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Fitoterapia 79 (2008) 515 – 523

FITOTERAPIA

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Ocimum basilicum ethanolic extract decreases cholesterol synthesis and lipid accumulation in human macrophages

Elena Bravo [a,*], Souliman Amrani [b], Mohammed Aziz [b], Hicham Harnafi [b], Mariarosaria Napolitano [a]

[a] *Department of Haematology and Medicine Molecular, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Roma, Italy*
[b] *Department of Biology, Faculty of Sciences, University Mohamed I, Oujda, Morocco*

Received 22 March 2008; accepted 5 May 2008
Available online 22 June 2008

Abstract

Macrophage lipid accumulation induced by low density lipoproteins (LDL) plays a pivotal role in atherosclerotic plaque development. Previous work showed that *Ocimum basilicum* extract, used as hypocholesterolemic agent by traditional medicine in Morocco, has hypolipidemic activity in rat acute hyperlipimidemia. This study investigated the effects of ethanolic extract of *O. basilicum* on lipid accumulation in human macrophages. As modification of LDL increase atherogenicity of the particles we evaluated the effects of the extract on LDL oxidation. The extract caused a dose-related increase of LDL-resistance to Cu^{2+}-induced oxidation. Furthermore, at the dose of 60 μg/ml, significantly decreases the accumulation of macrophage lipid droplets induced by modified LDL evaluated as by red-oil staining. Cholesterol esterification and triacylglycerol synthesis in the cells were not affected. Macrophage treatment with 60 μg/ml, but not 20 μg/ml, of the extract reduced newly synthesized unesterified cholesterol by about 60% and decreased scavenger receptors activity by about 20–30%, evaluated by the internalization of cholesterol carried by [^{3}H]CE-aggregated-LDL. The results suggest that *O. basilicum* ethanolic extract has the capability to reduce foam cell formation through the reduction of cholesterol synthesis and the modulation of the activity of surface scavenger receptors.

Keywords: *Ocimum basilicum*; Oxidation; Cholesterol; Foam cell; Scavenger receptors

1. Introduction

Growing interest is focused on preventing cardiovascular diseases through improved diet and on promoting research on botanical products and their physiological mechanisms. This is because most evidence indicates that life-style and in particular, nutrition habits, influence the development of atherosclerosis [1,2], the major cause of mortality and morbidity in western countries and an increasingly important health problem in developing countries [3].

Ocimum (O.) basilicum is an important medicinal plant and a culinary herb widely cultivated in many countries [4] that contains several antioxidants compounds [4–6] and displays a high antioxidant power [7,8]. *O. basilicum* extracts have been shown to display important effects at cellular level, including the platelet anti-aggregant property and

* Corresponding author. Tel.: + 39 06 4990 2766; fax +39 06 4938 7086.
E-mail address: elena.bravo@iss.it (E. Bravo).

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doi:10.1016/j.fitote.2008.05.002

inhibitory activity against HIV-1 reverse transcriptase [9,10]. In addition, infusions of *O. basilicum* are used in traditional medicine to decrease plasma lipid content in some Mediterranean areas such as the Eastern Morocco. Recent work in our laboratory has demonstrated that it has strong hypolipidemic action in a murine model of induced hyperlipidemia. The *O. basilicum* extract significantly lowered both plasma triglycerides (TG) and cholesterol in acute hyperlipidemia induced by Triton WR-1339 in rats [8]. However, the mechanisms underlying the hypolipidaemic effects of *O. basilicum* and the anti-atherogenic potential, if any, of this plant at cellular level remained unexplored.

A pivotal role in atherogenesis is played by the process of the foam cell formation. Foam cells are macrophages which have invaded the arterial tissue and scavenged modified lipoproteins in an unregulated manner, becoming engorged with lipid [11,12]. This is a very early stage of atherosclerotic fatty streak lesions development. Because of their crucial role in the vessel lesion development, macrophages have been recognized as a potential target for preventive and therapeutical intervention [11].

The objective of present work is to gain a deeper knowledge on the anti-atherogenic potential of *O.basilicum* at the cellular level. To this aim, this study investigated the effects of *O.basilicum* on both foam cell formation and lipid metabolism in human macrophages. The effects of *O. basilicum* on macrophage cholesterol and TG synthesis as well as scavenger receptor activity were investigated. The data from this study will be useful in promoting studies on the active components of this culinary/medical plant and evaluating its potential utilization for improving human health.

2. Experimental

2.1. General

[9,10(n)^{3}H]-oleic acid (specific activity: 9.2 Ci/mmol) and [4-^{14}C]-cholesteryl oleate (specific activity: 60 mCi/mmol) were obtained from NEN Life Science Products Inc. (Boston, MA, USA), [^{14}C]-cholesterol (specific activity: 56 mCi/mmol) and [2-^{14}C]-acetic acid sodium salt (specific activity: 57 Ci/mmol) were purchased from Amersham Pharmacia Biotech Inc. (Milan, Italy). Iscove's Modified Dulbecco's Medium (IMDM), foetal bovine serum (FBS), and Ficoll–Paque, penicillin and streptomycin were obtained from Hyclone Europe Ltd. Fatty acid-free bovine serum albumin (BSA), and all other chemicals and solvents were of analytical grade and purchased from Sigma Aldrich (Milan, Italy).

2.2. Plant

O. basilicum L. (Labiate), collected from Oujda city in Eastern Morocco, was identified by Prof. A. Khalil from the Biology Department, Sciences Faculty (Oujda Morocco) where a voucher specimen has been deposited (collection No. LO15).

2.3. Preparation of ethanolic extract

50 g of dried *O. basilicum* aerial parts mixed with 250 ml of ethanol was sonicated at 22–24 μ power for 30 min at 20 °C. The extract evaporated and filtered with Whatman paper n.3 was kept in sealed containers in the dark under nitrogen at room until use. For the experiments, the extract was dissolved in DMSO at the concentration of 100 mg/ml and centrifuged at 800 g for 15 min at 4 °C.The clear supernatant was stored for 2 weeks in aliquots at −20 °C. On the day of the experiments the extract aliquots were diluted to the final concentration in cell media before incubations with macrophages. Final concentration of DMSO in incubations with the macrophages never exceeded 0.001%.

2.4. Isolation and culture of human monocyte-derived macrophages (HMDM)

Monocytes were isolated by density gradient centrifugation from human buffy coats, as previously described [13]. Briefly, CD14 MicroBeads and LS Separation Columns (Miltenyi Biotec) were used for the positive selection of human monocytes from white blood cells, collected by layering on Ficoll–Paque. Monocytes were transferred to 16 mm dishes at a density of 8×10^{5} 10^{5} cells/ml and cultured in IMDM containing 15% FBS. The purity of isolated monocytes, and the