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Review

# Lycopene in atherosclerosis prevention: An integrated scheme of the potential mechanisms of action from cell culture studies

Paola Palozza *, Nadia Parrone, Rossella E. Simone, Assunta Catalano

*Institute of General Pathology, Catholic University, School of Medicine, L. Go F. Vito, 1 00168 Rome, Italy*

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ABSTRACT

Increasing evidence suggests that lycopene may protect against atherosclerosis, although, the exact mechanism(s) is still unknown. Because lycopene is an efficient antioxidant, it has been proposed for a long time that this property may be responsible for its beneficial effects. Consistent with this, the carotenoid has been demonstrated to inhibit ROS production *in vitro* and to protect LDL from oxidation. However, recently, other mechanisms have been evoked and include: prevention of endothelial injury; modulation of lipid metabolism through a control of cholesterol synthesis and oxysterol toxic activities; reduction of inflammatory response through changes in cytokine production; inhibition of smooth muscle cell proliferation through regulation of molecular pathways involved in cell proliferation and apoptosis. Focusing on cell culture studies, this review summarizes the experimental evidence for a role of lycopene in the different phases of atherosclerotic process.



## Introduction

Atherosclerosis is a chronic disease with a high health impact, since it contributes to more mortality and morbidity in the Western world than any other disorder. It is characterized by intimal lesions called atheromas or fibrofatty plaques, that protrude into the lumen, weaken the underlying media and undergo a series of complications [1]. Recently, it has been considered a chronic inflammatory response of the arterial wall initiated by an injury to the endothelium. Central to the pro-inflammatory hypothesis, there are several events, including endothelial injury, accumulation of lipoproteins, recruitment of blood monocytes and consequent transformation in foam cells, release of factors from activated macrophages, platelets or vascular cells that cause migration of smooth muscle cells from media into the intima [2]. The development of focal regions of chronic endothelial injury is usually subtle, with resultant endothelial permeability and increased leukocyte adhesion. It is well established that low-density lipoprotein (LDL)[1] plays a major role in macrophage foam cell formation, but modification of the particles, either chemically or by oxidation or aggregation, processes that occur within the artery wall, is necessary before extensive lipid accumulation is induced [1]. Uptake of native LDL (nLDL) via the LDL receptor (LDLr) is down-regulated when intracellular cholesterol levels begin to rise and thus does not lead to foam cell formation. The modified LDL, however, are taken up mainly by unregulated scavenger receptors such as scavenger receptor (SR) A and the class B receptor CD36, allowing large amounts of lipid to accumulate intracellularly [2]. Monocytes adhere to the endothelium early in atherosclerosis via the specific endothelial adhesion molecules induced on the surface of activated endothelial cells. Their adhesion is followed by migration into the intima and in their transformation into macrophages [2]. They avidly engulf

* Corresponding author. Fax: +39 06 3386446.
*E-mail address:* p.palozza@rm.unicatt.it (P. Palozza).

[1] *Abbreviations used*: LDL, low-density lipoprotein; LDLr, low-density lipoprotein receptor; nLDL, native LDL; SR, scavenger receptor; ROS, reactive oxygen species; CVD, cardiovascular disease; HDL, high-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; ABCA1, ATP-binding cassette A1; cav-1, caveolin-1; RhoA, Ras homolog gene family member A; PPAR, peroxisome proliferator activated receptor; LXR, liver X receptor; oxLDL, oxidative LDL; AAPH, 2,2′-diazobis-(2-amidinopropane)-dihydrocloride; $O_2^-$, superoxide anions; NF-kB, nuclear factor-kB; 7-KC, 7-ketocholesterol; IL-1β, interleukin-1β; 25-OHC, 25-hydroxycholesterol; MAPK, mitogen-activated protein kinase; ERK, extracellular regulated kinase; NADPH, nicotinamide adenine dinucleotide phosphate; hsp, heat shock protein; JNK, Jun N-terminal kinase; TNFα, tumor necrosis factor-α; CE, cholesterol esters; FeAOX-6, (±)-(*E*/*Z*)-2,5,7,8-tetramathyl-2(4,8,12-trimethyl-trideca-1,3,5,7,11-pentaenyl)chroman-6-ol; HMDM, monocyte-derived macrophages; acLDL, LDL modified by acetylation; aggLDL, LDL modified by aggregation; CRLPs, chylomicron remnant-like particles; AP-1, activator protein-1; iNOS, nitric oxide synthase; COX-2, cyclooxygenase; 5-LO, 5-lipoxygenase; PMNs, polymorphonuclear neutrophils; PMA, phorbol-myristate acetate; NO, nitric oxide; $NO_2^-$, nitrogen dioxide; $^1O_2$, singlet oxygen; IFN-γ, interferon-γ; IRF-1, interferon regulatory factor-1; STAT-1-α, signal transducer and activator of transcription-1-α; MDA, malondialdehyde; GJIC, gap junction intracellular communications; SMC, smooth muscle cells; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; TGF-α, transforming growth factor; PKC, protein kinase C; GJC, gap junction communication.

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lipoproteins, largely oxidized LDL, to become foam cells. These cells have a multifactorial role in the progression of atherosclerosis, owing to their production of a large number of secretory products, including reactive oxygen species (ROS), cytokines and growth factors, that may contribute to smooth muscle cell proliferation.

Lycopene is a carotenoid that is naturally present in tomatoes and tomato products. It is an open-chain hydrocarbon containing 11 conjugated and two non-conjugated double bonds arranged in a linear array [3]. Among the natural carotenoids, it is the most potent singlet oxygen quencher [4]. Recent epidemiological studies have shown an inverse relationship between the intake of tomatoes and/or lycopene levels in serum and adipose tissue, and the incidence of cardiovascular diseases (CDV) [5–9].

Several studies have reported that serum or tissue lycopene levels are inversely related to intimal wall thickness or lesions in the carotid artery and aorta, suggesting that lycopene may protect against the development of atherosclerosis [10–12]. Because lycopene is an efficient antioxidant [4], it has been proposed that this property may be responsible for its beneficial effects. In support of this, lycopene has been demonstrated to protect LDL from oxidation *in vitro*, and some dietary studies have shown that lycopene-containing foods increase resistance of LDL to oxidation *in vivo* [2,10]. On the other hand, plasma lycopene concentrations in smokers are not consistently lower than in non-smokers [10] as might be expected, suggesting that other mechanisms in addition to the antioxidant activity may be involved in its protective effects. This review summarizes the experimental evidence for a role of lycopene in the different phases of the atherosclerotic process in cell culture studies, and assesses the current state of knowledge regarding new possible mechanisms of action by which the carotenoid may be protective against this disease. These new insights not only increase our understanding of lycopene role in atherosclerosis process, but may also identify innovative therapeutic strategies to improve outcomes of individuals at high risk for CVD.

*Prevention of endothelial injury*

Chronic or repetitive endothelial injury is an important determinant of atherosclerotic process. In fact, it causes intimal thickening and, in the presence of high lipid diets, typical atheromas by inducing numerous endothelial genes with potential pro-inflammatory and pro-atherogenic activity and by causing increased endothelial permeability. Several factors seem to be involved in endothelial dysfunction. They include: endotoxins, hypoxia, specific endothelial toxins such as homocysteine, possible viruses or other infectious agents and products derived from cigarette smoke [13]. In particular, cigarette smoking impacts all phases of atherosclerosis from endothelial dysfunction to acute clinical events, increasing oxidation of LDL cholesterol, reducing blood levels of high-density lipoprotein-(HDL)-cholesterol, and enhancing blood levels of adhesion molecules and fibrinogen, joint factors which may lead to platelet aggregation and eventually to vascular spasm [14]. Recent experimental data support the hypothesis that lycopene may be protective against cigarette smoke exposure by limiting smoke-induced oxidative stress and by controlling molecular pathways involved in cell proliferation, differentiation, apoptosis and inflammation [15]. We have demonstrated that lycopene strongly inhibited cell growth in immortalized fibroblasts, exposed to cigarette smoke condensate, by arresting cell cycle progression and by promoting apoptosis [15]. The growth-inhibitory effects of the carotenoid were dose- and time-dependent and occurred at lycopene concentrations (0.5–2.0 μM) which are in the range of lycopene levels found in the serum of supplemented subjects [15]. In this study, the arrest of the cell cycle was independent of p53 and of 8-OH-dG DNA damage and related to a decreased expression of cyclin D1. On the other hand, the promoting effects of lycopene on apoptosis were due to an inhibition of cigarette smoke condensate-induced phosphorylation of Bad at $Ser^{136}$ [15].

*Modulation of lipid metabolism*

The mechanisms by which hyperlipidemia contributes to atherogenesis include chronic hyperlipidemia, particularly hypercholesterolemia, lipoprotein accumulation and oxidative modifications of both LDL and cholesterol by ROS generated in macrophages or endothelial cells.

*Modulation of cholesterol metabolism*

Hypercholesterolemia is one of the most important risk factors for atherosclerosis and lycopene has been suggested to have beneficial effects against such a disease, although the exact molecular mechanism is unknown. Cholesterol homeostasis is maintained through the coordinated regulation of pathways mediating cholesterol uptake, storage, *de novo* synthesis, and efflux. It is likely that the deregulation of these signals promotes foam cell formation [16,17]. The committed step in the biosynthesis of cholesterol and isoprenoids is catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which promotes the deacylation of HMG-CoA to mevalonate [18,19]. The activity of HMG-CoA reductase in animal cells has been shown to be sensitive to negative regulation by both sterols and non-sterol products of the mevalonate pathway [20,21]. Lycopene is a polyisoprenoid synthesized in plants from mevalonate via HMG-CoA reductase pathway. In plants, as well as in animal cells, HMG-CoA reductase is regulated by an end-product repression [22]. We recently found that lycopene was able to reduce the expression of HMG-CoA reductase in a dose- and time-dependent manner in THP-1 cells. The inhibition of HMG-CoA reductase by lycopene was also accompanied by a reduction in intracellular cholesterol levels [Palozza, unpublished results]. These data are in agreement with other observations showing that lycopene is able to reduce cholesterol levels in cultured macrophages [23] as well as in human subjects [24]. Fuhrman et al. [23] examined the effect of both lycopene and β-carotene on macrophage cholesterol metabolism in comparison with the effect of LDL cholesterol and of the cholesterol synthesis inhibitor, fluvastatin. In a macrophage cell line, *de novo* cellular cholesterol synthesis from [$^3$H] acetate, but not from [$^{14}$C] mevalonate, was suppressed following cell incubation with β-carotene or lycopene. Moreover, lycopene and β-carotene augmented the activity of the macrophage LDL receptor, similar to the effect of fluvastatin [23]. In agreement with these *in vitro* observations, dietary supplementation of lycopene to human subjects resulted in a significant reduction in plasma LDL cholesterol concentrations [8]. The mevalonate pathway produces numerous bioactive signalling molecules including farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP), which regulate transcriptional and post-transcriptional events that affect various biological processes, including changes in proteins involved in cholesterol efflux. Among these are ATP-binding cassette proteins, such as ATP-binding cassette A1 (ABCA1), and the caveolin family proteins, such as caveolin-1 (cav-1). We observed that lycopene dose-dependently induced the expression of both ABCA1 and cav-1 in THP-1 macrophages, favouring cholesterol efflux from macrophages through a potential mechanism involving Ras homolog gene family member A (RhoA) inactivation and subsequent increase in peroxisome proliferator activated receptor-γ (PPAR-γ) and liver X receptor-α (LXRα) activity [Palozza, unpublished results]. In agreement with this observation, other carotenoids, such as β-cryptoxanthin, induce ABCA1 and ABCG1 mRNAs and ABCA1 protein in macrophages [25]. These findings suggest that lycopene may modify cholesterol metabolism, and, consequently, foam cell formation.