ASCORBIC ACID AND ATHEROSCLEROSIS

Uraiwan Ketsawatsakul, Pravit Akarasereenont

Department of Pharmacology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, THAILAND

ABSTRACT

The role of ascorbic acid or vitamin C has been proposed in the prevention of atherosclerosis. The possibility is discussed that the antioxidant effect of ascorbic acid might be protective against and possibly propitious to atherosclerosis. Growing evidence suggests that oxidative modification of low-density lipoprotein (LDL) may be of particular importance in the pathogenesis of atherosclerosis because oxidized LDL exhibits proatherogenic effects. In addition to oxidative modification of LDL hypothesis, inflammatory process potentiated by cytokines also importantly contributes to the pathogenesis. These complex mechanisms presumably participate in endothelial injury resulting in impaired releasing factors, such as nitric oxide (NO) and prostanoids, taking part in abnormal vascular tone. Therefore, vasoactive substances produced from endothelium and their pathways may be modulated by both cytokines-and oxidized LDL-induced oxidative stress. Recently, antioxidants have been determined that can prevent LDL oxidation beneficial to the inhibition of atherosclerotic process. There are accumulating experimental, epidemiological, and clinical evidences of an association between antioxidant vitamin intake and reduced risk of coronary heart disease. In animal models, ascorbic acid has been shown to attenuate the oxidation of LDL and atherosclerotic lesions. Population studies suggest an inverse relationship between ascorbic intake and the development of atherosclerosis, although the effect has not yet been proven in clinical trials. A possible mechanism for the anti-atherogenic effect of ascorbic acid is the prevention of oxidation of LDL. Furthermore, the potential effects of ascorbic acid on the metabolism of NO and prostanoids as well as in the defense on monocyte adherence might particularly improve endothelial function in atherosclerosis. These finding should be pursued in basic research studies to elucidate its molecular biological mechanisms, additionally in clinical epidemiological studies of ascorbic supplementation in populations in order to verify a role of ascorbic acid for the practical use in clinical medicine.

Key words: vitamin, oxidized LDL, oxidative stress, antioxidants, free radicals, endothelial dysfunction.

Address correspondence and reprint requests to: Uraiwan Ketsawatsakul, Department of Pharmacology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, THAILAND. Tel: 4197569, 4115026

INTRODUCTION

Atherosclerotic development has been very likely multifactorial in the pathogenesis attributed to oxidative stress as well as cellular hyperadhesiveness, hyperaggregation and im paired formation of vasoactive substances, particularly prostacyclin (PGI_2), nitric oxide (NO) and endothelin (ET-1). Vascular endothe lium, responsive to a variety of pathophysiolo gic stimuli, plays a crucial role in maintaining vascular integrity in part by the synthesis and release of vasoactive mediators influencing vascular relaxation and contraction. Hence, dysfunction of endothelial cells is strongly pointed to be a pathogenetic role in the initia tion of atherosclerotic vascular disease and its clinical complications.[1-6]

The mechanisms account for initia ting atherosclerotic lesion have not been completely elucidated, however the oxidative modification of LDL hypothesis has been implicated as a significant cause. Resulting from the imbalance of subendothelial lipo proteins and oxidation potential, oxidation products, especially oxidized LDL (ox-LDL), set into motion the cascading of oxidative stress-related vicious cycles initiating foam cell formation and subsequently atherosclerotic plaque or atheroma formation. The precise mechanism of oxidative stress, which occurs when free radical formation exceeds the ability to protect them, within the vasculature is not well understood but is probably due to multi contributors.[7-10] Free radical-mediated oxida tion has been proposed as a mechanism by which LDL becomes modified in the vascular wall, leading to increased uptake by macro phages via the scavenger receptor pathways. Ox-LDL may promote atherosclerosis by addi tional mechanisms, including chemoattraction of monocytes and smooth muscle cells, cyto toxicity to endothelial and smooth muscle cells, inhibition of NO, and stimulation of smooth muscle cell proliferation.[10] Moreover, the damaging free radicals may cause either direct arterial wall injury or more may accele rate secondary processes including depletion of antioxidants (such as ascorbic acid or α-tocopherol), protein peroxidation, and activa tion of phagocyte-platelet-endothelial cell inter actions. Ox-LDL and free radicals probably both can inactivate NO.[11-12] Interestingly, adhe sion molecular gene expression and consequent mononuclear leukocyte recruitment may be induced by oxidative stress as an important intracellular signal in the pathogenesis.[13]

The inflammatory process, mediated by adhesion molecules, cytokines such as IL-1β and TNFα, and eicosanoids, also accounts for the observed endothelial damage leading to endothelial vasodilator dysfunction in athero sclerosis. Therefore, abnormal endothelial func tion has been caused by various mechanisms, including oxidative stress and cytokine-induced the expression of adhesion molecules on the endothelial cell surface[13-15], adhesion and migration of monocytes into the subendothelial space, platelet aggregation, additionally the impaired formation of NO and prostanoids, produced via the L-arginine and the cyclooxy genase (COX) pathway, respectively.[13-16]

Ascorbic acid or vitamin C, a water-soluble antioxidant has been shown as anti atherogen that can prevent LDL oxidation and help to preserve α-tocopherol (vitamin E) in lipoproteins. This strong reducing agent very effectively protects lipids in human plasma against peroxidative damage by scavenging oxygen-derived free radicals.[12,17-19] Also, the lower production rate of reactive oxygen species and regulation of intracellular redox state by ascorbic acid are probably effective not only in preventing oxidation of LDL but also by blocking intracellular redox-sensitive signal mediating adhesion molecule expression and presumably COX-2 pathway, additionally inhibitng free radicals-inactivated NO involved in atherosclerosis. Thus, ascorbic acid may improve endothelial vascular function in part by promoting NO[11,12,20] and prostanoid actions. Hence, in addition to the effect on cytotoxic ox-LDL, ascorbic acid presumably have the possibility to retard damaged endothelium by protecting against inflammatory mediators including cytokines, such as IL-1β, TNFα, and prostanoids.[21]

However, previous studies provide limited support for the hypothesis that the dietary antioxidants vitamin C may slow the progression of atherosclerosis. Thus, the anti atherogenic benefits of antioxidant ascorbic acid in human atherosclerosis remain unproved by clinical trials, and the long-term effects of mega-dose ascorbic acid are yet undefined.[22,23] An important clinical question is the extent to whether ascorbic acid could prevent or treat the detrimental effects of atherosclerosis. Standards of proof should be clarified since these will definitely provide valuable informa tion about optimal nutritional intakes as well as this antioxidant may become an additional treatment modality against atherosclerosis.

Pathogenesis of atherosclerosis

At the inner vascular surface in early atherosclerotic lesion, the endothelial cells are abnormal shape and function. Beneath the endothelium lies the fibrous caps composed mainly of extracellular collagen, fibrin, elastin, and proteoglycans, occasional lipid-laden macro phages (foam cells), and modified smooth muscle cells. Deeper within the vessel wall and beneath the fibrous cap are several layers of smooth muscle cells interspersed with macro phages and foam cells. Macrophages that process and internalize extracellular lipids, the LDL, are activated and changed to be foam cells, avidly accumulated and then initiating the fatty streak, the first atherosclerotic lesions and the probable precursor of atherosclerotic plaque.[4-8,24]

In vitro studies show that native (un modified) LDL is unlikely to contribute to the development of atherosclerotic lesions. The lipoprotein may be transformed into an athero genic agent through several mechanisms, inclu ding acetylation, nonenzymatic glycation, and oxidation. This suggests that the oxidative modification of LDL may be of particular significance[7,8] and much more atherogenic than native LDL in many ways because it is taken up rapidly by macrophages.[24,25]

Atheroma, a chronic inflammation process, teams with cells including vascular wall cells, such as endothelium and smooth muscle, as well as infiltrating leukocytes.[5,34,25] It contains ceroid pigment, a complex of oxidized lipids and protein. Transformation of the fatty streak into the mature plaque occurs with foam cell necrosis. Necrosis occurs when the influx of ox-LDL exceeds the capacity of the macrophage scavenger receptor to take up LDL. Thus, the ox-LDL concentration in the subendothelial space increases rapidly. Ox-LDL is cytotoxic to macrophages[5,8], smooth muscle cells[8,34], and endothelial cells[2,3,12]. As it poisons foam cells, cell necrosis releases intracellular ox-LDL plus lysosomal enzymes and free radicals that further injure adjacent cells and interstitial components, both directly and inducing an inflammatory response.[3,17-19] (Figure 1)

Ox-LDL can cause endothelial damage and platelet aggregation, adherence and activa tion. Platelet degranulation releases platelet-derived growth factor (PGRF) and secretory products that augment oxidative modification of LDL and its subsequent uptake by macro phages.

The abnormal functions of cells within atherosclerotic lesions account for the impaired endothelial vasodilator dysfunction.[20] The exact mechanisms mediated in the cellular regulation remain ill-defined. It has been shown the specific adhesion molecules on the surface of endothelial cells that may induce the adhesive interactions significant to the athero sclerotic initiation. Vascular cell adhesion mole cule-1 (VCAM-1), a mononuclear leukocyte-selective adhesion molecule cells during early atherosclerotic lesion development in certain animal models[16], expressed by endothelium precedes monocyte recruitment.[5,13-16] Addi tionally, cytokines, the protein mediators of inflammation and immunity may have parti cular relevance in regulation of many aspects of vascular pathology in atherosclerosis. Exam ples of cytokines known to localise in human atheroma are TNF-α[5,43] and IL-1β[5,13,14]. IL-1β derived from vascular smooth muscle and endothelial cells may initiate local immune and inflammatory responses as well as potentiate the expression of adhesion molecules and chemoattractant cytokines, e.g. IL-8 or IL-1 itself, that can then recruit the phagocytes.[14] Thus, both ox-LDL and inflammatory media tors may evoke the expression by endothelial cells of the adhesive proteins that capture leukocytes and recruit them to the sites of lesion initiation.[5]

Moreover, a significant role in the atherogenesis is the inflammatory components that involve prostanoids, especially PGI_2, the major product in the endothelial cell, that also crucially modulates vascular tone in part by acting on endothelial and smooth muscle cells, platelet and leukocytes. Since PGI_2 is a potent inhibitor of platelet aggregation, leukocyte activation and adhesion, vascular smooth muscle contraction, migration and growth, and cholesterol ester accumulation in vascular cells, participating in hypersensitivity and suggested to be agonists for pro-thrombotic and pro-atherosclerotic process. Consequently, COXs which exist as COX-1 and COX-2, the central enzymes regulating the prostanoid synthesis from membrane-derived arachidonic acid, are also substantial in the pathogenesis. Particularly, COX-2, the inducible enzyme that can be rapidly up-regulated by a variety of biochemical stimuli, such as LPS[2,3], cyto kines[40], growth factor[36,37], and possibly ox-LDL.[15,16]

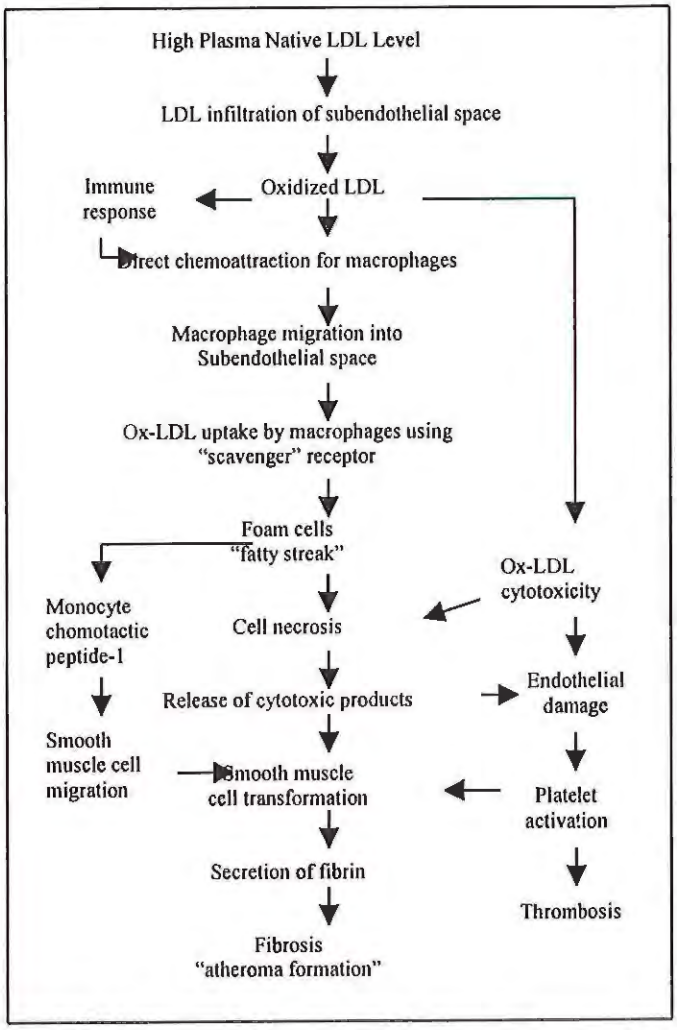

Figure 1 Postulated pathway of atherogenesis

Hence, changes in the endothelial function are particularly important since the endothelium regulates vascular tone by releasing the major vasoactive mediators including PGI_2, ET-1 and NO involved in relaxation and contraction, in coagulation and thrombus formation and in growth inhibition and stimulation.[2,3,26] This suggests that the damaged endothelial cells and subsequently impaired formation of vasoactive mediators deteriorate the regulation of vasomotor tone in coronary artery disease.

Oxidation and atherosclerosis

Reactive oxygen species: origins and consequences

It is now well-established that free radicals, such as superoxide ($O_2^{\bullet -}$), peroxyl radical ($HOO^{\bullet}$), lipid oxyl and peroxyl radicals derived from polyunsaturated fatty acids (PUFAs), hydroxyl radical ($HO^{\bullet}$), and other reactive oxygen species (such as H_2O_2) are continuously produced in vivo.[7,27] The term reactive oxygen species (ROS) is a collective one that includes not only oxygen-centered radicals such as $O_2^{\bullet -}$ and $OH^{\bullet}$ but also some nonradical derivatives of oxygen, such as hydrogen peroxide (H_2O_2), singlet oxygen Δg, and hypochlorous acid (HOCL).[24] $OH^{\bullet}$ is a very reactive species that can attack all biologi cal molecules and membrane proteins, usually setting off free-radical chain reaction by abstracting hydrogen from adjacent fatty acid side chains and so propagating the chain

reaction of lipid peroxidation.[19,28-30] Both $O_2^{\bullet-}$ and H_2O_2 are less harmful than $OH^{\bullet}$ because of far less reactive but can cause cellular damage if they are produced in excess.

The human body has a multipli city of different antioxidant defenses. However, anti oxidant defenses are not 100% effective. Consequently, depletion of antioxidant defenses and/or rises in ROS production can tip the ROS-antioxidant balance and cause oxidative stress, which may result in tissue injury, including damage to DNA, lipids and proteins in the human body.[30]

The relative importance of damage to different molecular targets in producing cell injury or death by imposing oxidative stress also depends on what degree of stress occurs, by what mechanism it is imposed, for how long, and the nature of the system stressed. For example, lipid peroxidation appears to be a highly significant consequence of oxidative stress in injured human arterial walls. How ever, most cells can tolerate mild oxidative stress, which often leads to increased synthesis of antioxidant defense systems.[24]

Oxidative modification of lipoproteins

The free radical chain reaction of LDL oxidation plays an important role in the progression of atherosclerosis.[29] (Table 1) Although, the oxidation hypothesis of athero sclerosis has not yet been definitely proved, evidence that oxidation is critical to athero sclerosis is substantial. Ox-LDL has been found in human atherosclerotic lesions, and increased titers of autoantibodies against ox-LDL are presented in plasma of patients with atherosclerosis. Studies using the antioxidants give further evidence of its support.[30,31]

In vitro studies, LDL can be oxidative ly modified by incubation with endothelial cells, smooth muscle cells or monocytes-macrophages in the presence of trace amounts of transition metal ions such as copper or iron as it is completely inhibited by metal chela tors.[18]

Lipid peroxidation is a chain reaction that proceeds in three stages. (Figure 2) Firstly, in the initiation phase, an oxygen free radical generated elsewhere (macrophage, polymorpho nucleocyte, etc.) reacts with a PUFA to produce a lipid radical ($L^{\bullet}$).[10-12,32] Secondly, in the pro pagation phase, the lipid radical reacts rapidly with molecular oxygen to form a peroxyl radical ($LOO^{\bullet}$), a chain reaction that is able to attack another PUFA molecule. Although the initial peroxyl radical is converted to a hydroperoxide (LOOH), this process produces a new carbon-centered peroxyl radical. Lastly, the propagation process continues and accele rates, consuming PUFAs and producing a corresponding quantity of hydro-peroxide. The chain reaction does not stop until the chain-carrying peroxyl meets and combines with another peroxyl radical to form inactive products.[1,27]

Stage 1. $L\text{-}H + OH^{\bullet} \rightarrow H_2O + L^{\bullet}$

2. $L^{\bullet} + O_2^{\bullet} \rightarrow LOO^{\bullet}$

3. $LOO^{\bullet} + LOO^{\bullet} \rightarrow$ inactive products

Figure 2 Lipid peroxidation stage.

It is postulated that ox-LDL can inhibit endothelium relaxations and promote endothelium contractions; the consequences are alteration in vascular tone leading to vasospasm and thrombus formation, generally found in patients with coronary artery disease.

Interestingly, peroxides and oxidized LDL might accelerate cyclooxygenase (COX) and lipoxygenase-catalyzed reactions in endo thelium, leading to enhanced formation of eicosanoids.[16,29,36] Eicosanoids, especially the cyclooxygenase metabolites of arachidonic acid, are also thought to play a role in the pathogenesis of atherosclerosis. The studies have demonstrated that the expression of COX-2 mRNA in cultured endothelial cells could be induced by ox-LDL possibly through the action of lipid hydroperoxides[37-39], addition ally the increase in COX-2 expression, known to occur under pathological conditions[2,40], was correlated with an increase in free radical catalyzed products of arachidonic acid, 8-epi prostaglandin $F_{2\alpha}$[38,39], the isoeicosanoids which may be formed by either COX-1 or COX-2.[39] Thus, oxidative stress caused by ox-LDL could have profound effects on vascular function by a variety of mechanisms including the imba lance of prostaglandins.[36-38]

Inactive Nitric Oxide (NO)

It is documented that NO is formed in endothelial cells, phagocytes and many other cell types, from a guanidino nitrogen of L-arginine by an oxidation reaction which can be catalyzed by NO synthase. Since NO has ability to relax vascular smooth muscle and inhibit aggregation and adhesion of platelets[1-3], therefore it is thought to play a central role in vascular homeostasis. Specifically in the role of atherogenesis, NO possibly inhibits throm bosis, cytokine-induced VCAM-1 expression, leukocyte adhesion to endothelium, and smooth muscle proliferation and migration. The ineffective action of NO has been found in atherosclerotic patients.[41]

Table 1 The potential atherogenic properties of ox-LDL.

1. Foam cell formation.[33]	The accumulated ox-LDL in macrophages that express abundant scavenger receptors for modified LDL results in foam cell formation.[33]
2. The exertion of chemotactic and cytotoxic activities.[18]	Ox-LDL stimulates the monocyte to penetrate into the subendothelial space of the arterial wall by directly itself and by indirectly mediating the expression of the VCAM-1 expressed on endothelial cells.[5,13] Thus, ox-LDL-induced the intimal accumulation of monocytes and smooth muscle cells as well as itself cytotoxicity can cause endothelial and smooth muscle cell damage.[33-35]
3. The production of damaging free radicals.[7]	Ox-LDL could involve releasing extracellular $O_2^{\bullet-}$ and H_2O_2 by endothelial cells, which then interact with transition metal ions, to form damaging species such as $OH^{\bullet}$, significantly starting off lipid peroxidation.[25-27]
4. The impaired production and inactivation of NO.[2,3,6]	Ox-LDL can interfere with the intracellular availability of L-arginine and inactivate NO that result in altering the endothelium response to NO, thus potentially inhibiting arterial relaxation.[2,35]
5. The stimulation of ET-1 secretion.[1,26]	ET-1 is a potent vasoconstrictor possibly involved in a variety of cardiovascular diseases.[1,2] The expression of ET-1 mRNA can be induced by ox-LDL in cultured aortic endothelial cells.[1,2,26]

In endothelial vascular dysfunction, inactive NO has been attributed in part to vascular oxidative stress since NO is readily inactivated by superoxide anion ($O_2^{\bullet-}$), mostly produced by phagocytes, subsequently genera ting peroxynitrite anion ($ONOO^-$)[10-12,42] :

$$O_2^{\bullet-} + NO \rightarrow ONOO^-$$

Additionally, $O_2^{\bullet-}$ can act as a vaso constrictor and $ONOO^-$ might not only be toxic itself but also it might decompose to give $OH^{\bullet}$.

Consequently, the decreased bioavai lability of NO may be due to either decreased production by damaged endothelium or increased degradation by oxygen-derived free radicals (especially $O_2^{\bullet-}$).[20]

Additonal effects of oxidation

In the previous studies, in addition to ox-LDL, cytokines, e.g. IL-1β and TNFα, have been shown to exert oxidative stress, probably by inducing the synthesis of oxygen radicals in both lymphoid and nonlymphoid cells[43], consequent leading to oxygen radical induced injury of the vascular endothelium. It has been established that cytokines-induced oxidative stress may modulate intracellular regulatory signal by redox-sensitive signal

transduction pathways involved in athero sclerosis. Hence, an increase in the endothelial cell oxidative state may sensitize the vascula ture to otherwise physiologic signals resulting in abnormally elevated expression of adhesion molecules such as VCAM-1 and other gene products involved in the inflammatory response including COX-2 and iNOS expres sion.[16,37,43]

The role of antioxidant defense in endothelial vascular dysfunction

The endogenous oxygen species produced by the cells present in the arterial wall may cause oxidative damage to cellular components altering endothelial cell function as a crucial role in atherogenesis. Recent findings have suggested a possibility for pharmacological and nutritional antioxidants in the prevention of atherosclerosis.[23,24,44,45]

Antioxidants can be defined as sub stances whose presence in relatively low concentrations significantly inhibits the rate of oxidation of that substrate. The available for therapeutic use can be conveniently divided into natural (physiological) antioxidants nor mally present in the body, and synthetic compounds with antioxidant activity.[44]

Some antioxidants are synthesized in the human body including enzymes (such as superoxide dismutase, catalase, and glutathi one peroxidase) and nonenzymatic antioxidant free radical scavengers (such as α-tocopherol, ascorbic acid, β-carotene). These native tissue antioxidants form a synergistic, multilevel defense system against free radical injury.[7] Removal of excess $O_2^{\bullet-}$ by intracellular super oxide dismutase (SOD) enzymes is an im portant physiological antioxidant defense mechanism.[26] In endothelial cells, both catalase and glutathione peroxidase enzymes are involved in removal of hydrogen peroxide (H_2O_2). Large amounts of H_2O_2 are cytotoxic to cell types, and endothelial cells are no exception because endothelial cells can be killed by high concentrations of H_2O_2 by mechanisms that involve damage to DNA and proteins caused by free radicals and an increased concentration of intracellular free calcium ions.[25] However, low concentrations of H_2O_2 are efficiently dealth with by catalase and glutathione peroxidase, the enzymes that metabolise H_2O_2 in the endothelium.

As a result of superoxide that can be generated by endothelial and phagocytic cells, whether endothelial cells release superoxide all the time in vivo, or whether only after an insult (such as ischemia and reperfusion) is unknown. Because of the damaging superoxide that reacts quickly with NO to form $ONOO^-$, consequently superoxide antagonises the vaso dilatory action of NO as well as superoxide dismutase, an intracellular antioxidant enzyme that scavenges superoxide, prolongs the life of NO.[11,12,20]

Moreover, oxidative stress-modulated intracellular redox sensitive regulatory mecha nisms in the endothelial cell may play a crucial role in differentially modulating the expression of the genes participated in the inflammatory responses such as VCAM-1, affecting mono nuclear leukocyte accumulation, including COX-2 , the inducible enzyme that produces prostanoids and iNOS, regulating NO synthe sis.

Therefore, both generally and locally in tissue, the balance between free radical production and the multilevel defense system may be critical. In according to the evidence that endogenous antioxidants do not complete ly prevent ROS-induced oxidative stress in the human body, consequently efficient repair systems are needed and supplementing natural antioxidant defenses may retard oxidative damage.[27,30]

It is hypothesized that dietary antioxi dants may prevent development and slow progression of atherosclerosis by several species. LDL oxidation has been established to be an important step in the atherogenesis, it may be beneficial that enhancing the endoge nous antioxidant defense systems within the LDL particle might decrease oxidation of LDL and slow the atherogenic process without producing undesirable effects.[22,23] Additional role of antioxidants has been expanded on the notion of its effect as a potential intracellular regulatory signal that modulates the expression of significant inflammatory genes, such as IL-1β and TNFα.[16,37] In according to a therapeu tically important feature of antioxidants in atherosclerosis that may be due to direct alterations in the metabolism and function of endothelial, smooth muscle, and inflammatory cells[43], consequently, in particular, the endo genous defense systems may be improved by micronutrient supplementation with lipophilic antioxidants such as α-tocopherol and β-carotene, or by supplementing the aqueous phase antioxidant capacity with ascorbic acid. In several animal species, the dietary admini stration of various antioxidant compounds reduces the susceptibility of LDL to oxidation

and retards the development of atherosclero sis.[45-48] Epidemiologic data also suggests that decreased levels of micronutrient antioxidants are associated with an increased frequency of cardiovascular disease.

Ascorbic acid (vitamin c) as antioxidant defense

Ascorbic acid is the main water-soluble chain-breaking antioxidant in human plasma, reacts with superoxide, hydroxyl radi cals, and singlet oxygen, additionally sparing other endogenous antioxidants (especially α-tocopherol) from comsumption.[48] It is the only endogenous antioxidant in plasma capable of completely inhibiting oxidative modification of LDL by aqueous peroxyl radicals. Indeed, it has been shown that the antioxidant capacity of the LDL particle can be accounted for by α-tocopherol. There is, however, a synergistic interaction between ascorbic acid and α-toco pherol, and α-tocopherol can be regenerated from the corresponding radical ($T^{\bullet}$) by ascor bic acid.[27-30]:

$$T^{\bullet} + \text{ascorbate} \rightarrow TH + \text{ascorbate}^{\bullet}$$

The ascorbate free radical, semi dehydro-ascorbate, is a rather stable and almost unreactive metabolic intermediate between ascorbic and dehydroascorbic acid. Two molecules of ascorbate free radical can be converted by disproportion into ascorbate and dehydroascorbate. Thus, propagation of free radical reaction is stopped without any toxic free radical remainder.[17] In the previous studies, ascorbic acid can compensate for reduced level of lipophilic antioxidants.[10,11,17,35]

The current evidence suggests that ascorbic acid more effectively prevents lipid peroxidation than any other endogenous antioxidant in plasma and LDL, including α-tocopherol, the major lipid-soluble antioxidant in humans.[18-20] The effect of ascorbic acid may be due to interference with either LDL oxida tion or a process that is induced by ox-LDL.

It is possible that low levels of ascor bic acid in the arterial wall may predispose LDL to oxidation, which could promote athero sclerosis. Also, the results of several epidemio logic studies support a role for low plasma ascorbic acid levels in atherosclerosis. A significant inverse relationship was found between plasma ascorbic acid and coronary artery disease mortality[3] as well as human subjects with low ascorbic levels have been reported to have higher amounts of lipid peroxides in plasma than do subjects with high ascorbic acid levels. Moreover, previous studies have suggested that ascorbic acid could be improve endothelial vasomotor dysfunction in hypercholesterolemic patients.[27]

Interestingly, in human endothelial cells stimulated to generate radicals, ascorbic acid recently has been shown to inhibit vascular cell adhesion molecule-1 (VCAM-1) induction. VCAM-1, which can be stimulated by ox-LDL and inflammatory mediators including IL-1β, lipopolysaccharide (LPS), and TNFα[5,14,37], can enhance monocyte adhesiveness to endothelium and subsequently induces inflammatory responses in the vessel wall involved in atherosclerosis.

Furthermore, one crucial pathogenesis responsible for atherosclerosis is the inducible COX-2 exerting abnormal prostanoid produc tion.[16,21,36] It has been documented that the antioxidant scavenger may modulate in the signaling pathway, induced by IL-1β and TNFα, that influences, either directly or indirectly, on some eukaryotic initiation factors regulating COX-2 and iNOS expres sion in rat mesangial cells.[37] This also has been supported by our recently work showing that ascorbic acid could inhibit IL-1β-induced COX-2 expression in cultured human umbili cal vein endothelial cell (HUVEC)[21]. There fore, the endothelial vascular dysfunction mediated by the imbalance prostanoids would be ameliorated by ascorbic acid. It is possible that ascorbic acid may be a novel approach for intervention in cytokine-induced inflammatory processes in atherosclerosis.[16,37]

How ascorbic acid might impact on athero-sclerosis.

<u>On LDL oxidation:</u>

Ascorbic acid has an inhibitory effect on LDL oxidation by either scavenging peroxy radicals directly or acting synergistically with the α-tocopherol endogenous to the particle, additionally by regenerating the reduced form of α-tocopherol back to the active tocopherol form. The LDL protected by ascorbic acid was not taken up and degraded by macrophages via the scavenger receptor mechanism.[18]

Ascorbic acid treatment has been found to increase LDL resistance to oxidation in vitro in rats that have an iron overload because ascorbic acid has a significant inhibitory effect on LDL oxidation mediated

by metal ion, such as coppper ion.[9-11,18] Ascorbic acid also preserved the endogenous lipophilic antioxidants within the LDL particles (α- and γ-tocopherol, and β-carotene) during oxidation with copper.[2]

On endothelium-derived relaxing factor (or NO) action:

Ascorbic acid has been shown to be an efficient scavenger of $O_2^{\bullet-}$, including HOCL and $ONOO^-$.[17,19,22-24] This ability provides one possible explanation for the beneficial effects of ascorbic acid on endothe lial function since superoxide anion has ability to react rapidly with NO and limit the biologi cal activity of NO.[11,12,20,25]

Ascorbic acid may also improve endothelial function by the role in the regula tion of intracellular redox state, by sparing intracellular glutathione from oxidative degradation. Glutathione is an important source of intracellular reduced thiols and can be degraded by oxidation to glutathione disulfide. Under conditions of increased oxidative stress, depletion of reduced thiol leads to decreased synthesis of NO in cultured endothelial cells. Thus, prevention of glutathione oxidation and therefore increase of the availability of reduced thiol by intracellular ascorbic acid could improve NO action.[27,43]

On additional actions:

In according to a significant initiating mechanism in atherosclerosis is the increased interaction of monocytes with endothelial cells activated to synthesize adhesion molecules, interestingly, ascorbic acid may have ability to inhibit VCAM-I induction and monocyte adhesion in damaged human endothelium in the mechanism by which ascorbic acid interferes with P-selectin translocation to the endothelial cell surface in response to radicals.[35] Additionally, it has been established in the activated endothelial cell that VCAM-1 gene expression by cytokine and noncytokine inducers is regulated by a redox-sensitive signal transduction pathway sensitive to inhibition by antioxidant, potentially regula ting intracellular redox state[43], possibly as ascorbic acid.

Furthermore, COX pathway evidently is also a source of free radical $O_2^{\bullet-}$, which can potentiate endothelium-dependent contractions either by inactivating NO or by direct effects on vascular smooth muscle[3]. Additionally, the effect of free radicals initiating lipid hydropero xide on membrane-derived arachinodic acid is relevant to impaired prostanoid synthesis. In another important evidence, reactive oxidative intermediates (ROI)-mediated intracellular redox state has also influenced COX-2 expres sion.[35] These suggest that vascular oxidative stress can modulate COX-2 pathway. Since ROI produced by the mitochondrion, a site where excessive production of radicals apparently occur during oxidative stress, may mediate TNF and IL-1 signaling and up-regulate the proinflammatory genes of COX-2 and iNOS, therefore the scavenging antioxi dants may involve in redox-sensitive inhibition of cytokine-induced COX-2 and iNOS expres sion.[37] In according to the beneficial effects of ascorbic acid to detoxify reactive oxygen species and to regulate endothelium intracellular redox state, hence ascorbic acid may interfere COX-2 pathway by inhibiting ox-LDL-induced lipid hydroperoxide and by interfering with ROI-modulated intracellular redox state resulting in COX-2 expression.[35,37]

Considering oxidative stress, elicited by ROI releasing from damaged endothelium, that can be induced by cytokine and ox-LDL, therefore ascorbic acid may be prospective to atherosclerosis not only in the role of ox-LDL-induced but also in cytokines-stimulated endo thelial injury. Thus, it has been postulated that a potential molecular linkage between the redox state-modulated signaling pathways of the vascular endothelial cell and abnormal expression of the genes responsible for inflam matory process, e.g. VCAM-1, COX-2 and iNOS[35], is possibly notable for oxidation as a significant intracellular regulatory signal observed in damaged endothelium early in the pathogenesis of atherosclerosis. In particular, ascorbic acid would be specific regulatory factors that tranduce metabolic signals (i.e., oxidation) into nuclear regulatory signals (i.e., expression of adhesion molecule genes) that may have significant therapeutic implications in atherosclerosis.[43]

Recommended intakes of ascorbic acid

Worldwide recommended intakes for ascorbic acid are 30-100 mg/day[7,8,24], which can achieve plasma ascorbic acid levels approximately 30-150 μmol/L[28,45], based on maintenance of adequate body reserves that preclude classical scorbutic symptoms.[7,8,24] In smokers receiving up to 1.5 g/day of supple mental ascorbic acid, serum lipids were protected from oxidation.[7] Several epidemio

logic studies concluded that plasma concentra tions of ascorbic acid approximately 50 μ mol/L are associated with decreased risk of cardiovascular disease.[10,19,46] At such concen trations, easily achievable by diet alone, can strongly inhibit LDL modification.[10] Similar to the other antioxidants, There is little evidence of toxicity for even multigram daily doses of ascorbic acid.[19,29] However, large doses of 1-5 g/day may cause diarrhea, nausea and vomiting. Large doses may also result in precipitation of oxalate, cysteine, or urate renal stones if the urine becomes acidic during therapy. Oxalate also accumulates in patients with chronic renal failure and those receiving long-term hemodia lysis, and these individuals should be cautioned against taking large doses of ascorbic acid.[8] Ascorbic acid can be prooxidant in vitro rather than antioxidant in the presence of transition metal ions, mixing ascorbic acid with iron ions can cause $OH^{\bullet}$ generation and lipid peroxida tion.[18,30,31] Thus, ascorbic acid is contra indicated in patients with hemochromatosis and other conditions characterized by iron overload.[8]

Although higher ascorbic acid intakes may be recommended for certain groups such as smokers and those under a variety of stresses, the present data suggests that antioxidant protection is best served by the variety of antioxidant substances found primarily in fruit and vegetables[24], additionally dietary guide lines are better for a public health strategy than recommendation of specific nutrients intakes.[22,48]

CONCLUSION

Since various mechanisms evidently contribute to atherogenesis, in studying isolated human plaque, one has access only to a single time point in what is a complicated disease process with ongoing, stage-dependent events. It has been establishd that endothelium, a crucial vascular structure, produces mediators and vasoactive substances regulating vascular tone apparently impaired in atherosclerotic patients, consequently endothelial damage is strongly thought to initiate atherosclerotic lesion.[1-3] Oxidative modification of LDL hypo thesis as well as cytokines-modulated inflam matory process account for endothelial injury in the pathogenesis.[7-12] The reactions involving free radicals, particularly the oxidation of LDL in vessel walls, seem to be an important early step in atherogenesis because ox-LDL promotes the development of foam cells and endothelial injury before the establishment of organized arterial plaque.[4-8,46,47] The several mechanisms have been shown that ox-LDL and inflamma tory mediators, such as IL-1β and TNFα, are major contributors to the adherence of mono cytes to endothelium.[6,13-16] Moreover, both monocytes and macrophages within the vessel wall can generate superoxide and hydrogen peroxide. Peroxynitrite is formed by the interaction of superoxide and NO resulting in inactive NO and consequent endothelial vasodilator dysfunction.[11,20,41,42] Also, the imbalance prostanoids, regulated by COX-2, crucially participating in vascular tone dysfunction, can be induced by inflammatory mediators. Apparently, in addition to cytokines, ox-LDL-stimulated oxidative stress presumably mediates COX-2 pathway leading to abnormal prostanoid synthesis detected in atherosclerotic patients.[16,36-39] Thus, it is very difficult to discern the possible sequence of factors contributing to oxidative stress, inflammatory processes and endothelial damage. However, if oxidative LDL hypothe sis is true, antioxidant substances are likely to exert their effects at the earliest stages of the atherogenic process. Theoretically, the role of antioxidants should be evaluated with benefit to the development of preclinical disease.[22-24,27] A large body of experimental and epidemio logical data indicates that antioxidant vitamins may be able to reduce atherosclerosis.[45-48] For the most part, three antioxidants have been studied: ascorbic acid, α-tocopherol, and β-carotene. All three have been shown to diminish the susceptibility of LDL to oxidation in vitro. However, the role of dietary antioxidants in human atherosclerotic disease remains inconclusive, additionally epidemio logical data concerning the role that these substances may play in atherosclerotic disease are in-consistent with regard to both plasma levels and dietary intake.[7,8]

Ascorbic acid, an outstanding antioxi dant in human plasma, effectively protects against oxidative stress, caused by ox-LDL and possibly cytokines, e.g. IL-1β.[17-20] On account of its actions in scavenging free radicals, ascorbic acid can increase LDL resistance to oxidation, potentially preventing lipid peroxidation as well as regulate intra cellular redox state, possibly improving NO activity.[20,28] Furthermore, it may also inhibit ox-LDL and cytokines-stimulated monocyte adhesion.[35,41] In additional interesting effect of ascorbic acid is that it may modulate in the signaling pathway induced by cytokines that

up-regulate COX-2 expression[16,37], supported by the evidence of decreased COX-2 expres sion inhibited by ascorbic acid in IL-1β-treated HUVEC.[21] Thus, this suggests that ascorbic acid not only prevents the oxidation of LDL but itself alone may maintain the function of endothelial cells by other mechanisms, including inhibiting inflammatory process responsive to cytokines and COX-2, that should be explored for further advantage. However, how much is optimal is another unanswered question. Laboratory findings may not have relevance to free-living humans. Observational epidemiologic studies can not exclude the possibility that people who consume antioxidant-rich diets or who take vitamin supplements also share other lifestyle or dietary practises that actually justify their lower disease rates.[25] The only way to determine reliably whether antioxidants play any role in reducing the risk of cardiovascular disease is to conduct large-scale[22,44,45], randomized controlled clinical trials in asymp tomatic and symptomatic individuals before public health recommendations concerning the use of ascorbic acid supplementation for coronary heart disease prevention can be made.

REFERENCES

1. Haller H. Endothelial function: general considerations. *Drugs* 1997; 53 (suppl.): S1-S10.
2. Luescher TF, Tanner FC. Endothelial regulation of vascular tone and growth. *A J H* 1993; 6: 283S-293S.
3. Vane JR, Botting RM. Regulatory mecha nisms of the vascular endothelium: an update. *Pol J Pharmacol* 1994; 46: 499-521.
4. Henderson A. Coronary heart disease: over view. *Lancet* 1996; 348 (suppl.): S1-S4.
5. Libby P. Atheroma: more than mush. *Lancet* 1996; 348 (suppl.): S4-S7.
6. Zeither AM. Endothelial vasodilator dys function: pathogenic link to myocardial ischaemia or phenomenon? *Lancet* 1996; 348 (suppl.): S10-S12.
7. Meyers DG, Maloley PA. *Pharmaco-therapy* 1993; 13(6): 574-582.
8. Odeh RM, Cornish LA. *Pharmaco-therapy* 1995; 15(5): 648-659.
9. Suarna C, Dean TR, May J, et al. Human atherosclerotic plaque contains both oxidized lipids and relatively large amounts of α-tocopherol and ascorbate. *Arterioscler Thromb Vasc Biol* 1995; 15: 1616-1624.
10. Martin A, Frei B. Both intracellular and extracellular vitamin C inhibit atheroge nic modification of LDL by human vascular endothelial cells. *Arterioscler Thromb Vasc Biol* 1997; 17: 1583-1590.
11. Heitzer T, Just H, Munzel T. Antioxidant vitamin C improves endothelial dysfunc tion in chronic smokers. *Circulation* 1996; 94: 6-9.
12. Plotnick GD, Corretti MC, Vogel RA. Effect of antioxidant vitamins on the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high-fat meal. *JAMA* 1997; 278: 1682-1686.
13. Dinarello CA. Biologic basis for interleu kin-1 in disease. *Blood* 1996; 87: 2095-2147.
14. Schoenbeck U, Mach F, Bonnefoy J, et al. Ligation of CD40 activates interleukin 1β-converting enzyme (caspase-1) activi ty in vascular smooth muscle and endo thelial cells and promotes elaboration of active interleukin 1β. *J Biol Chem* 1997; 272: 19569-19574.
15. Maier JAM, Barenghi M, Bradamante S, et al. Modulators of oxidized LDL-induced hyperadhesiveness in human endothelial cells. *Biochem Biophys Res Commun* 1994; 204: 673-677.
16. Topper JN, Cai J, Falb D, et al. Identifi cation of vascular endothelial genes differentially responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. *Proc Natl Acad Sci* 1996; 93: 10417-10422.
17. Freyschuss A, Xiu R, Zhang J, et al Vitamin C reduces cholesterol-induced microcirculatory changes in rabbits. *Arterioscler Thromb Vasc Biol* 1997; 17: 1178-1184.
18. Retsky KL, Freeman MW, Frei B. Ascor bic acid oxidation product(s) protect human low density lipoprotein against atherogenic modification: anti-rather than prooxidant activity of vitamin C in the

presence of transition metal ions. *J Biol Chem* 1993; 1304-1309.
19. Halliwell B. Ascorbic acid: hype, hoax, or healer? *Am J Clin Nutr* 1997; 65: 1891-1892.
20. Ting HH, Timimi FK, Haley EA, et al. Vitamin C improves endothelium-depen dent vasodilation in forearm resistance vessels of humans with hypercholesterole mia. *Circulation* 1997; 95: 2617-2622.
21. Ketsawatsakul U, Akarasereenont P, Techatraisak K, et al. Effects of ascorbic acid on the expression of cyclooxygenase isoforms in interleukin-1β treated human umbilical vein endothelial cells. Abstract book, 38th Siriraj Scientific Congress, Siriraj Hospital, 2nd –6th March 1998; p162-163.
22. Hennekens CH, Gaziano JM, Manson JE, et al. Antioxidant vitamin-cardiovascular disease hypothesis is still promising, but still unproven: the need for randomized trials. *Am J Clin Nutr* 1995; 62 (suppl.): S1377-S1380.
23. Kritchevsky SB, Shimakawa T, Tell GS, et al. Dietary antioxidants and carotid artery wall thickness: the ARIC study. *Circulation* 1995; 92: 2142-2150.
24. Jacob RA, Burri BJ. Oxidative damage and defence. *Am J Clin Nutr* 1996; 63: S985-S990.
25. Halliwell B. Free radicals and vascular disease: how much do we know? *BMJ* 1993; 307: 885-886.
26. Chen T, Tseng H, Yang J, et al. Effect of antioxidant in endothelial cells exposed to oxidized low density lipoproteins. Life science 1998; 62: 277-282.
27. Halliwell B. Antioxidants in human health and disease. *Annu Rev Nutr* 1996; 16: 33-50.
28. Levine GN, Frei B, Koulouris SN, et al. Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease. *Circulation* 1996; 93: 1107-1113.
29. Darley-Usmar V, Halliwell B. Blood radicals: reactive nitrogen species, reactive oxygen species, transition metal ions, and the vascular system. *Pharmaceu tical Research* 1996; 13: 649-657.
30. Halliwell B, Chirico S. Lipid peroxida tion: its mechanism, measurement, and significance. *Am J Clin Nutr* 1993; 57 (suppl.): S15-S25.
31. Halliwell B. Antioxidant characteriza tion: methodology and mechanism. *Biochem Pharmcol* 1995; 49: 1341-1348.
32. Iuliano L, Colavita AR, Camastra C, et al. Protection of low density lipoprotein oxidation at chemical and cellular level by the antioxidant drug dipyridamole. *Br J Pharmacol* 1996; 119: 1438-1446.
33. Halliwell B. Oxidation of low-density lipoprotein: questions of initiation, propa gation, and the effect of antioxidants. *Am J Clin Nutr* 1995; 61 (suppl.): S670-S677.
34. Naito M, Hayashi T, Iguchi A. New approaches to the prevention of athero sclerosis. *Drugs* 1995; 50: 440-453.
35. Weber C, Erl W, Weber K, et al. Increased adhesiveness of isolated monocytes to endothelium is prevented by vitamin C intake in smokers. *Circulation* 1996; 93: 1488-1492.
36. Wohlfeil ER, Campbell WB. 25-hydroxy cholesterol enhances eicosanoid produc tion in cultured bovine coronary artery endothelial cells by increasing prosta glandin G/H synthase-2. *Biochim Biophys Acta* 1997; 1345: 109-120.
37. Tetsuka T, Baier LD, Morrison AR. Antioxidants inhibit interleukin-1-induced cyclooxygenase and nitric-oxide synthase expression in rat mesangial cells. *J Biol Chem* 1996; 271: 11689-11693.
38. Klein T, Reutter F, Schweer H, et al. Generation of the isoprostane 8-epi-prostaglandin $F_{2\alpha}$ in vitro and in vivo via the cyclooxygenases. *J Pharmacol Exp Ther* 1997; 282: 1658-1665.
39. Delanty N, Reilly M, Pratico D, et al. 8-Epi $PGF_{2\alpha}$: specific analysis of an isoeico sanoid as an index of oxidant stress in vivo. *Br J Clin Pharmacol* 1996; 42: 15-19.
40. Akarasereenont P. The inducible isoform of cyclo-oxygenase (COX-2): new target for antiinflammatory therapy. *Thai J Pharmacol* 1997; 19: 33-42.
41. Vita JA, Frei B, Holbrook M, et al. L-2-oxothiazolidine-4-carboxylic acid reverses endothelial dysfunction in patients with coronary artery disease. *J Clin Invest* 1998; 101: 1408-1414.
42. Eiserich JP, Hristova M, Cross CE, et al. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxi dase in neutrophils. *Nature* 1998; 391: 393-397.

43. Marui N, Offermann MK, Swerlick R, et al. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an anti oxidant-sensitive mechanism in human vascular endothelial cells. *J Clin Invest* 1993; 92: 1866-1874.
44. Eiserich JP, Vliet A, Handelman GJ, et al. Dietary antioxidants and cigarette smoke-induced biomolecular damage: a complex interaction. *Am J Clin Nutr* 1995; 62 (suppl.); s1490-s1500.
45. Azen SP, Qian D, Mack WJ, et al. Effect of supplementary antioxidant vitamin intake on carotid arterial wall intima-media thickness in a controlled clinical trial of cholesterol lowering. *Circulation* 1996; 94: 2369-2372.
46. Maxwell SRJ. Prospects for the use of antioxidant therapies. *Drugs* 1995; 49: 345-361.
47. Jialal I, Fuller CJ. Oxidized LDL and anti oxidant. *Clin Cardiol* 1993; 16 (suppl.): S6-S9.
48. Duell PB. Prevention of atherosclerosis with dietary antioxidants: fact or fiction? *J Nutr* 1996; 126: 1067-1071.