

CURCUMIN AND PGV-0 SUPPRESS IN VITRO OSTEOCLASTOGENESIS AND INDUCE APOPTOSIS OF OSTEOCLAST CELLS

Edy Meiyanto

CCRC-Faculty of Pharmacy, Gadjah Mada University
Email: edy.meiyanto@gmail.com

ABSTRACT

Curcumin is known could inhibit the activation of NF- κ B, which is important in osteoclast differentiation and activation. The aims of this research were to conduct the effect of curcumin and Pentagamavunon-0 or PGV-0 [2,5-bis-(4'-hydroxy-3'-methoxybenzylidene)-cyclopentanone], on osteoclast differentiation and apoptosis. Osteoclast differentiation was carried out using Osteoclast Differentiation Factor (ODF) induction model in Raw 264.7. The evident of osteoclast was determined microscopically using inverted microscope after TRAP staining. The apoptosis of osteoclasts were determined microscopically using confocal microscope. The results showed that PGV-0 and curcumin at the concentration of 5 μ M and 10 μ M, respectively inhibited osteoclast formation by 50 –80%. PGV-0 and Curcumin induced apoptosis on osteoclast cells at the concentration of 10 μ M and 20 μ M respectively. Those effects were dose dependent.

Key words: Curcumin, PGV-0, osteoclast differentiation, apoptosis

INTRODUCTION

Osteoclastogenesis is a differentiation process of osteoclast formation from monocyte-macrophage lineage (Meiyanto, et al., 2001). The formation and activation of osteoclast cells played a pivotal role in bone remodeling to maintain bone mass and bone density. The over formation and activation of osteoclast cells caused osteoporosis, a disease characterized by a progressive loss of bone mass and density. Osteoporosis is a common disease in elderly causing bone fracture and sometimes mortal (Troen, 2003). Over osteoclast formation and activation were also usually found in metastasize cancers. Lung cancer and breast cancer were found to metastasize into bone by inducing osteoclast formation and activation (Fohr, 2003). Therefore the interruption of osteoclast formation and activation were an important strategy to overcome the diseases.

In the bone, osteoclast formation began from initiation of stromal cell induced by RANK-RANKL signaling cascade (Troen, 2003). RANK is a transmebran receptor of stromal cell that can interact with RANKL (refer to ODF:

osteoclast differentiation factor) expressed by osteoblast cells as a transmembrane protein. Complex RANK-RANKL induced signal transduction through MAP Kinase signaling pathway leading to activation of a subset of transcription factors including NF- κ B. NF- κ B played an important role not only in the initiation phase but also in the maturation and activation of osteoclast cells (Yasuda, et al., 2004). RANK-RANKL signaling cascade can be inhibited by a decoy receptor, called OPG (osteoprotegerin) that can be expressed by osteoblast cells in the present of estrogen (Gori, et al., 2000). Osteoclast differentiation can also directly be inhibited by estrogen thorough inhibition of MAPK signaling pathway (Taitelbaum and Steven, 2000).

In the *in vitro* model, osteoclastogenesis can be prepared by using sODF induced-macrophage cells system (Meiyanto, et al., 2001). Soluble (s) ODF was an extra cellular domain of ODF which could bind to RANK and induced osteoclastogenesis through the same pathway of complex RANKL-RANK resulting in the activation of NF- κ B (Suda, et al., 1999). This model is suitable for studying of the molecular mechanism of osteoclastogenesis as well as for finding of anti-osteoclastogenesis substances.

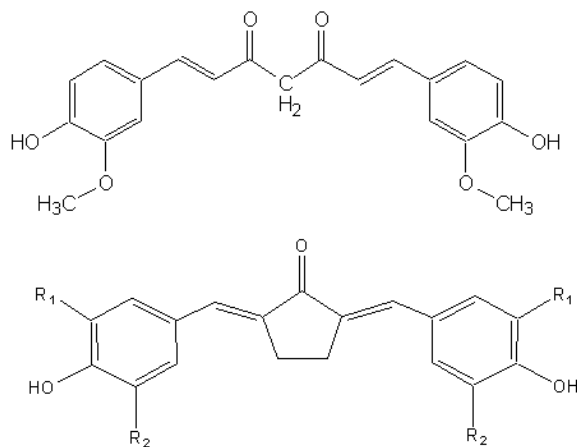


Figure. 1. Molecular structure of curcumin (upper) and PGV-0 (bottom)

Curcumin was also known as NF- κ B inhibitor that may play a role in the interruption of osteoclast differentiation (Bharti, et al., 2004). PGV-0 was an curcumin analogue (Figure. 1) exhibiting better pharmacological activities than curcumin, such as cytotoxic effect to some cancer cells (Meiyanto, et al., 2006), anti-inflammatory (Anonymous, 1999), and modulate some genes expression related to cell cycle and apoptosis evidence (Nurulita and Meiyanto 2005; Meiyanto, et al., 2006). Curcumin has been tested to induce apoptosis in rat osteoclast model (Ozaki, et al., 2000). This research was to investigate the inhibitory effect of curcumin and PGV-0 on the initiation phase of osteoclastogenesis and the apoptotic effect on mature osteoclast.

MATERIAL AND METHOD

Materials

Soluble (s) ODF and RAW264.7 mouse monocyte/macrophage line cells were kindly gift from Prof Tatsuo Takeya (Nara Institute of Science and technology). Curcumin and PGV-0 were obtain from Curcumin Research Center, Faculty of Pharmacy UGM.

Method

Cell culture, and osteoclastogenesis. RAW264 cells were maintain in in Eagle medium supplemented with 10% fetal calf serum and 1% nonessential amino acids (GIBCO-BRL). Osteoclastogenesis was performed by using the method as described previously (Meiyanto, et al., 2001) with little modification. Briefly, 1×10^4 of the cells were cultured for 24 hours with 10 % fetal calf serum then add 100 ng/mL sODF. Osteoclast cells appeared at the day 2 after ODF stimulation, then the medium was changed with the new one and the new ODF was added and osteoclast will be confluent at the day 4 until the day 7 before the cells undergo apoptosis (Figure. 2).

The inhibitory and apoptotic effect of curcumin and PGV-0. Curcumin or PGV-0 at the concentration of 20 μ M and 10 μ M respectively was added to the culture

together with sODF and the formation of osteoclasts was observed at the day 4 using confocal microscope (LSM) and light microscope to observe tartrate resistant acid phosphatase (TRAP) positive cells (4). To observe the apoptotic effect of curcumin and PGV-0 on the osteoclast cells, the confluent osteoclasts at the day 4 were treated with curcumin or PGV-0 at the concentration of 20 μ M and 10 μ M respectively then the death cells were observed at the day 5 by using confocal microscope.

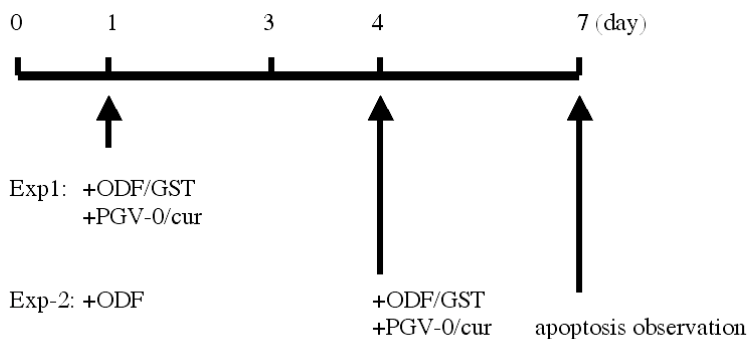


Figure. 2. Experimental design and treatment. ODF was used to stimulate osteoclast formation added at indicated time, whereas curcumin/PGV-0 was added at the day 1 for inhibitory experiment (exp-1) or the day 4 for apoptosis induction experiment (exp-2).

RESULT AND DISCUSSION

Results

Osteoclast formation

The system for *in vitro* osteoclastogenesis has been established by Meiyanto, et al., 2001 by ODF stimulation of arrested RAW 264 cells. In this research, osteoclastogenesis was stimulated by ODF of RAW cells without starvation. The formation of multinucleated cells begins at the day 3 after the first ODF stimulation and become bigger and giants at the day 4. The cells indicated osteoclast characteristic as they are TRAP positive. The cells observed at the day 2, that mostly still solely grew, were also TRAP positive, indicated that they already become competent for osteoclastogenesis (Figure. 3). This system was proven at least 3 times with the same results (data not shown).

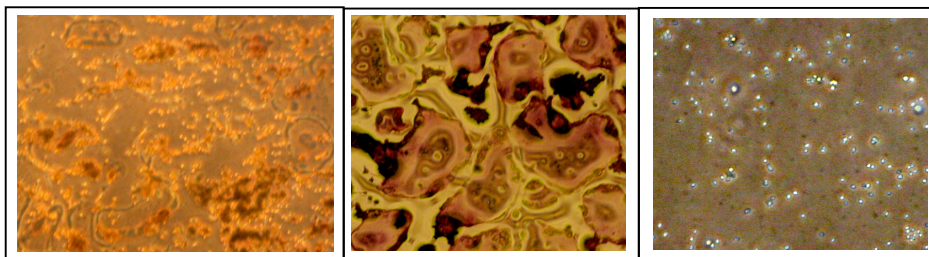


Figure 3. TRAP positive cells. Two days after ODF stimulation, RAW 264 cells become TRAP positive (red color) (left panel) and at the day 4 the cells form multinucleated giant TRAP positive cells indicate as osteoclast (middle panel), whereas the control cells (GST stimulation) are TRAP negative (right panel).

Inhibition of osteoclastogenesis by curcumin and PGV-0

For investigation of inhibitory effect of curcumin and PGV-0, the growing Raw 264.7 cells were treated with curcumin or PGV-0 at three level concentrations together with ODF. The result showed that the differentiation into osteoclasts can be effectively inhibited by curcumin and PGV-0 at the concentration of 20 μM and 10 μM respectively. At the respective concentration, curcumin and PGV-0 inhibited osteoclast formation by 70 and 80% respectively. Curcumin and PGV-0 were able to inhibit osteoclast formation at the concentration of 10 μM and 5 μM respectively (Figure. 4). These results indicated that PGV-0 performs stronger inhibitory effect than curcumin on osteoclastogenesis.

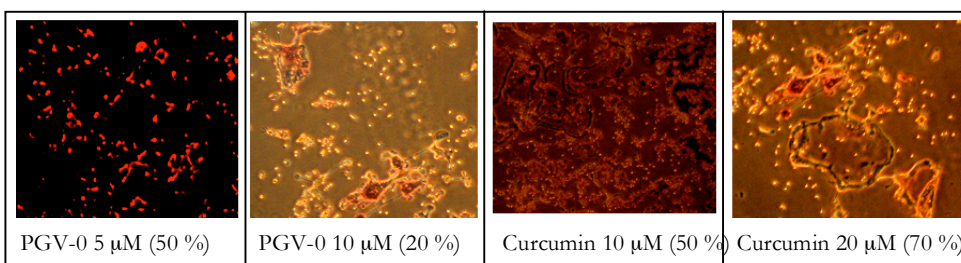


Figure 4. Inhibitory effect of curcumin and PGV-0 on osteoclastogenesis. Raw 264.7 (1×10^4) were cultured under ODF stimulation in the medium containing curcumin or PGV-0 at the indicated concentration. Photographs were taken for TRAP positive polynuclei cells (osteoclasts) with original magnification of 100 X.

Curcumin and PGV-0 induce apoptosis on osteoclast cells

In the previous study, curcumin treatment of the cells caused morphological changes in osteoclast indicated as apoptosis (Ozakim, et al., 2000). PGV-0 (10 μ M) induced apoptosis when incubated with osteoclast cells. The apoptotic cells were observed in 24 hour after treatment. In this experiment, PGV-0 also exhibited more effective to induce apoptosis than curcumin (Figure. 5).

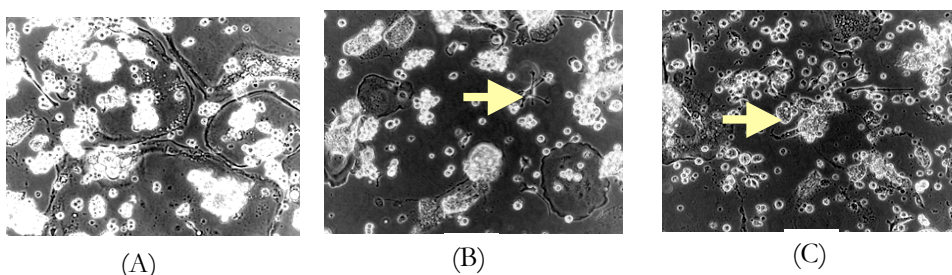


Figure. 5. Curcumin and PGV-0 induce apoptosis on osteoclast cells. Osteoclasts were formed after 4 days culture of ODF induced Raw 264.7 cells and followed by curcumin/PGV-0 treatment. The cells morphology was observed using confocal microscope and photographed in the magnification of 100 x. (A) vehicle , (B) 10 μ M PGV-0, (C) 20 μ M Curcumin. Arrow indicates apoptotic cells.

DISCUSSION

PGV-0 is an analogue substance of curcumin that more rigid and flat in the chemical structure than curcumin. This chemical characteristic may contribute in the inhibitory activity of kinases, enzymes that play roles in signal transduction including activation of NF- κ B. Since curcumin exhibited inhibitory effect to NF- κ B activation through IKK inhibition (Bharti *et al*, 2004), PGV-0 perhaps inhibited osteoclastogenesis through such mechanism. In the other experiment using T47D, PGV-0 also reduced the expression of BCL2, a protein that was expressed through NF- κ B activation (Meiyanto, et al., 2006).

In this experiment, PGV-0 performed stronger both inhibitory effect on osteoclast differentiation and apoptosis induction on mature osteoclasts than curcumin. The stronger inhibitory effect of PGV-0 than curcumin on osteoclast differentiation suggested that PGV-0 is more potent to inhibit NF- κ B activation

than curcumin. This effect may be affected by the chemical structure characteristic such as more rigid and flat that increase the affinity to bind the kinases (Knockarert, et al., 2002).

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