Inflammation in Atherosclerosis: New Opportunities for Drug Discovery

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Abstract: Many lines of evidence indicate that inflammation is the ultimate cause of atherosclerosis; high cholesterol levels cause atherosclerosis through mechanism of inflammation. Drugs designed to address inflammatory aspects of atherosclerosis will likely be more effective than current therapies in treating and preventing coronary artery disease.

INTRODUCTION

Atherosclerosis is the underlying condition of coronary artery disease (CAD), the leading cause of death in most parts of the world. A disease of venerable history, atherosclerosis was originally viewed as a natural degenerative occurrence of the aging process. In 1815, the same year when cholesterol was discovered but not yet correlated to atherosclerosis, the London surgeon Joseph Hodgson published a monograph on vascular disease, claiming that inflammation was the underlying cause of atherosclerosis [1]. In 1858, the German pathologist Rudolf Virchow, publishing the first in-depth study on the atherosclerotic lesion, found inflammatory cells in the plaque, concluded that atheroma was a product of an inflammatory process within the intima, and proposed that local intimal injury was the initiating stimulus of atherosclerosis [2]. This inflammation hypothesis, however, was ignored for nearly a century and a half because the cholesterol theory, put forward much later, became overwhelmingly "convincing."

Cholesterol was discovered in 1815 from human gallstones by the French chemist M. E. Chevreul [3], but its causal relationship with atherosclerosis was not established until the Russian scientist Alexander Ignatowski, in 1908, demonstrated that high-fat diets promoted atherogenesis in rabbits. Anitschkow and Chalotow subsequently, in 1913, found cholesterol present in the atherosclerotic plaque and at higher concentrations in the blood of animals fed high-fat diets [4]. Cholesterol was also confirmed to be present in human atherosclerotic lesions around this time [5]. Thereafter, and especially in the last several decades, a tremendous amount of research was carried out to understand the mechanism of hypercholesterolemia in causing atherosclerosis; experimental and epidemiological results strongly pointed to one conclusion: the more cholesterol there is in the blood, the more rapidly atherosclerosis develops [6]. More importantly, at least from a direct life-saving perspective, research efforts to find remedies for hypercholesterolemia have resulted in one of the major biomedical achievements in the 20th century – the discovery of β-hydroxy-β-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors, or statins, as safe and effective cholesterol-lowering drugs. The fact that statins, in clinical practice, have dramatically and substantially reduced mortality due to CAD inversely supports the notion that hypercholesterolemia causes atherosclerosis, at least in the patients that responded to the therapy. Pooled results of numerous clinical trials suggest that for every 10% reduction in blood cholesterol levels by statins, mortality due to CAD is reduced by at least 15% [7].

THE CHOLESTEROL CONTROVERSY

Several longitudinal studies, the Framingham Study being the best known, have definitely established total serum cholesterol concentration as an independent risk factor, among others, for CAD, therefore correlating cholesterol with CAD epidemiologically [8]. However, although total cholesterol levels can be predictive of CAD when one large group is compared with another large group, they are insufficient on their own in determining risk of CAD on an individual basis [9]. In the first 26-year follow-up of the Framingham Study, the total cholesterol distributions of the subjects with incidence of CAD and those free of CAD fall under two overlapping, bell-shaped curves. The overlapping range, 150-300 mg/dl, covers 90% of all individuals included in the study, demonstrating that 90% of the total cholesterol levels measured were useless by themselves for predicting risk of CAD in a general population [9]. Moreover, 35% of CAD occurred in people with total cholesterol levels between 150 and 200 mg/dl, a range considered desirable by the National Cholesterol Education Program, and a similar percentage of people with total cholesterol levels between 230 and 300 mg/dl did not have any incidence of CAD in the follow-up of the Framingham Study [9]. Other studies also suggest that cholesterol does not correlate with the degree of atherosclerosis at autopsy or on angiography and has no exposure-response correlation with atherosclerotic progression [10].

CONTEMPORARY VIEW ON ATHEROSCLEROSIS

Atherosclerosis was firmly believed to be a disease of cholesterol for nearly a century until the end of the last millennium, when many lines of evidence gradually cast doubt on the theory. In 1999, Russell Ross, drawing on numerous pathophysiologic observations in humans and animals and reinforcing Virchow's original response-to-injury hypothesis made over 100 years ago, bluntly branded atherosclerosis an *inflammatory disease* in the New England Journal of Medicine [11].

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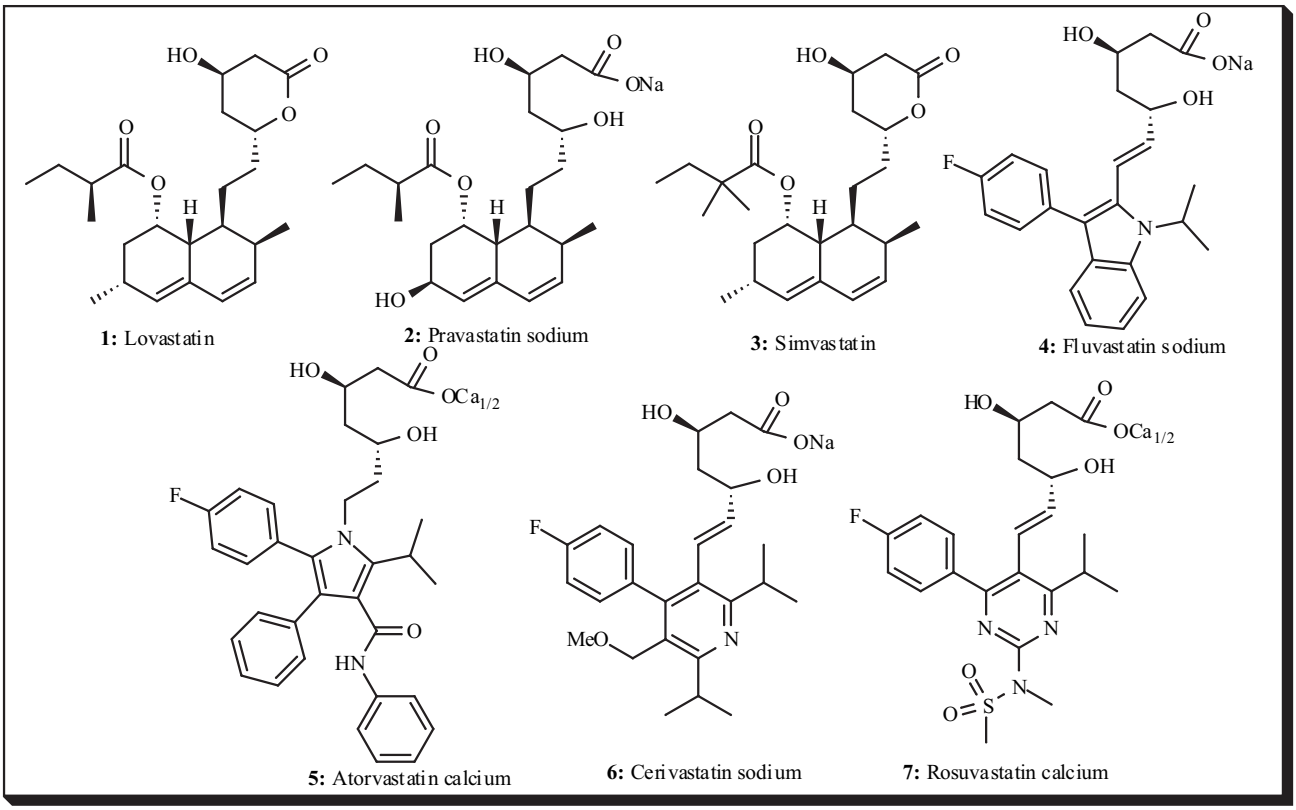
According to Ross's theory, initial injury of the arterial endothelium results in endothelial dysfunction, which in turn leads to compensatory responses that cause changes in homeostatic properties of the endothelium, such as increased adhesiveness and permeability of the endothelium, and formation of vasoactive molecules, cytokines, and growth factors. Such inflammatory responses stimulate recruitment of monocytes to the endothelium, and migration and proliferation of smooth muscle cells to form the initial atherosclerotic lesion. As inflammation continues to develop, macrophages and lymphocytes migrate from the blood and multiply in the lesion. Activation of these cells leads to further release of hydrolytic enzymes, cytokines, and growth factors, which can induce further damage and eventually lead to focal necrosis. Such cycles of accumulation of monocytes, migration and proliferation of smooth muscle cells, and formation of fibrous tissues lead to further enlargement and restructuring of the lesion, so that it becomes a core of lipid and necrotic tissues covered by a fibrous cap composed of extracellular matrix – a so called advanced lesion, or plaque, which, at some point, may intrude into the lumen and affect blood flow. Clinical data have shown that biological quality and function of the plaque is more crucial for a consistent clinical outcome than sheer size or degree of stenosis caused by the plaque [12], for 86% of fatal myocardial infarction (MI) happened with arteries less than 70% stenosed, while only 14% of fatal cases happened with arteries over 70% stenosed [13, 14].

The above is a simplified description of the pathophysiologic mechanism of atherosclerosis. In fact, it is a lot more complicated and also involves other inflammatory components; the role of cholesterol, an important one, is discussed below. However, each and every step in the development of atherosclerosis is one of escalating inflammatory advancement and therefore atherosclerosis is an inflammatory disease.

CHOLESTEROL DOES MATTER

Cholesterol itself is insoluble in aqueous solutions. It, in free alcohol or ester form, is complexed to other lipids and proteins to form lipoproteins, which are freely soluble in blood. Lipoproteins are categorized, according to their densities, into low-density lipoprotein (LDL), high-density lipoprotein (HDL), and others. In normal persons, about two-thirds of the total cholesterol is carried in LDL. Scientific data show that LDL contributes to the development of atherosclerosis and HDL protects against atherosclerosis; they are termed "bad cholesterol" and "good cholesterol," respectively. Brown and Goldstein discovered, in 1983, that macrophages took up native LDL at a rate insufficient to load them with cholesterol, and therefore proposed that circulating LDL must undergo some kind of structural modification before it becomes fully proatherosclerotic [15]. The uptake of modified LDL by macrophages can be viewed as an inflammatory response of macrophages to invading pathogenic lipoproteins in the arterial wall [16]. Modifications of LDL can occur through oxidation, aggregation, enzymatic modification, or complexing with immunoglobulins. The best studied of these and the only one supported by *in vivo* data is the oxidative modification hypothesis put forward by Steinberg and coworkers [17-20].

In contrast to native LDL, the uptake of oxidized LDL by macrophages is dramatically enhanced, leading to foam cell formation and contributing to the pathophysiology of both the initiation and progression of the atherosclerotic



lesion by many proinflammatory mechanisms. There is now a large body of experimental work in animal models that strongly support an important role for oxidative modification of LDL [19]. For example, the LDL extracted from atherosclerotic tissues of both animals and humans has been shown to exhibit all of the properties of oxidized LDL prepared *in vitro* [21]. Here, however, the key question is whether the oxidized LDL hypothesis, based on a wealth of evidence from a variety of animal species including nonhuman primates, will equally apply to the human disease of atherosclerosis. There are good reasons to believe it may, since structure, composition, and sequence of events in progression of lesions in animal models are very similar to human lesions and oxidized LDL has been recovered from human lesions [19]. Another school of thought contends that the types of modified lipids and proteins extracted from human atheroma do not necessarily correspond to the ones derived from lipoproteins oxidized *in vitro*, and therefore, the relevance of the LDL oxidation hypothesis to human atherosclerosis remains unproven [22].

Regardless of the validity of the LDL oxidation hypothesis, it is generally accepted that LDL first undergoes some kind of modification and then is taken up by macrophages which in turn form foam cells. This whole process and its enormous consequences are pro-inflammatory. Therefore, cholesterol does matter to atherosclerosis, but through mechanisms of inflammation. And there is no longer a "cholesterol controversy" [23].

THE STATIN TESTAMENT

Statins are the most commonly prescribed agents for the treatment of hypercholesterolemia and hence the prevention of CAD. Currently, there are five statins widely used clinically (**1**-**5**). In addition, cerivastatin (**6**) has been withdrawn due to side effects after a short period of use, and a newcomer, rosuvastatin (**7**), may enter various markets in the world soon, as it has been approved in a few countries.

Although statins lower serum cholesterol levels in hypercholesterolemic patients effectively and almost ubiquitously, it has been observed that in all the statin trials published before 1998 improved outcomes were obtained only with around 30% of the patients [24]. Since serum cholesterol level was not the primary inclusion criterion in major statin trials, there could be some patients whose cholesterol levels were not elevated. However, even after this normocholesterolemic population is excluded there would still have been a high percentage of patients that failed to receive benefit from statins in these trials. This probably explains why CAD is still the leading cause of death today after extensive prescription of statins for over a decade. Moreover, this also contributes to the increasing suspicion that high cholesterol levels are not the ultimate cause of atherosclerosis.

In the patient population wherein statins do exert benefit, on the other hand, the beneficial effects cannot all be attributed to cholesterol lowering alone. The non-lipid-related, pleiotropic effects of statins have been widely observed [25-28]. The mechanism of action of the statin class of cholesterol-lowering drugs is the inhibition of HMG CoA reductase necessary for mevalonate synthesis, a rate-limiting step in the biosynthesis of cholesterol. Blocking such a very early step of the lengthy process of cholesterol biosynthesis, statins inhibit the production of many kinds of isoprenoids which would be normally formed in the more than 20 steps thereafter. These isoprenoids carry out various biological functions and blocking their production could have profound changes in these functions, especially those that are inflammation-related. Moreover, inhibition of the production of these isoprenoids might trigger physiological feedback mechanisms, further complicating the ultimate effects of statins. Theoretically, therefore, statins could exert far-reaching biological/pharmacological effects beyond those caused by the lowering of cholesterol levels.

Smooth muscle cell proliferation in the arterial wall is a key step in the atherosclerotic progression process as discussed above. Several statins have been shown to be able to inhibit smooth muscle cell proliferation both *in vitro* and *in vivo* [26]. Compared to healthy subjects, platelets from hypercholesterolemic patients are activated [29] and statins improve platelet function in such subjects [26, 30, 31]. It is worth noting, however, that statins failed to show significant effect in the prevention of restenosis in which smooth muscle cell proliferation and platelet hyperactivity are believed to be involved, although drug availability to the local area of artery, where restenosis takes place, could be a concern; statin-coated stents seem to have worked for the prevention of restenosis [32]. Other non-lipid effects of statins are discussed below.

ADHESION MOLECULES

According to the response-to-injury theory supported by experimental data, the first response of the endothelium to initial injury is endothelial dysfunction to induce expression of adhesion molecules which, in turn, recruit circulating monocytes from the blood stream. Adhesion of monocytes to the endothelial cell surface is a multistep process involving primary adhesion and rolling, secondary firm adhesion, and finally transmigration. E-selectin mediates early and reversible events, while vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) regulate later and irreversible steps, leading to firm attachment and subsequent diapedesis of monocytes [33].

The role of VCAM-1 and ICAM-1 in mediating permanent attachment of monocytes to the endothelium surface, makes them rational targets for atherosclerosis drug discovery. However, since the counter-receptor of ICAM-1, lymphocyte function-associated antigen-1 (LFA-1), is expressed on the surface of neutrophils, chronically interfering with ICAM-1 or LFA-1 may be associated with an increased susceptibility to severe infections [34]. The counter-receptor of VCAM-1, very late antigen-4 (VLA-4), is not expressed on neutrophils. More importantly, as Cybulsky *et al*. reported [35], although both VCAM-1 and ICAM-1 are expressed in the regions predisposed to atherosclerosis and at the periphery of established lesions, and ICAM-1 is even expressed more broadly, VCAM-1 plays a dominant role in the initiation of atherosclerosis. Data from directly comparing the roles of VCAM-1 and ICAM-1 in atherosclerosis in mice that express VCAM-1 at levels less than 10% of wild type indicate that deficiency of

VCAM-1 significantly diminishes early lesion formation throughout the aorta. ICAM-1 deficiency did not influence early foam cell lesion formation either alone or with VCAM-1 deficiency [35]. Other experimental data also corroborate that VCAM-1, but not ICAM-1, plays a dominant role in the pathology of atherosclerosis [36] and therefore is a new target for atherosclerosis drug discovery. Many other lines of evidence confirm the involvement of VCAM-1 in the initiation and progression of atherosclerosis. For example, the aortic endothelium of rabbits fed an atherosclerotic diet expressed VCAM-1 after only one week on the diet but before the first appearance of intimal macrophages, and lesions composed of macrophages developed when the rabbits had been on the diet for three weeks or longer [37]. Several statins have been shown to be able to reduce adhesion of human monocytes to endothelial cells [38].

CHEMOKINES

The chemoattractant cytokines, or chemokines, are small disulfide-linked polypeptides, and are potent chemoattractants for leukocytes such as T cells, natural killer (NK) cells, monocytes and macrophages [39]. Much of the current interest in the role of chemokines in atherogenesis arose from studies performed on monocyte chemoattractant protein-1 (MCP-1) [40]. The expression of MCP-1 in human atherosclerotic lesions has been clearly demonstrated [41, 42]. MCP-1 triggered firm adhesion of monocytes to activated endothelium and caused rapid arrest of human monocytes rolling on endothelial cells under flow conditions [43]. Further indirect evidence for MCP-1 being a potential player in atherogenesis came from the fact that treatment of human endothelial cells with oxidized LDL induced MCP-1 secretion [44]. Ablation of the gene encoding MCP-1 in apoE knockout mice results in a marked reduction in the size of atherosclerotic lesions [45], strongly suggesting that MCP-1 plays a non-redundant role in monocyte recruitment and/or macrophage retention in atherosclerotic lesions. Statins have been shown to inhibit the expression of MCP-1 [46].

C-REACTIVE PROTEIN

C-reactive protein (CRP) is a hepatically derived pentraxin that plays a key role in the innate immune response and is now understood to be a marker of atherosclerosis [47]. To date, over a dozen prospective epidemiological studies carried out among individuals with no prior history of CAD demonstrate that a single, non-fasting measure of CRP is a strong predictor of future vascular events [48-51]. The relationship between a patient's baseline level of CRP and future CAD risk has proven independent of age, smoking, cholesterol levels, blood pressure, and diabetes. CRP levels have long-term, as long as 20 years, predictive value [52].

It has been demonstrated in a clinical trial that the magnitude of risk reduction in coronary events attributable to pravastatin was substantially greater among those with evidence of inflammation than those without inflammation measured by CRP levels. The study also found that pravastatin lowered CRP levels significantly and in a manner unrelated to the effect of the drug on cholesterol levels, providing strong evidence that statins have important anti-inflammatory effects and, indirectly, inflammation plays a major role in atherosclerosis [53, 54]. The CRP-lowering effect of pravastatin has been confirmed in a much larger trial, and several other statins have been shown to have similar effects [22].

Strikingly, lovastatin reduced coronary event rates among those with lower levels of LDL cholesterol and above-median levels of CRP, but did not benefit those with below-average LDL levels and below-average CRP levels [55]. This further confirms that inflammation is more crucial than cholesterol levels for atherosclerosis. Since half of all heart attacks and strokes in the U.S. occur among individuals with normal cholesterol levels, these data provide novel biological insights about some patients who may be at high risk due to elevated CRP levels. New guidelines drawn by the American Heart Association and the Centers for Disease Control and Prevention issued on Monday, January 27, 2003 urged doctors to consider testing millions of Americans at moderate risk of heart disease for signs of inflammation in the bloodstream (Associated Press). It is also worth noting that a large number of patients with clinical CAD events do not have elevated CRP levels; CRP is not a universal marker for CAD.

OXIDATIVE STRESS

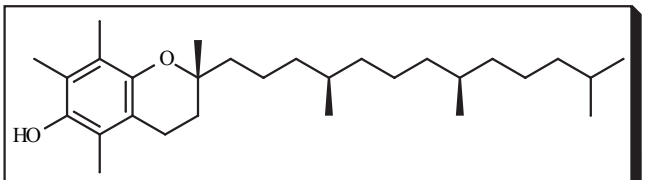
In order to survive, eukaryotic organisms rely on atmospheric oxygen to oxidize organic fuel molecules to provide energy. This process inevitably generates reactive oxygen species (ROS) as side products. Evolution has developed intrinsic enzymes with antioxidant properties, e.g., superoxide dismutase and glutathione peroxidase, to quench ROS. In healthy subjects, the quenching is seamless and balanced. Under certain circumstances, however, ROS could be over-produced or the quenching can become insufficient, leaving net excess amount of ROS in the system. Such abnormal levels of ROS will then cause various oxidative damages – oxidative stress, which leads to inflammatory consequences.

Oxidative stress is believed to be involved in aging [56] and the pathophysiology of numerous diseases such as Alzheimer's disease [57]. With regard to atherosclerosis, oxidative stress may exert far-reaching effects [58, 59] – e.g., modulation of protein kinase activity and gene expression – beyond LDL oxidation discussed above. Despite the high volume of data suggesting oxidative stress as the culprit for diseases including atherosclerosis, the oxidative hypothesis is not yet proven. The ultimate proof ought to be a clinical one – i.e., an antioxidant working effectively in treating a disease in humans [60, 61].

VITAMIN E

Vitamin E (α-tocopherol, **8**) is one of the best known antioxidants. Numerous large observational and epidemiological studies suggest a lower incidence of CAD in people with a higher intake of vitamin E either from diet or from supplements, confirming the preventative effect of vitamin E against CAD [62]. Furthermore, it has been clearly shown that vitamin E decreases lipid peroxidation and platelet aggregation, and functions as a potent anti-

inflammatory agent in man [63]. However, among four large clinical trials of vitamin E on CAD in different populations, two showed a reduction in both cardiovascular death and nonfatal MI, meeting primary endpoints, while one only met the secondary endpoint and the fourth failed completely [19, 63]. It seems that the negative results outweigh the positive outcome in these studies so that there is no basis now for recommending vitamin E supplementation to patients with CAD [20]. Moreover, Vitamin E may blunt the effectiveness of hypolipidemic therapy with statins and niacin in patients with CAD [64].



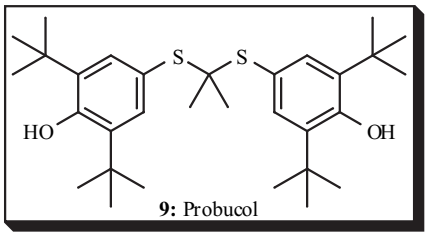
8: Vitamine E

Understandably, the levels of ROS are much higher in a disease state, especially oxidative stress-related diseases, than in a healthy condition. Vitamin E is a stoichiometric antioxidant; for each molecule of ROS one molecule of vitamin E is needed to quench it. Vitamin E cannot reach high enough concentrations to quench all the ROS molecules in a disease state. This could be the reason why vitamin E works prophylactically, but not therapeutically for CAD. Vitamin E has been successfully used in combination with doxorubicin, an anticancer drug, to quench the harmful free radicals generated by the latter [65]. In this case probably the free radicals do not accumulate to high concentrations. In addition, that vitamin E cannot reach various biological targets efficiently, as discussed below, could also contribute to its inconsistent behavior.

PROBUCOL

Probucol (**9**), though discovered and marketed once as a lipid-lowering agent, only has moderate LDL-lowering effect compared to the newer class of lipid-lowering drugs – the statins, but lowers HDL levels significantly and, in some patients, causes QTc prolongation. These could be the reason(s) why it was later withdrawn from most markets in the world. However, the strong and unique antioxidant property of probucol has been widely recognized, and the compound has been used as an antioxidant research tool in numerous publications [66]. Besides, and more importantly, the antioxidant property of probucol has been employed to guide new drug discovery (see below).

Probucol effectively inhibits the oxidative modification of LDL independently of its cholesterol-lowering effect [67]. LDL isolated from plasma of animals or patients treated with probucol is highly resistant to oxidative modification and minimally recognized by macrophages [68, 69]. Although both are known antioxidants and, especially, vitamin E is 10-100 times more potent an antioxidant than probucol in terms of chemical reactivity toward oxygen radicals in homogeneous solution, probucol protects LDL from oxidation slightly more efficiently than, or at least as efficiently as, vitamin E [70]. This could be due to the fact that the molecule of probucol is more lipophilic than vitamin E and therefore can enter the lipophilic core of LDL more readily [71]. The antioxidant BHT (2,6-di-tert-butyl-4-methylphenol), structurally similar to probucol with the lipophilic tert-butyl groups, is also more efficient than vitamin E in protecting LDL from oxidation [72].



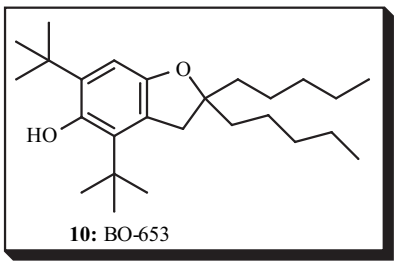
9: Probucol

The majority of many studies have shown that treatment of hypercholesterolemic animal models with probucol leads to suppression of atherosclerosis in several species [19, 66]. In contrary to an earlier study, probucol showed significant–as much as pravastatin did–reduction in carotid artery intima-media thickness after two years of treatment, independently of its LDL- or HDL-lowering effect, and lowered incidence of clinical cardiac events in a trial [73]. Probucol has also been shown to be able to substantially reduce luminal narrowing after angioplasty in humans [74]. The striking ability of probucol to prevent restenosis is most likely due to its antioxidant effect [66]. Interestingly and paradoxically, multivitamins including vitamin E did not show any efficacy and combination of probucol with such multivitamins exerted less beneficial effect than probucol alone in preventing restenosis in humans [74]. This blunting effect of (presumably) vitamin E further distinguishes probucol from vitamin E in their clinically relevant antioxidant properties.

BO-653

BO-653 (**10**) is an antioxidant designed by Chugai Pharmaceuticals based on probucol and vitamin E, meant to retain the advantages and overcome the shortcomings of both antioxidants [75]. Several key factors were taken into consideration in the design of BO-653: the 5-hydroxy group taken from both probucol and vitamin E as antioxidant source, the 4,6-di-tert-butyl groups taken from probucol to make the compound more lipophilic, and the 2,3-dihydrobenzofuran unit derived from a vitamin E analog to increase the antioxidant activity.

The chemical reactivity of BO-653 towards a peroxyl radical was less than that of vitamin E, probably due to the steric effect of the bulky t-butyl groups at the ortho-positions, which hinders the access of a peroxyl radical to the phenolic hydrogen. However, the antioxidant potency of BO-653 against lipid peroxidation was superior to that of

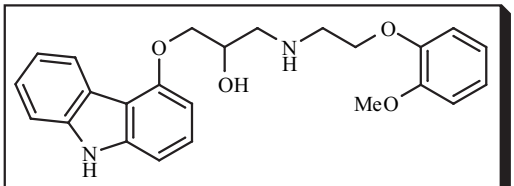


10: BO-653

vitamin E [76-78]. In a study on the antioxidant activities of BO-653 against the oxidative modification of human LDL, BO-653 was consumed faster than vitamin E, and retarded consumption of vitamin E. Vitamin E was not consumed until most BO-653 was consumed. The formation of lipid hydroperoxides was effectively inhibited until almost all BO-653 was consumed. The superior antioxidant potency of BO-653 over vitamin E is likely due to the increased lipophilicity of the molecule and enhanced stability of its radical, both of which help it reach deep into the core of LDL particles [78]. BO-653 has shown efficacy in animal models of atherosclerosis and restenosis; in some species the effects were superior to those of probucol. Also, it did not lower HDL levels in animals [71]. It is currently in clinical trials for the treatment of atherosclerosis, which will eventually determine whether its advantageous antioxidant property, lipophilicity, and anti-atherosclerotic effect make it a better drug than either probucol or vitamin E, from which it was designed.

CARVEDILOL

Angiotensin-converting enzyme inhibitors and β-adrenergic receptor blockers are now the drugs of choice in the therapy of heart failure. Carvedilol (**11**) is a non-selective β_1- and β_2-receptor blocker with selective α_1-adrenergic receptor-blocking activity. The cardioprotective activity of carvedilol is not equally shared by other β blockers, so there must be other mechanism(s) involved in its action. Oxygen radicals have been implicated in myocardial damage during ischemic insults leading to heart failure and carvedilol has been shown to be a potent antioxidant [79].

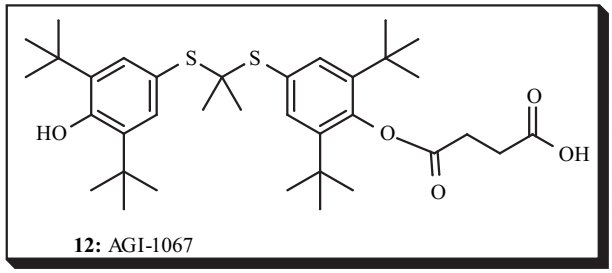


11: Carvedilol

In electron paramagnetic resonance studies, carvedilol inhibited the electron adduction of oxygen radicals in a concentration-dependant manner in both lipid and aqueous environments. In cardiac membranes prepared from swine ventricular tissue, carvedilol significantly inhibited lipid peroxidation, whereas other drugs in the class, such as propranolol, pindolol, labetalol, or atenolol, were devoid of antioxidant effects at concentrations 100-fold higher than those of carvedilol. In brain homogenates subjected to oxygen radical stress sufficient to deplete the endogenous antioxidant vitamin E, carvedilol was observed to inhibit vitamin E depletion in a dose-dependant fashion. Carvedilol is about 10 times more potent than vitamin E as an antioxidant [79]. The antioxidant effects of carvedilol have also been confirmed in animals and humans [80]. Carvedilol is metabolized to several hydroxylated compounds which are significantly more potent antioxidants than the parent drug, SB 211475 being one of them [81]. Therefore, the antioxidant effect of carvedilol observed *in vivo* results not only from the parent drug but also from one or more of its metabolites. Unlike vitamin E, probucol or BO-653, carvedilol is not a phenolic antioxidant though its metabolites are phenols.

MULTI-FUNCTIONAL APPROACHES

Based on the rationale that compounds with proper monosubstitution at one of the phenol groups of probucol may retain the beneficial antioxidant and lipid-lowering properties of probucol but have an improved safety profile due to the inability of such compounds to form spiroquinone metabolites [82] that might cause toxicity such as QTc prolongation, AtheroGenics set out to design anti-inflammatory properties into such compounds as effective *multifunctional* drugs for atherosclerosis [83]. AGI-1067 (**12**) is a clinical compound derived from this endeavor. It exhibits many of the *in vitro* properties desirable in a molecule to treat atherosclerosis, that probucol lacks. Like probucol, AGI-1067 functions as a potent antioxidant. However, unlike probucol, AGI-1067 selectively and potently inhibits inducible VCAM-1 and MCP-1 expression, and inhibits human aortic smooth muscle cell proliferation. AGI-1067 inhibited the progression of atherosclerosis and lowered LDL levels in animals with neutral or elevating effects on HDL levels [84]. AGI-1067 is not metabolized to probucol in animals and humans, so it is not a prodrug of probucol [83]. In a phase II trial, AGI-1067 improved lumen dimensions of reference segments of coronary artery after angioplasty, suggesting a direct positive effect on atherosclerosis, and did not cause QTc prolongation [85]. It is entering phase III studies, which will determine the real merit of this novel, multifunctional drug in CAD patients.



12: AGI-1067

PERSPECTIVES

Throughout human history, atherosclerosis was not the major cause of death, although it has been known since ancient times. Rather, infection and famine were the two major causes, toward both of which evolution has developed innate mechanisms of defense: high potentials of inflammation to guard against infection and insulin resistance to preserve energy in case of food shortage. We humans are living in a totally new era since a little while ago in historical terms; there are no injuries caused by other creatures and there is plenty of food, at least in most parts of the world. However, the evolutional "hangover" of inflammation and insulin resistance will stay with us until evolution has developed new mechanisms according to our new environment, probably in millions of years. On the other hand, the fact that we are not forced to exercise as much as our ancestors who hunted for animals all day long, makes the whole situation even worse. Therefore, search for

therapies to overcome inflammation and insulin resistance is a very crucial task in drug discovery for a long period of time to come.

LDL undergoes some kind of modification, no matter an oxidative one or not, and then gets taken up by macrophages and participates in the whole inflammatory process of atherosclerosis formation and progression. Nowadays, the question is not whether inflammation plays a crucial role in atherosclerosis, rather it is whether cholesterol exerts any additional effect beyond participating in the inflammatory process of atherosclerosis. A definite answer could come from specially designed "statins" that do not have any cholesterol-lowering effect. However, such "statins" would not come by easily. On the one hand, the pharmaceutical industry would not be interested in such compounds because it is still believed that cholesterol lowering at least does not do any harm, rather might do good. Perhaps only academia supported by public funds can take such endeavors to answer a very important scientific question. On the other hand, since the numerous anti-inflammatory effects of statins come most likely from the blockage of the cholesterol biosynthetic pathway, though not directly from cholesterol lowering, these beneficial effects might be gone if the cholesterol lowering mechanism of statins is removed. In other words, statin analogs without the HMG CoA reductase-inhibiting pharmacophore may not have any anti-inflammatory effects at all. Anyway, the current statins were not designed as anti-inflammatory drugs; drugs designed according to inflammatory parameters relevant to atherosclerosis, statin-related or not, may become much better drugs to treat atherosclerosis.

Roughly two-thirds of cholesterol is synthesized in the body and the rest comes from food intake. Statins block the former route and now there is a new drug (ezetimibe) [86] to block the latter. A combination of both therapies is more effective than either one in lowering cholesterol levels [87]. However, it remains to be seen whether deeper cholesterol lowering using such a combination exerts any additional benefit to CAD patients as compared to current statin therapy. This in a sense will also prove whether cholesterol levels are relevant or not to atherosclerosis.

Although oxidative stress is widely believed to be involved in the pathology of many disorders including atherosclerosis, antioxidants, meant to correct oxidative stress, have not proven to have any therapeutic value. Most antioxidants used to date are stoichiometric antioxidants and can not reach high concentrations necessary to combat ROS in a disease state. Also, some may not have the right physical properties such as lipophilicity to enter various biological entities. Future antioxidants, ones that deploy intrinsic antioxidant enzymes in a catalytic fashion and/or ones that can be readily available to disease sites, may become useful drugs.

Because of biological redundancy and different mechanistic facets and stages of a disease, multifunctional drugs might work better than therapies that only address one single mechanism of a disease. Aspirin probably would not be as effective and widely used as it actually is, if it were only an anti-inflammatory or anti-platelet agent, but not a dual-function one. Some drugs of serendipitous multi-function, such as aspirin and carvedilol have been used effectively and safely in the clinic. The long-term efficacy and safety of newly designed multi-functional drugs for atherosclerosis remains to be seen.

ACKNOWLEDGEMENTS

The author thanks Drs. C. Kunsch, J. Sikorski and C. Sundell for constructive advice toward this manuscript.

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