with their use

The results of large, prospective epidemiological studies have supported a protective role for antioxidant vitamins in cardiovascular diseases.[14] In spite of the most sophisticated statistical approaches, such observational studies are inherently limited by their inability to control for the effects of unknown or unmeasured confounders. Indeed, persons with greater intake of antioxidant vitamins through food sources or supplements are likely to differ from others in important ways that may alter the risk of cardiovascular diseases, such as lifestyle factors or other dietary habits. In contrast to the epidemiological studies, results of randomized clinical trials with antioxidant vitamins have been disappointing (Table 2).

Figure 1: All traditional risk factors for coronary heart disease (CHD) increase oxidative stress and in turn activate redox-sensitive signaling pathways and transcription factors.[2]

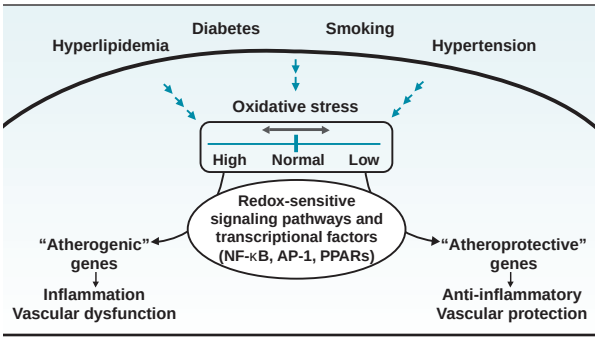


Table 2: List of neutral cardiovascular trials with vitamin E

- GISSI Prevention
- HOPE
- HPS
- MVP
- WAVE
- HATS

An excess risk for cancer and cardiovascular mortality was observed with beta-carotene in the large Alpha-Tocopherol/Beta Carotene (ATBC)[15] and Carotene And Retinol Efficacy Trial (CARET)[16] studies.

For vitamin E, the results of the Cambridge Heart Antioxidant Study (CHAOS) were initially encouraging in secondary prevention.[17] CHAOS was a British double-blind, placebo-controlled, randomized trial of 2002 British patients with angiographically-proven CAD. Patients assigned to vitamin E (400 to 800 U daily) had a 47% reduced risk of the combined primary endpoint of cardiovascular death and nonfatal myocardial infarction (MI). This risk reduction was due to a significant benefit for nonfatal MI, but there was a nonsignificant 18% excess of cardiovascular deaths in the vitamin E group.

However, recent results from 3 other major secondary prevention trials with vitamin E, in which more than 40,000 patients were randomized, were not supportive.[18-20] The Gruppo Italiano per lo Studio della Sopravivenza nell'Infarcto miocardico (GISSI) prevention trial was an open-label, randomized trial assessing dietary supplements of vitamin E (300 mg daily) and n-3 polyunsaturated fatty acids in 11,324 patients who had a recent MI.[18] The Heart Outcomes Prevention Evaluation (HOPE) study of over 9000 high-risk vascular patients tested both an angiotensin-converting enzyme (ACE) inhibitor, as well as vitamin E (400 U daily), in a factorial design for the prevention of cardiovascular morbidity and mortality.[19] The Heart Protection Study (HPS) recruited >20,000 patients at high risk of atherosclerosis-related events because of a past history of MI or other evidence of atherosclerosis, diabetes mellitus, or hypertension.[20] The patients were randomly assigned in a 2- by -2 factorial design to receive simvastatin alone, antioxidant vitamins (600 mg vitamin E, 250 mg vitamin C and 20 mg beta-carotene daily), the combination of simvastatin and vitamins, or placebo. Vitamin E did not offer cardiovascular protection in either the GISSI, HOPE or HPS trials.[18-20]

Important problems associated with the use of vitamins, such as their potential pro-oxidant effects, have been offered as explanations for the neutral clinical trial results.[21,22] Alpha-tocopherol itself becomes a radical when it scavenges a free electron. Because the tocopherol radical is relatively unstable, it can become a donor of free radicals or act as a pro-oxidant. Bowry et al[21] showed that, with high concentrations of vitamin E, lipid peroxidation is actually faster in the presence of alpha-tocopherol, and lipid peroxidation is propagated within LDL particles by reaction with the tocopherol radical. This may

Table 3: Comparison of the characteristics of AGI-1067 and probucol

Activities	AGI-1067	Probucol
• Phenolic antioxidant	+++	+++
• VCAM-1 expression inhibitor	+++	–
• MCP-1 expression inhibitor	+++	–
• E-selectin expression inhibitor	+++	–
• SMC proliferation inhibitor	+++	–
• LDL-lowering	+++	+/-
• HDL-lowering	+/-	+++
• Cellular permeability	++	–
• Potential to prolong QTc interval	–	++
• Stability	++	+
• Anti-atherosclerotic models		
– Monkeys	+++	+
– Rabbits	+++	+
– LDLr-KO mice	+++	+
– ApoE-KO mice	+++	–
• Inhibition of LPS-induced TNF-α and IL-1ß release from HAECs and hPBMCs	+++	–

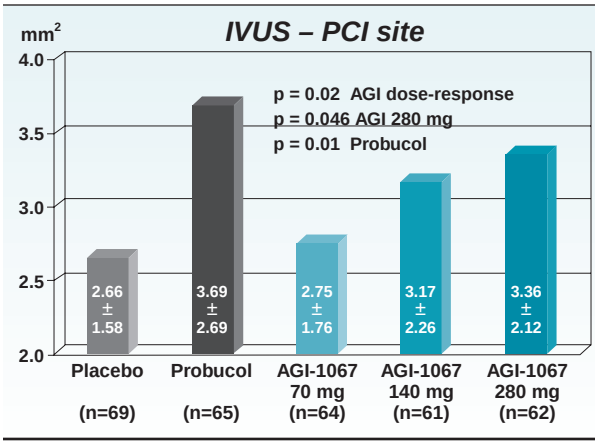
explain why high-dose alpha-tocopherol worsens endothelial-dependent vasodilation, whereas a low-dose improves it in cholesterol-fed rabbits.[22] These problems associated with vitamin E supplementation may explain the negative results of the vitamin arms of the GISSI, HOPE, HPS, WAVE and MVP trials.[18-20,23,24] Moreover, antioxidant vitamins negated in part the beneficial effects of lipid-lowering agents in patients with CAD in the recently published HDL Atherosclerosis Treatment Study (HATS),[25] as well as in the MVP trial.[24,26,27]

Beneficial effects of powerful synthetic antioxidants

Animal studies have shown a beneficial effect of antioxidants on both neointimal formation and arterial remodeling after balloon angioplasty.[28-32] More recently, the antioxidant probucol was shown to promote the regeneration of functional endothelium in balloon-injured rabbit aortas.[33] Small clinical studies, along with the MVP and PART trials, have shown that probucol started before balloon angioplasty reduces coronary restenosis.[13,24,26,27,34-38] However, prolongation of the QT interval and lowering of high density lipoprotein (HDL)-cholesterol with probucol remain potential long-term safety concerns. An agent that would offer antioxidant properties similar to probucol, but with more favorable safety and pharmacokinetic profiles, would have significant potential for the prevention of both restenosis and atherosclerosis progression. The recently developed antioxidant and vascular protectant AGI-1067 appears to fulfill these criteria and was therefore assessed in the Canadian Antioxidant Restenosis Trial (CART-1).[39]

AGI-1067, the mono-succinic acid ester of probucol, is a phenolic antioxidant member of a novel class of agents termed "vascular protectants."[40] It has strong antioxidant properties equipotent to those of probucol and anti-inflammatory properties.[4,41] AGI-1067 also exhibits greater water solubility and cell permeability compared with probucol. AGI-1067 has the ability to selectively block the expression of oxidation-sensitive inflammatory genes

Figure 2: Primary endpoint result in CART-1[39]



that code for VCAM-1 and MCP-1 through an NF-kappa B independent mechanism (Table 3). Although AGI-1067 and probucol are equipotent antioxidants, they produce different effects on VCAM-1 expression in human aortic endothelial cells. AGI-1067 produces a concentration-related decrease in TNF-alpha stimulated VCAM-1 expression in the concentration range of 2.5 to 10 µmol/L while probucol was shown to have no effect at concentrations as high as 100 µmol/L.[41] It is notable that ICAM-1 expression is not inhibited by AGI-1067, which demonstrates that this agent is not a global inhibitor of inflammatory response genes. Because ICAM-1 is the counter ligand to neutrophils, immune surveillance by these cells should not be affected by inhibiting only VCAM-1. AGI-1067 is also a powerful inhibitor of smooth muscle cell proliferation in experimental studies.

Effects of AGI-1067 and probucol on restenosis and atherosclerosis

CART-1 was a double-blind, double-dummy, multicenter trial in which 305 patients[39] scheduled to undergo elective percutaneous coronary intervention (PCI) with or without stent placement (85% with received stents) were randomly assigned to placebo, probucol 500 mg twice daily (as a positive control), AGI-1067 70 mg, 140 mg or 280 mg once daily. Patients were treated for 2 weeks prior to and 4 weeks after PCI in this phase 2 trial. The primary endpoint in CART-1, the minimal lumen area at the site of percutaneous coronary intervention (PCI) on follow-up intravascular ultrasound (IVUS) was on average:

- 2.66 mm^2 in the placebo group
- 3.69 mm^2 with probucol
- 2.75 mm^2 for AGI-1067 70 mg
- 3.17 mm^2 in the AGI-1067 140-mg group, and
- 3.36 mm^2 with AGI-1067 280 mg ($p < 0.05$ for larger lumen with AGI-1067 280 mg and probucol versus placebo)[39]
- there was a significant dose-response relationship of AGI-1067 ($p = 0.02$, Figure 2).

The volumetric assessment with IVUS supported these results.[39] The early benefits of AGI-1067 and probucol after PCI raise the possibilities that countering oxidative stress may rapidly improve endothelial function[42] and/or that these agents may produce changes in plaque content[43] that contributed to an improved response to PCI.

Importantly, volumetric (3-D) changes of non-intervened coronary reference segments away from the PCI site, were also

Figure 3: Changes in reference segments, away from the PCI site, at 6 months of follow-up (patients had stopped receiving study medications for 5 months at the time of follow-up intravascular ultrasound examination).[39]

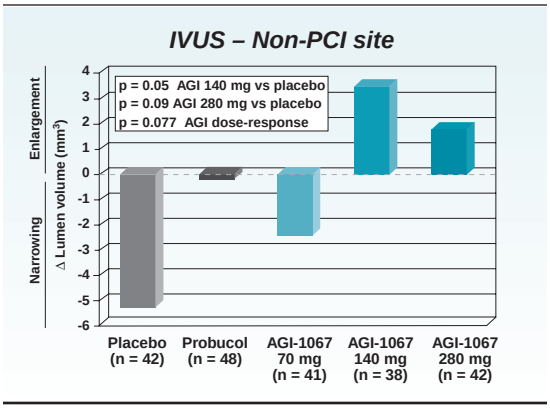
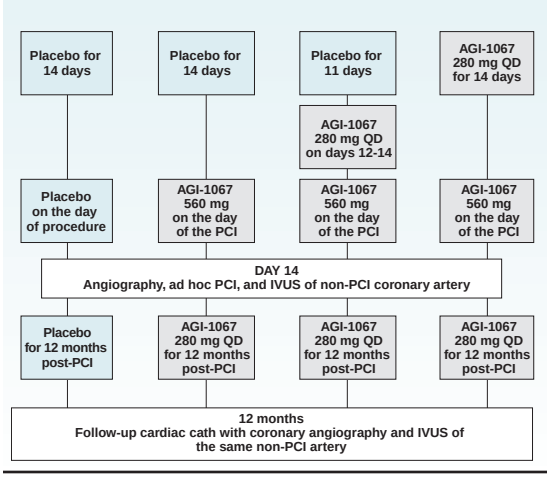


Figure 4: Study design of the CART-2 multicenter trial[44]



evaluated with IVUS in CART-1.[39] The mean changes in lumen volume (follow-up minus baseline) in the reference segments were:

- –5.3 mm^3 in the placebo group
- –0.2 mm^3 for probucol,
- –2.4 mm^3 with AGI-1067 70 mg,
- +3.5 mm^3 with AGI-1067 140-mg dose
- +1.8 mm^3 with AGI-1067 280-mg group
- p = 0.05 for AGI-1067 140 mg versus placebo; p = 0.077 for dose-response relationship).

This improvement in non-PCI site coronary artery lumen may represent the first clinical evidence of vascular protection with AGI-1067.[44] This effect was due to trends for both the inhibition of negative remodeling and reduction of plaque burden, processes which, when unchecked, may contribute to progressive coronary artery narrowing (Figure 3).

These clinical results are supported by demonstration of atherosclerosis prevention by AGI-1067 in all tested animal models,[41] including the apo-E knockout and LDL receptor-deficient mice and the hyperlipidemic primate.

The pre-clinical and clinical results obtained with AGI-1067 are concordant with those in the Fukuoka AtheroSclerosis Trial (FAST), in which probucol induced regression of carotid atherosclerosis in 246 asymptomatic hypercholesterolemic patients.[45] In this small study, the incidence of major cardiac events was also significantly lower with probucol than in the control group (2.4% versus 13.6%, $p<0.05$). The results of the older PQRST trial appear to be discordant with those described here, but the design of that study raised several previously discussed and important issues.[46] The primary endpoint was lumen volume of femoral arteries using three-dimensional reconstruction of angiograms, an approach rarely used in other clinical trials. The choice of the femoral location for assessment is also questionable, in light of the preferential effect of probucol on younger lesions in the proximal thoracic aorta compared to the more advanced iliac lesions in experimental atherosclerosis in non-human primates.[47] In addition, probucol was given to all patients (including those in the placebo group) for 2 months during the pre-randomization phase, which represents another problematic design feature considering that probucol accumulates in tissues for prolonged periods.

In CART-1, AGI-1067 did not prolong QT interval like probucol[44] and has now entered the next phase of testing of the antioxidant/anti-inflammatory hypothesis.

Rationale and design of the Canadian Atherosclerosis and Restenosis Trial (CART-2)

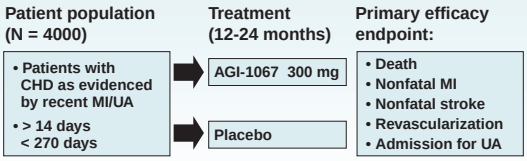
Based on pre-clinical and clinical data, and also on its beneficial effects in preventing low-density lipoprotein (LDL)-lipid oxidation, blockage of monocyte adhesion, and recruitment to inflamed endothelium through inhibition of endothelial adhesion molecule expression, it is hypothesized that AGI-1067 will significantly slow progression of atherosclerosis and may even induce regression in CART-2. Therefore, CART-2 is assessing the value of AGI-1067 for the reduction of both atherosclerosis progression in non-PCI vessels (measured with intravascular ultrasound[48]) and post-PCI restenosis after 12 months of treatment (Figure 4).

Considering that oxidative stress and inflammation may persist for a prolonged period after stenting,[49] treatment with AGI-1067 for the entire period of risk after PCI (instead of only 4 weeks in CART-1) may result in enhanced protection against luminal renarrowing. This hypothesis is also being tested in CART-2.[50] Although restenosis reduction with AGI-1067 started 2 weeks before PCI was the initial proof of concept in CART-1, this strategy cannot be applied to patients undergoing non-elective PCI. An other objective of CART-2 is therefore to extend the initial results from CART-1 and to determine whether pre-treatment with AGI-1067 for a shorter duration, combined with prolonged treatment after PCI, will prevent post-PCI restenosis.

The Aggressive Reduction of Inflammation Stops Events (ARISE) Trial

Despite improvements in imaging modalities, visualization of morphological details indicative of plaque stabilization or activity is not yet possible with IVUS[48] and the predictive value of anatomic changes for future clinical

Figure 5: Study design of the ARISE multinational, multicenter trial.



UA = unstable angina

events is not yet known. The greatest impact AGI-1067 may have clinically is in the prevention of plaque rupture and subsequent cardiovascular morbidity and mortality. Modifications resulting from the administration of AGI-1067, which stabilize the plaque but do not result in overall plaque volume changes, are only evaluable with a properly conducted clinical events trial. Therefore, the ARISE trial was designed to evaluate the potential utility of AGI-1067. The primary objective of ARISE is to determine whether long-term treatment with AGI-1067 will prevent major cardiovascular events in modernly-managed patients with CAD. Since the beneficial effects of AGI-1067 would likely be related to its potent antioxidant and anti-inflammatory properties, another key objective is to determine the effects of AGI-1067 on markers of inflammation and oxidation. ARISE represents a unique opportunity to test, in a definitive fashion, the antioxidant/anti-inflammatory hypothesis.[51]

ARISE is a multicenter, double-blind, randomized, placebo-controlled trial involving approximately 200 study sites (Figure 5). Approximately 4000 patients with a recent diagnosis of CAD (unstable angina or MI) and one additional risk factor will be enrolled in the U.S., Canada, Europe, and South Africa. Patients will be randomized to AGI-1067 or placebo in a 1:1 ratio. The primary study endpoint will be the combined incidence of cardiovascular mortality, resuscitated cardiac arrest, non-fatal MI, non-fatal stroke, need for coronary revascularization, and urgent hospitalization for angina pectoris with objective evidence of ischemia. The study will be complete when at least 1160 patients have experienced a primary event or, when all patients have been treated for at least 12 months, whichever occurs first.

Conclusion

Medical advancements made over the past decade have resulted in a greater ability to detect and treat atherosclerotic disease. Despite these medical advances, cardiovascular disease originating from atherosclerosis inflicts a large burden in terms of life expectancy, quality of life, and societal costs. Based on the aging of the population and trends towards environmental and lifestyle factors that increase the risk for atherosclerosis, it is anticipated that this burden will not dissipate in the near future. There is, therefore, a need for a novel pharmacological intervention that would provide further cardiovascular protection in patients with atherosclerosis, over and above the protection offered by other medications available, including statins. In light of its favorable effects, two important and large clinical trials are ongoing with AGI-1067. CART-2 and ARISE are respectively evaluating its value on atherosclerosis progression and clinical endpoints.

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Editor's Note:

I am pleased to be Co-Chairing the ARISE trial along with Dr. Tardif. Although ARISE is well up and running, there is still an opportunity for experienced investigators to join. If you are interested in being considered for one of the few remaining clinical sites, please contact my office (617-732-5681).

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