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Antioxidant Vitamins in Atherosclerosis – Animal Experiments and Clinical Studies

Witaminy antyoksydacyjne w miażdżycy – doświadczenia na zwierzętach i badania kliniczne

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Abstract

Atherosclerotic heart diseases are universal problems in modern society. Oxidative damage to lipids is a primary cause of atherosclerosis. There are many choices for treatment, but no definite recommendations to prevent the occurrence of the disease. There is a relationship between atherosclerotic risk factors and increased vascular production of reactive oxygen species (ROS). Oxidized low-density lipoproteins (LDL) and ROS may directly cause endothelial dysfunction by reducing endothelial nitric oxide (NO) bioavailability. Vitamin E can to some degree prevent the consequences of oxidized LDL, and vitamin C provides NO synthase activity. Although prolonged use of vitamin A, C, and E supplementation in pharmaceutical forms has been proven to be effective in preventing atherosclerosis in animal experiments, this has not yet been demonstrated in clinical trials with human beings. It should be taken into account that the evidence has been gathered from young/adult experimental animals with early stages of atherosclerosis and from in-vitro studies, while most of the clinical trials have involved older patients with late stages of the disease. Prolonged use of vitamins in the diet has not yet been recommended in human beings. There is some indication that a diet rich in antioxidant fruit and vegetables may be beneficial in the prevention of cardiovascular events (*Adv Clin Exp Med* 2012, 21, 1, 115–123).

Key words: antioxidant, vitamin A, C, E, folate, flavonoid, atherosclerosis.

Streszczenie

Miażdżycy układu krążenia jest powszechnym problemem współczesnego społeczeństwa. Oksydacyjne uszkodzenie lipidów jest główną przyczyną miażdżycy. Istnieje wiele możliwości leczenia, ale nie ma określonych zaleceń, jak zapobiec wystąpieniu choroby. Istnieje związek między czynnikami ryzyka miażdżycy a wzrostem wydzielania reaktywnych form tlenu (ROS) przez naczynia. Utlenione lipoproteiny o małej gęstości (LDL) i ROS mogą bezpośrednio powodować dysfunkcję śródbłonną, zmniejszając biodostępność tlenku azotu (NO) w śródbłonnku. Witamina E może do pewnego stopnia zapobiegać skutkom utlenionych LDL, a witamina C zapewnia aktywność syntazy NO. Chociaż długotrwałe stosowanie suplementacji witamin A, C i E w postaci farmaceutycznej okazało się skuteczne w zapobieganiu miażdżycy u zwierząt doświadczalnych, to jeszcze nie sprawdzono tego w badaniach klinicznych na ludziach. Należy wziąć pod uwagę, że dowody zostały zebrane w badaniach na młodych i dorosłych zwierzętach doświadczalnych na wczesnych etapach choroby oraz w badaniach *in vitro*, podczas gdy w większości badań klinicznych brali udział starsi pacjenci na zaawansowanym etapie choroby. Długotrwałe stosowanie witamin w diecie nie jest jeszcze zalecane u ludzi. Istnieją pewne przesłanki, że dieta uwzględniająca bogate w przeciwutleniacze owoce i warzywa może być korzystna w zapobieganiu incydentom sercowo-naczyniowym (*Adv Clin Exp Med* 2012, 21, 1, 115–123).

Słowa kluczowe: przeciwutleniacze, witaminy A, C, E, kwas foliowy, flawonoidy, miażdżycy.

Cardiovascular disease (CVD) is a universal problem in modern society. Atherosclerosis is the most important manifestation of CVD. The treatment and prevention of CVD are perennial subjects of investigation in cardiovascular medicine. Investigations of treatment choices have been targeted for millennia and successful advances have been achieved in recent decades. Thanks to developments in the treatment of CVD, human beings live longer than ever before. Current knowledge includes effective percutaneous and/or medical management of coronary disease and heart failure [1]. The beneficial effects of medical treatments may be related to their known antioxidant properties [2]. Drugs that may have antioxidant properties include nitric oxide (NO), calcium channel blockers, statins, dobutamine and some angiotensin-converting enzyme (ACE) inhibitors.

The occurrence of any CVD could be associated with dietary components. According to recent literature, there are no definite guidelines for preventing the occurrence of atherosclerosis. Routine supplementation with antioxidants such as vitamins (A, C, and E) and minerals (zinc and selenium) has not yet been recommended for human beings, although beneficial effects in preventing cardiovascular risk factors have been shown in animal experiments [3]. The efficacy of natural antioxidants in human patients with cardiovascular risk factors has not been fully explained with regard to pathophysiological features. The antioxidative effects of fruit and vegetable intake are being investigated in human diseases, especially cardiovascular events [4, 5]. Bourassa and Tardif wrote that "a diet rich in antioxidant macro-nutrients, particularly fruits and vegetables, is recommended for all individuals, and some types of diets such as the Mediterranean diet have been shown to be highly beneficial in the prevention of cardiovascular events in patients with coronary heart disease" [3]. However, evidence that fruit and vegetable consumption prevents any atherosclerotic event remains limited.

Atherosclerotic risk factors are associated with increased oxidative stress, implicated in the vascular production of lipid oxidation and reactive oxygen species (ROS; superoxide, hydrogen peroxide, and their metabolites) [6]. ROS, known to be cytotoxic and mutagenic agents, induce an oxidative stress response. Landmesser and Drexler noted that "hypercholesterolemia, hypertension, diabetes and smoking increase the vascular production of ROS, in particular superoxide" [7]. It has recently been demonstrated in experimental designs for smoking that vitamin E and plant extracts (flavonols, tocopherols and carotenoids) can protect the heart from nicotine-induced oxidative stress [8].

In this review, the authors focus first on possible mechanisms for the occurrence and prevention of atherosclerosis. Reports of the use of antioxidants for treatment and prevention in both animal and human studies have been reviewed. There have been numerous reports about antioxidants in experimental animals, human epidemiological observations and clinical trials for almost half a century. Here, the most relevant papers published within the last two decades have been reviewed, in order to present the current state of knowledge.

The Oxidation Hypothesis for the Occurrence of Atherosclerosis

Endothelial dysfunction that results in atherosclerosis may be associated with vessel-wall thickening as a result of a build-up of fatty materials and plaques. The mechanisms of oxidative stress and antioxidants in patients with atherosclerosis have been evaluated in extensive animal experiments and clinical research. There is a relationship between atherosclerotic risk factors (ARF) and increased vascular production of ROS; the most important ARFs are heredity, age, hypertension, dyslipidemia, diabetes and smoking [7]. Increased vascular production of ROS, particularly superoxide, has been shown in in-vitro studies [9, 10]. ROS and reactive nitrogen species (RNS) are closely linked to the disease process. Incomplete scavenging of ROS and RNS influences the mitochondrial lipid cardiolipin, stimulates the release of mitochondrial cytochrome c and finally activates the intrinsic death pathway [6]. Local generation of RNS may contribute to vascular tissue injury. Therefore, ROS and RNS participate as signaling molecules that regulate diverse pathophysiological signaling pathways. High levels of ROS are potent inducers of the intrinsic apoptotic pathway and tissue injury in pathophysiological conditions as an integral part of atherosclerotic plaque stabilization. The most important sources of ROS production associated with CVD pathology are the mitochondrial respiratory chain, nicotinamide adenine dinucleotide phosphate oxidases, xanthine oxidase, lipoxygenase, uncoupled nitric oxide synthase and myeloperoxidase [11].

Oxidative damage to lipids is a primary cause of atherosclerosis [7]. Lipid peroxidation directly produces oxidative stress initiated in the presence of hydroxyl radicals, resulting in the production of malondialdehyde [12]. To elucidate the potential mechanisms of lipid oxidations, all types of oxidative systems have been suggested as contributing to the process. Oxidized low-density lipoprotein

(LDL) is present in atherosclerotic plaques and is involved in the transition of stable atherosclerotic lesions into active lesions. The precise mechanisms involved have not been fully explained, but oxidation of LDL is believed to play a role in the initiation of atherosclerosis, leading to LDL uptake by macrophages and foam-cell formation, but becoming less important in the later stages of the condition [13, 14]. Incubation of macrophages with oxidized LDL causes intracellular cholesterol ester accumulation. Increased oxidative stress, evidenced by lipid peroxidation, has been found in human patients with ARF [15, 16]. Gul et al. found that the “rat heart has sufficient antioxidant enzyme capacity to cope with exercise-induced oxidative stress, and adaptive changes in antioxidant enzymes due to endurance training are limited” [17].

It is obvious that LDL oxidation alone does not explain the complex mechanism of oxidative stress and atherosclerosis. A mechanism involving oxidized LDL and NO has been proposed regarding the occurrence of atherosclerosis [6]. Oxidized LDL stimulates the release of interleukin-1 from endothelial cells and macrophages [18]. In addition, NO causes vasodilatation and inhibits the effects of cytokines and adhesion molecules related to atherosclerosis [19]. As Landmesser and Drexler noted: “Whereas oxidized LDL may contribute to endothelial dysfunction [7] oxygen radicals may directly cause endothelial dysfunction, i.e. by reducing endothelial NO bioavailability. In particular, superoxide reacts rapidly with NO, resulting in formation of peroxynitrite and loss of NO’s bioactivity [7]. ROS, and especially peroxynitrite, can oxidize tetrahydrobiopterin, a critical co-factor for endothelial NO synthase” [7, 20, 21, 22]. In the occurrence of atherosclerosis and heart ischemia, NO bioavailability plays an important role in preventing endothelial dysfunction and protecting against oxygen radicals. Borutaite et al. found that “NO rapidly protects the ischemic heart from apoptosis and mitochondrial dysfunction via PKG-mediated blockage of mitochondrial permeability transition and cytochrome c release” [23]. Thus the oxidation of LDL apparently triggers atherogenesis, which leads to atherosclerosis [24, 25]. The pathogenesis of atherosclerosis is further related to inflammation, immune response and the proliferative process. Endothelial denuding injury leads to platelet aggregation and releases platelet-derived growth factor, which triggers the proliferation of smooth muscle cells forming the nidus of the atherosclerotic plaque in the arterial intima, implicating inflammatory changes in the development of the disease [26]. The proposed mechanisms described above are presented in simplified form in Figure 1.

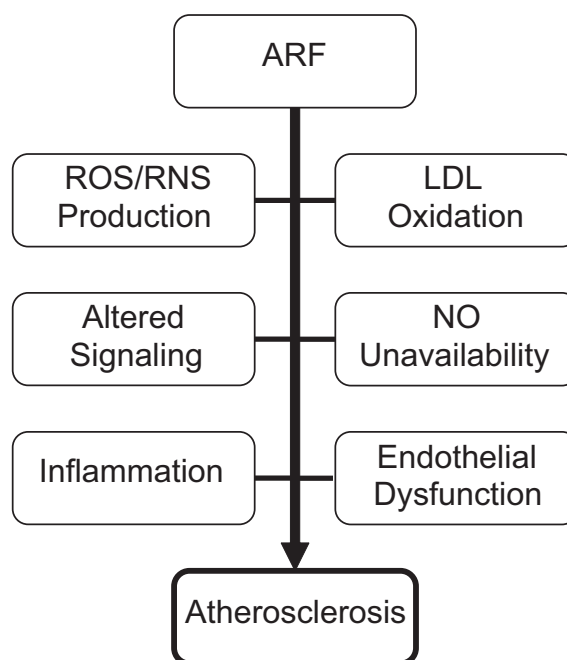


Fig. 1. A proposed mechanism for the occurrence of atherosclerosis. ARF: atherosclerotic risk factors; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; LDL: low-density lipoprotein; NO: nitric oxide. ARFs are heredity, age, hypertension, dyslipidemia, diabetes and smoking

Ryc. 1. Proponowany mechanizm występowania miażdżycy. ARF – czynniki ryzyka miażdżycy, ROS – reaktywne formy tlenu; RNS – reaktywne formy azotu; LDL – lipoproteiny o małej gęstości; NO – tlenek azotu. ARFs to dziedziczenie, wiek, nadciśnienie tętnicze, dyslipidemia, cukrzyca, palenie tytoniu

The Antioxidation Hypothesis for the Prevention of Atherosclerosis

LDL oxidation occurs early in CVD and is known to precede foam-cell formation. Supplementing treatment with antioxidants might be helpful to patients. Vitamin E reduces the consequences of oxidized LDL by decreasing monocytes’ ability to bind to endothelial cells [27, 28]. E-selectin has been proposed as an important factor in the development of the inflammatory process underlying atherothrombosis. The expression of E-selectin on the endothelium is decreased by vitamin E in cultured human endothelial cells [27]. However, Upston et al. found that “dietary vitamin E supplementation of rabbits after arterial injury significantly increases both the aortic levels of α -tocopherol and the overall content of cis/trans isomers. These data are fully consistent with α -tocopherol acting as a hydrogen donor

during lipid oxidation *in vivo* and suggest that α -tocopherol does not prevent lipoprotein lipid oxidation in the diseased vessel wall" [29]. Contrary to the effects of vitamin E as a chain-breaking and peroxy-radical-trapping antioxidant, α -tocopherol inhibits radical chain formation as a strong pro-oxidant for LDL [30]. Therefore, a great deal of attention has been paid to vitamin E as a suppressor of LDL lipid oxidation, since it is the main antioxidant in human lipoproteins [30].

Vitamin C has also been investigated in studies on the prevention of atherosclerosis. The following proposals have been put forward regarding the mechanisms of vitamin C in preventing atherosclerosis: First, vitamin C has been shown to prevent apoptosis caused by cytokines in cultured endothelial cells [31]. It also decreases the release of micro-particles derived from endothelial cells and suppresses proapoptotic activity in congestive heart failure patients *in vivo* [32]. Second, vitamin C stimulates all types of collagen synthesis by specific hydroxylase enzymes [33]. Endothelial cell proliferation is, in part, associated with the synthesis of type IV collagen [34]. Thus, a lack of vitamin C prevents the generation of type IV collagen in cultured endothelial cells [35]. Third, vitamin C protects the vascular endothelium by enhancing endothelial NO synthase. Endothelial NO synthase activity is inhibited by ROS that oxidize and deplete the co-factor tetrahydrobiopterin [36]. Therefore, vitamin C prevents the loss of NO synthase activity by maintaining tetrahydrobiopterin [37]. Glutathione monoethyl ester, but not ascorbic acid, exerted protective effects against ischemia-reperfusion injury. Interestingly, the protective effects of glutathione monoethyl ester are enhanced by co-administration with vitamin C in rat hearts subjected to ischemia and reperfusion [38]. Chronic zidovudine administration increases blood pressure and promotes cardiovascular damage through a NAD(P)H oxidase-dependent mechanism that involves protein kinase C. Vitamin C combats these adverse effects in the cardiovascular system in rats [39]. The oxidized LDL leads to increased platelet-endothelial cell adhesion, which can be prevented by superoxide dismutase (SOD) and catalase [40]. In fact, the vascular extracellular expression of SOD is stimulated by NO [41].

Antioxidant Vitamins in Animal Experiments

Animal experimental studies are presented in chronological order. Freyschuss et al. found that "butylated hydroxytoluene, a synthetic analog of vitamin E, effectively inhibited the accumulation

of intimal smooth muscle cells and the development of intimal thickening of the aorta in hypercholesterolemic rabbits after a balloon catheter-induced injury indicating antioxidants may modify intimal response to injury" [42]. Palace et al. reported: "Dietary supplements of vitamin E [α -tocopherol] can sustain better cardiac function subsequent to [myocardial infarction in rats]. Antioxidant vitamin levels in the myocardium or in storage organs and not in plasma may be better indicators of myocardial oxidative stress" [43]. As already noted, co-administration of vitamin C enhances the protective effects of glutathione monoethyl ester in isolated rat hearts following ischemia and reperfusion [38].

Narang et al. reported that dietary palm olein oil, which is rich in monounsaturated fatty acid and antioxidant vitamins, "protected rat heart from oxidative stress associated with ischemia-reperfusion injury" [44]. Das et al. found "a role for c-Src [a family of proto-oncogenic tyrosine kinases] in postischemic cardiac injury and dysfunction and demonstrate direct cardioprotective effects of [the tocotrienol-rich fraction of palm oil (TRF)]. The cardioprotective properties of TRF appear to be due to inhibition of c-Src activation and proteasome stabilization" [45]. Carlson et al., using a rat model, reported: "Antioxidant vitamin therapy [vitamin C, vitamin E, vitamin A and zinc] abrogated myocardial inflammatory cytokine signaling and attenuated sepsis-related contractile dysfunction, suggesting that antioxidant vitamin therapy may be a potential approach to treat injury and disease states characterized by myocardial dysfunction" [46].

Hypertension is directly regulated by the kidneys and cardiovascular system and adversely affects these organs. Tian et al. found that in salt-sensitive rat model of hypertension, vitamin C and vitamin E treatments "decreased renal inflammatory cytokines and chemokines, renal immune cells, NF- κ B, and arterial pressure and improved renal function and damage" [47]. As noted above, chronic zidovudine administration promotes cardiovascular damage, and vitamin C has been found to combat this effect in rats [39]. Diabetes mellitus may initiate increased myocardial vulnerability to ischemia-reperfusion injury and pro/antioxidant imbalance. Resistance to ischemia-induced ventricular arrhythmias and levels of endogenous antioxidants (α -tocopherol) have been found to be increased in diabetic rat myocardium [48]. In a study by Averill et al., dietary antioxidants (vitamin E, vitamin C and beta carotene) and genistein (an isoflavone) "do not inhibit the progression of established atherosclerotic lesions in [the innominate arteries of] older apolipoprotein E-deficient

mice” [49]. Contrary to previous reports on studies with experimental animals, Herrera et al. found that antioxidants do not cause a change in total plasma cholesterol, body weight, average area of the lesion or media, or changes in lesion composition. On the other hand, “co-administration of dexamethasone with antioxidant vitamins [vitamin C and vitamin E] improves survival and partially restores vascular dysfunction” in newborn rats, while dexamethasone alone “has detrimental effects on survival and peripheral vasoconstrictor function” and antioxidant vitamins alone decreased vasodilator capacity [50].

It should be noted here that none of the studies reported above used older animals in the experimental designs.

Antioxidant Vitamins in Human Clinical Trials

Human clinical trials are presented in chronological order. In 2000 patients with coronary atherosclerosis randomized to vitamin E (400 or 800 mg) or placebo, vitamin E treatment has significantly reduced the rate of non-fatal myocardial infarction, with beneficial effects after one year of treatment and with no difference in mortality rate [51]. However, because of imbalances in several baseline characteristics in that study, randomization may have failed to produce truly comparable groups. In another study 34,486 older women with no cardiovascular disease completed a questionnaire that included questions about intake of vitamins A, C and E; 242 of the women died of coronary heart disease, and the questionnaire indicated that increasing dietary vitamin E intake might help to prevent coronary heart disease, but that increased intake of vitamins A and C does not lower the risk of death from coronary heart disease [52]. In an epidemiological study covering several nations, vitamin C levels have been found to be low in Indians and Malays compared with Chinese, and it was concluded that vitamin C may have a role in reducing the risk of coronary heart disease [53].

In a large-scale prospective study of over 1 million adult Americans, Watkins et al. investigated the relationship between the use of multivitamins and death from CVD [54]. Their observations, contrary to previous data, “provide limited support for the hypothesis that multivitamin use in combination with vitamin A, C, or E may reduce heart disease and cardiovascular disease mortality, but add to concerns raised by randomized studies that some vitamin supplements may adversely affect male smokers” [54]. Furthermore, the Heart

Outcomes Prevention Evaluation (HOPE) study found that “400 IU of vitamin E administered daily for four to six years had no beneficial effects on cardiovascular outcomes in a high-risk population of [older] patients” [55].

As noted above, LDL oxidation occurs early in the development of CVD – it is known to precede foam-cell formation, and supplementation with antioxidants might be helpful to patients. Vitamin E prevents lipoprotein lipid oxidation in the diseased vessel wall during lipid oxidation [29]. In a food-frequency questionnaire study in older men with no history of CVD or diabetes, Wannamethee et al. compared fruit and vegetable intakes and dietary vitamin C with C-reactive protein, an acute phase reactant, and tissue plasminogen activator antigen, a marker of endothelial dysfunction. It was found that “vitamin C has anti-inflammatory effects and is associated with lower endothelial dysfunction” in the study group [56]. In an in vitro study using human aortic smooth muscle cells, Ivanov et al. suggested that “the [nutrient mixture] of ascorbic acid, tea phenolics, and selected amino acids has potential in blocking the development of atherosclerotic lesions by inhibiting atherogenic responses” [57]. Carrero et al. reported that among men who had undergone myocardial infarction and were adhering to a cardiac rehabilitation program, daily intake of a combination of nutrients (fish oil, oleic acid, folic acid, and vitamins B and E) decreased plasma C-reactive protein, suggesting that these nutrients reduce coronary heart disease risk factors [58]. However, in another study, high intake of carotenoids, but not vitamin E, decreased circulating C-reactive protein [59]. Hozawa et al. examined the relationship between circulating carotenoids and inflammation, oxidative stress, endothelial dysfunction and smoking, and found that circulating carotenoids are associated with smoking, with an attenuated relation between dietary intake and the risk factors for coronary diseases [60].

The Coronary Artery Risk Development in Young Adults (CARDIA) Study noted that “hostility is a personality trait associated with the increased risk of coronary heart disease”, and evaluated “whether the associations of carotenoid concentrations with hostility were modified by smoking” [61]. The authors suggested that “low levels of some serum carotenoids [alpha-carotene, beta-carotene, beta-cryptoxanthin and zeaxanthin/lutein] may be on the causal pathway in the association of hostility and cardiovascular risk” [61]. In addition, geographical and ethnic factors may have a significant role in CVD. Baghwat et al. found a strong relationship between genetic factors and ischemic heart disease in rural Indian

patients, among whom there is a high incidence of hyperhomocysteinemia [62].

Consumption of Dietary Antioxidants

Approximately two decades ago, consumption of dietary antioxidants (vitamin E and vitamin C) was found to be associated with reduced CVD mortality rates, suggesting that antioxidant vitamins have potent therapeutic effects [63, 64]. However, a meta-analysis of randomized clinical trials on vitamin E treatment carried out by Vivekananthan et al. consistently revealed a lack of beneficial effects, and no support for routine use [65]. No statistically significant association has been observed between dietary intake of vitamin C, vitamin E and beta-carotene and mortality rate [4]. However, as noted above, greater intake of fruits and vegetables has been shown to lower risk factors, suggesting that consuming fruits and vegetables is a sound health recommendation [4, 56]. The consumption of fruits and vegetables rich in flavonoids and antioxidants should be investigated in future studies on lowering the risk of atherosclerosis and markers of inflammation and oxidative stress. Recently, Holt et al. evaluated the effects of increased consumption of fruit and vegetables on markers of inflammation and oxidative stress in adolescents, suggesting that consuming

five or more servings of fruits and vegetables per day can be beneficial for cardiovascular health [5]. More randomized and controlled clinical trials investigating the association between vitamins and coronary heart disease are needed.

Conclusions

There are several hypotheses regarding the occurrence and prevention of atherosclerosis. Oxidized LDL appears to contribute to endothelial dysfunction by reducing endothelial NO bioavailability and producing ROS. Vitamin E can to some degree prevent the consequences of oxidized LDL, and vitamin C provides NO synthase activity. Prolonged use of vitamin supplementations in pharmaceutical forms is not currently recommended in human beings although beneficial effects have been shown in animal experiments. It should be taken into account that the evidence comes from studies on young/adult experimental animals with early stages of atherosclerosis and from *in-vitro* studies, while most of the clinical trials have included older patients in the late stages of CVD. Therefore, the currently available evidence needs to be confirmed in clinical trials. Consumption of fruit and vegetable diets rich in antioxidant nutrients can be recommended for all individuals, and there is some indication that such a diet can be beneficial in the prevention of cardiovascular events.

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