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Quercetin accelerates TNF-α-induced apoptosis of MC3T3-E1 osteoblastic cells through caspase-dependent and JNK-mediated pathways

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Abstract

The bioflavonoid, quercetin, is believed to inhibit bone loss by regulating many systemic and local factors including hormones and cytokines. However, our previous findings revealed that quercetin did not inhibit but facilitate the tumor necrosis factor (TNF)-α-mediated apoptosis of MC3T3-E1 osteoblastic cells. Therefore, this study was carried out to examine the cellular mechanisms for how quercetin accelerates TNF-α-mediated apoptosis, and to determine whether the accelerating effect of quercetin is a general effect in osteoblastic cells. Quercetin promoted the TNF-α-induced apoptosis of MC3T3-E1 cells through both the mitochondrial-mediated and caspase-dependent mechanisms. Quercetin also augmented the TNF-α-mediated apoptosis by activating c-Jun N-terminal kinase (JNK) with the attendant activation of activator protein-1, where the nuclear translocation of c-Jun protein appeared to be a critical event responsible for the accelerating action of quercetin. However, TNF-α-mediated apoptosis and its acceleration by quercetin were not observed in primary osteoblasts. These results strongly suggest that quercetin accelerates TNF-α-mediated apoptosis of osteoblasts through caspase-dependent and JNK-mediated pathways, and that the cellular responses of osteoblasts to TNF-α and/or quercetin might differ according to their origins.
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Keywords: Quercetin; TNF-α; Osteoblastic cells; Apoptosis; Caspase; JNK

1. Introduction

Bone cells along with their interactions are sensitive to growth factors, hormones, and cytokines (Katagiri and Takahashi, 2002). Especially, tumor necrosis factor-alpha (TNF-α), which is produced mainly by activated macrophages, bone marrow cells and monocytes, plays a key role in the regulation of bone metabolism. For example, TNF-α stimulates osteoblasts to secrete other inflammatory cytokines such as interleukin (IL)-1 and IL-6, as well as prostaglandin E_2 (PGE_2) and TNF-α itself, which acts directly on osteoclasts to cause bone resorption (Franchimont et al., 1997; Jilka, 1998; Glantschnig et al., 2003). TNF-α also induces the apoptosis of osteoblasts (Chua et al., 2002; Suh et al., 2003). Therefore, it is believed that an increase in the TNF-α level and a decrease in the number of osteoblasts might be responsible for bone loss in vivo.

Recently, there has been a global trend towards the use of natural bioactive compounds to regulate bone metabolism (Choi and Koo, 2003; Gallagher et al., 2004). A dietary flavonoid, quercetin, has been highlighted as the bioactive substance, because of its biological, pharmacological, and medicinal

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activities. Accumulating evidence suggests that quercetin inhibits bone loss by affecting osteoclastogenesis and regulating a variety of systemic and local factors such as hormones and inflammatory cytokines (Wattel et al., 2003; Woo et al., 2004). However, the precise mechanisms by which quercetin regulates osteoblastic activation are not completely understood, particularly in the presence of inflammatory cytokines. Moreover, recent findings suggest that quercetin does not inhibit TNF-α-induced cytotoxicity and apoptosis in osteoblastic MC3T3-E1 cells but accelerates these processes (Son et al., 2006a). This led us to postulate that the effects of quercetin on bone cells can differ according to the types of cells studied and the conditions of experimental models, i.e., in vivo and iv vitro (Arjmandi et al., 1996; Horcajada-Molteni et al., 2000).

We have investigated the precise role of quercetin on osteoblasts in the presence or absence of TNF-α. Our previous data suggested that Fas activation and poly (ADP ribose) polymerase (PARP) cleavage are closely related to TNF-α-induced apoptosis and its acceleration by quercetin. However, the precise mechanisms on the promoting action of quercetin are still unclear. This study further examined the cellular mechanisms how quercetin accelerates TNF-α-mediated apoptosis in osteoblastic MC3T3-E1 cells. Our data shown in this study strongly suggests that quercetin accelerates the TNF-α-induced apoptosis of the cells through caspase-dependent and c-Jun NH_2-terminal kinase (JNK)-mediated pathways.

2. Materials and methods

2.1. Chemicals and laboratory wares

Unless otherwise specified, all the chemicals and laboratory wares were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Falcon Labware (Becton-Dickinson, Franklin Lakes, NJ, USA), respectively. Quercetin (Q0125; Sigma Chemical Co.) or z-VAD-fmk (FK009; ICN, OH, USA), z-FA-fmk (FK029), z-IETD-fmk (FK023) and z-LEHD-fmk (FK022) were dissolved in ethanol or dimethylsulfoxide (DMSO) immediately before use. Mitogen-activated protein kinase (MAPK) inhibitors, PD98059 (2-(2-Amino-3-methoxyphenyl)-4H-1-benzopyran-4-one), SB203580 (4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole) and SP600125 (Anthra[1,9-cd]pyrazol-6 (2H)-one), were purchased from TOCRIS (MI, USA). The final ethanol or DMSO concentration did not exceed 0.5% (v/v) in any of the experiments.

2.2. Cell culture and treatment

Murine osteoblastic MC3T3-E1 cells (ATCC, CRL-2593) were cultured in α-minimum essential medium (α-MEM) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA) and antibiotics, as previously described (Son et al., 2006a). After the cells had reached 80% confluence, the culture medium was replaced with a fresh medium that was supplemented with quercetin (0–10 μM), TNF-α (10 ng/ml), MAPK and/or caspase inhibitors, and incubated for various times (0–72 h). In addition, NIH/3T3 cells, Jurkat cells, and primary calvarium osteoblasts were incubated in MEM supplemented with 10% FBS and antibiotics, and processed for analyzing whether the accelerating effect of quercetin on TNF-α-mediated apoptosis is a general effect in osteoblastic cells or whether it is restricted to MC3T3-E1 cells. The primary osteoblasts were prepared from the mouse calvaria of 1-day-old BALB/c mice by repeated digestion of them with 0.05% trypsin and 0.1% collagenase. This experiment was approved by the Chonbuk National University Committee on Ethics in the Care and Use of Laboratory Animals. Control cells in all experiments contained 0.5% DMSO and/or ethanol, which did not affect the cellular responses to quercetin and/or TNF-α.

2.3. Apoptosis detection

The apoptosis-mediated death of MC3T3-E1 cells was analyzed using a FACS Calibur® system (Becton Dickinson, San Jose, CA, USA) after propidium iodide (PI)- or Annexin V/PI double-staining, as previously described (Son et al., 2006a).

2.4. Caspase activity assay

The activity of caspases was assessed using the colorimetric CaspACE assay system (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, after treatment with 10 ng/ml TNF-α and various concentrations of quercetin (1–10 μM) for various times, the cells were resuspended in a lysis buffer provided by the manufacturer. Aliquots of cell lysates were incubated with 200 μM of each substrate, Ac-DEVD-chromophore *p*-nitroaniline (pNA) and Ac-IETD-pNA specific for caspase-3 and -8, respectively, in the assay buffer at 37 °C for 4 h. The absorbance of the cleaved product was read at 405 nm using a SpectraCount™ ELISA reader.

2.5. Cytofluorimetric analysis of mitochondrial membrane potential ($\Delta\Psi m$)

MC3T3-E1 cells were treated with various concentrations of quercetin in the presence of TNF-α (10 ng/ml), MAPK and/or caspase inhibitors, and stained with 50 nM 3, 3′-dihexyloxacarbocyanine iodide ($DiOC_6$; Molecular Probes, Eugene, OR) for 20 min at 37 °C. The fluorescence related to $\Delta\Psi m$ was measured using a FACS Calibur® system.

2.6. Electrophoretic mobility shift assay (EMSA)

Nuclear proteins were prepared as described elsewhere (Maulik et al., 1998), and quantified using the Bradford method (1976). DNA-protein binding reactions were carried out for 30 min at room temperature using 10–15 μg of the protein in a 20-μl volume containing 1 μg/ml bovine serum albumin, 0.5 μg/μl poly (dI-dC), 5% glycerol, 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM Tris–Cl (pH 7.5), 50 mM NaCl, 5 mM $MgCl_2$, 30,000 cpm of [α-^{32}P] dCTP-labeled oligonucleotides, and the Klenow fragment of DNA polymerase. The samples were separated on 6% polyacrylamide gels, dried and exposed to X-ray films

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