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Beneficial role of the phytoestrogen genistein on vascular calcification☆

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Abstract

Although soy phytoestrogen are proposed to prevent or improve postmenopausal vascular and bone diseases, the currently available data are controversial and unclear. In this study we evaluated the molecular and biochemical action of genistein on the cellular events involved in vascular calcification. Rat monocytes, aortic vascular cell and osteoblasts cultures in vitro exposed to Gen were employed. Gen down regulated the expression of cell adhesion molecules involved in stable leukocyte attachment. Using flow cytometry we found that the PE significantly diminished monocyte integrins CD11b, CD11c and CD18 expression either under basal and pro-inflammatory environment. At endothelial level, Gen also reduced Intercellular Adhesion Molecule 1 mRNA expression. On vascular muscle cells, the PE markedly reduced cell proliferation and migration. When vascular calcification was studied, muscle cells transdifferentiation into osteoblasts like cells was evaluated. Cells were cultured in osteogenic medium for 21 days. The expression of alkaline phosphatase and the presence of calcified nodules in the extracellular matrix were selected as features of muscle transdifferentiation. Calcified muscle cells exhibited higher levels of alkaline phosphatase activity and enhanced deposition of calcium nodules respect to native cells. Both osteoblastic markers were significantly reduced after Gen treatment. In contrast to this anti-osteogenic action, on bone cells Gen promoted osteoblasts growth, enhanced alkaline phosphatase activity and increased matrix mineralization. Its mitogenic action on osteoblasts directly depends on nitric oxide endothelial production stimulated by the PE. The data presented suppose a beneficial role of Gen on bone and vascular cells, with a cross link between both systems.

Keywords: Genistein; Osteoblasts; Monocytes; Vascular cells; Vascular calcification

1. Introduction

Soy is the major source of plant derived phytoestrogens (PE). This term is generally used to define non-steroidal compounds that are either of plant origin or metabolically derived from plant precursors. There are mainly three subtypes of PE: isoflavones, lignans and coumestans. Due to their structural similarity with 17 beta estradiol (E_2), these compounds have estrogen like functions and may exhibit estrogen agonist or antagonist activity [1]. They act through interaction with estrogen receptors (ER). Although the overall evidence reported in the literature suggests that PE are less potent estrogens than the natural E_2, these plant derived compounds exhibit higher affinity for ERα and ERβ [2]. Especially, the isoflavones genistein (Gen) and daidzein have higher binding preference for ERβ. It has been also reported that PE modulate signaling pathways associated with cell growth and proliferation through activation of membrane G-protein coupled estrogen receptors (GPERs) [3]. Indeed epigenetic alterations of target genes expression has been proposed as a possible mechanism of the potential beneficial role of Gen in cancer [4].

Soy PE intake may reduce the risk of cardiovascular disease (CVD) upon the sudden loss of ovarian function during menopause. This hypothesis is supported by the low rates of CVD in Asian populations where the diet is particularly rich in soy [5]. Although some experimental and clinical studies show that in postmenopausal women isoflavones exhibit potential beneficial features against chronic diseases such as CVD and osteoporosis, the currently available data are controversial and insufficient [2]. Additional work is required for a complete comprehension of the risk/benefit of FE administration on menopause women health.

Bone and vascular homeostasis depend on cellular, endocrine and metabolic signals that flow bidirectionally between both systems. Bone never forms without vascular interactions. Indeed, skeletal trauma and impaired skeletal healing are commonly associated with altered vascular function [6]. Arterial calcification is also associated with osteoporosis, especially in postmenopausal women [7]. Both bone mineralization and vascular calcification are cell mediated

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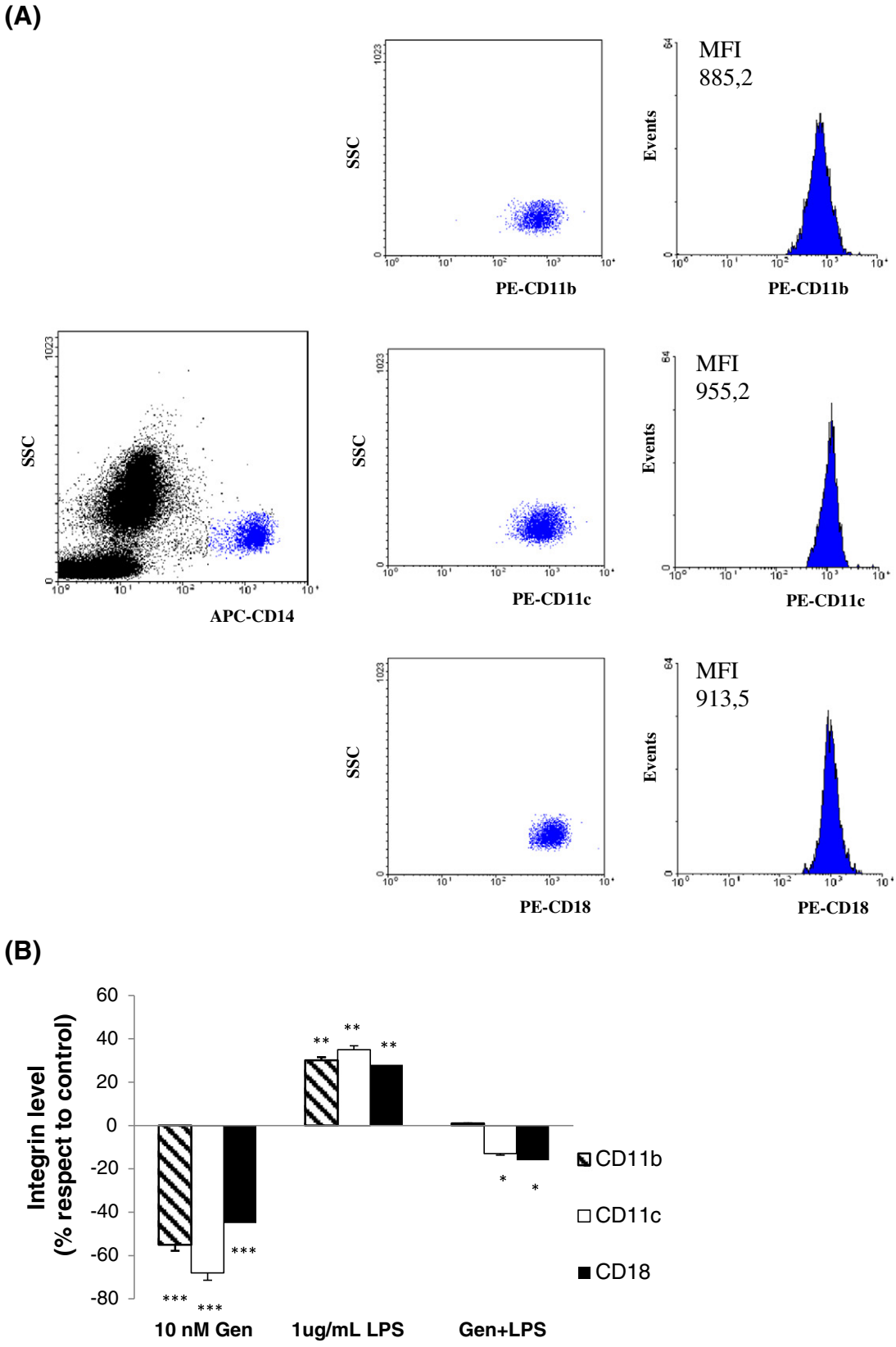

Fig. 1. Gen regulation of cell molecule adhesion expression. A-B: monocytes were incubated with 1 μg/ml LPS 2 h or 10 nM Gen 24 h in absence or presence of LPS added during the last 2 h of Gen treatment. Panel A shows light scatter profiles and CD14 positivity of selected blood monocytes; dot plot and histogram of CD11b, CD11c and CD18 expression in control samples. Values express the MFI. Panel B shows the quantification data expressed as % respect to control (MFI: 885,2±33, 955,2±25 and 913,5±25, CD11b, CD11c and CD18 respectively), Results represent the mean ± SD of three separated experiments in which each experimental condition have been performed by quatriplicate (n=4). *P<.05; **P<.01; ***P<.001 vs. control. Panel C: ECs were incubated for 24 h with 10 nM Gen, 1 μg/ml LPS or Gen plus LPS (added during the last 19 h of Gen treatment). A representative gel photograph of PCR amplification products is shown. The expected band size for ICAM-1 and GAPDH product are indicated. Bars show the relative intensity of each band determined by densitometric analysis. Data are presented as ICAM-1 mRNA relative to GAPDH mRNA. Results represent the mean ± SD of three separated experiments. *P<.0 vs. control **P<.01 vs. LPS.