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Bilberries exert an anti-atherosclerotic effect in lipid-loaded macrophages

Research Article

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Abstract: We hypothesized that the mechanism responsible for the anti-atherosclerotic action of bilberry extract (BE) is linked to its antioxidant and anti-inflammatory potential, and investigated its direct effect on the regulation of apolipoprotein E (apoE) and cholesteryl ester transfer protein (CETP) secretion from lipid-loaded macrophages. Human THP-1 macrophages were loaded with lipids by incubation with human copper-oxidized LDL (oxLDL) and then exposed to different concentrations of BE (1-5 μg mL^{-1}) obtained from bilberries (mechanically homogenized and solubilized in ethanol). Cellular and secreted proteins, the phosphorylation level of NF-κB and protein kinase A (PKA) were quantified by Western blot and gene expression was evaluated by Real-time PCR. The results showed that BE induced in lipid-loaded macrophages has: (i) an antioxidant effect by reducing the expression of NADPH oxidase subunits, p22^{phox}, p47^{phox} and NOX4, (ii) an anti-inflammatory effect by diminishing the secretion of CRP, MCP-1 and IL-1β and (iii) cholesterol efflux by increasing the secretion of apoE and CETP and by reducing cellular cholesterol content. BE exerted these effects by inhibition of NF-κB and activation of PKA signaling pathways. Our study supports BE therapeutic administration to decrease oxidative and inflammatory stress by a molecular mechanism regulated by NF-κB and PKA signaling pathways in lipid-loaded macrophages.

Keywords: *Apolipoprotein E • Atherosclerosis • Bilberry extract • Cholesteryl ester transfer protein • C-reactive protein • Inflammation • Interleukin 1-β • Macrophage • NADPH oxidase*

1. Introduction

Emerging scientific evidence indicates that consumption of a flavonoid-rich diet may prevent the risk of developing atherosclerosis, due to the ability of these compounds to inhibit oxidative stress and inflammation [1]. These processes are of major importance in atherogenesis because they stimulate oxidized LDL (oxLDL)-induced cholesterol accumulation in macrophages and the resulting foam cells in the arterial wall are the hallmark of atherosclerosis [2]. The generation of increased intracellular reactive oxygen species (ROS) was associated with oxLDL uptake by macrophages [3]. Moreover, oxLDL is known to induce the expression of NADPH oxidase subunits NOX4/p22^{phox} and NOX2/p47^{phox}/p22^{phox} in human smooth muscle cells [4] and macrophages [5,6], thus generating intracellular oxidative stress.

Macrophages are key mediators in the immune response and their physiological protective function is harmfully activated in the atherosclerotic process [7]. Macrophages contribute to plaque formation and development by internalizing oxLDL and converting into cholesterol-rich foam cells. Many inflammatory mediators, such as interleukins (IL-1β, IL-6), monocyte-chemoattractant protein (MCP)-1 and C-reactive protein (CRP), have genes targeted by the nuclear factor (NF)-κB [8], a transcription factor that is essential in orchestrating the inflammatory responses to a wide range of insults and is involved in the pathogenesis of cardiovascular diseases [9].

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