NOVEL ACTION OF QUINOLONES ON OSTEOCLAST-LIKE CELLS

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ABSTRACT — Quinolones have a broad antibacterial spectrum against Gram-negative and Gram-positive bacteria. The compounds, however, have a few adverse effects, such as convulsion and toxicity to articular cartilage. We observed that some quinolones such as Nalidixic acid, Ofloxicin, and Norfloxicin have osteoclast-inducing effects. All quinolones we tested produced tumor necrosis factor-alpha and prostaglandin E2, and have potency as osteoclast inducers to cultured cells. These results suggest that some quinolones affect osteoclast induction or activation, and this may be related to the production of tumor necrosis factor-alpha (TNF-alpha) and prostaglandin E2 (PGE2).

KEY WORDS: Quinolones, Osteoclast, Tumor necrosis factor-alpha, Prostaglandin E2 production, Toxicity

INTRODUCTION

New quinolones, especially fluoroquinolones, have a broad antibacterial spectrum against Gram-negative and Gram-positive bacteria, and are a good choice for urinary infection (Smith and Lewin, 1988; Cohen et al., 1988). The compounds, however, have a few adverse effects, such as convulsion and toxicity to articular cartilage. It is known that the convulsion is induced when the drug is used with nonsteroidal anti inflammatory drugs (NSAID), and the mechanisms of quinolone-induced convulsion may be related with the GABAA receptor in the central nervous system (Tsutomi et al., 1994). On the other hand, the mechanisms of toxicity to articular cartilage remain unclear. In this paper, we investigated the effects of quinolones on bone cells, and we found that the drugs have osteoclast-inducing effects.

MATERIALS AND METHODS

Chemicals

Nalidixic acid (NA), Ofloxicin (OFLX), and Norfloxicin (NFLX), and the following chemicals were purchased from commercial sources: minimal essential medium alpha (alpha-MEM), 1-alpha, 25(OH)2 vitamin D3 (Sigma, St. Louis); Mouse tumor necrosis factor-alpha (TNF-alpha) ELISA kit (Genzyme, USA); PGE2 RIA kit (NEN Research Products, Japan). Other chemicals were of the highest purity available from commercial sources.

Preparation of osteoblast-like cells

In this experiment, we used ddY mouse (Japan SLC, Inc.). Cells were isolated from calvariae of 17-day-old fetal mice using phosphate buffer saline (PBS) containing 0.1% collagenase and 0.2% dispase. The isolated cells were cultured in alpha-MEM, containing 10% of heat-inactivated fetal bovine serum and 1% of non-essential amino acids (Takahashi et al., 1988).

Characterizations of osteoblast-like cells

Isolated cells from calvariae were cultured for 8 days, and the cells were fixed and identified with osteoblast-like cells by means of alkaline phosphatase staining (Jeffree, 1970), type I collagen staining using the ABC method (Hsu et al., 1981a, Hsu et al., 1981b), and von Kossa staining.

Preparation of spleen cells

Spleen cells were isolated from 7 w male mice. Isolated cells were separated using nylon wool column (Kuribayashi, 1976). Nylon wool-binding cells were collected, and treated with both Th1.1 antibody and complement. Prepared cells did not contain T cells.

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Co-culture system

Osteoblast-like cells (10⁶ cells/ml, 1 ml/well) were cultured in the alpha-MEM and maintained at 37°C in a humidified atmosphere of 5% CO₂ and 95% air for 7 days on a 12-well culture plate until confluent (Takahashi et al., 1988). Prepared spleen cells were added to the full-seeded-osteoblast-like cells, and co-cultured for some periods between 1 and 11 days.

Measurement of tartrate-resistant acid phosphatase positive cell numbers

Ten nM of 1-alpha,25(OH)₂ vitamin D₃ or 0.1, 1, 2, 5, 10, 100 μM of NA, OFLX, and NFLX were added in the co-culture system, and incubated up to 7 days. After that, cells were fixed and stained with tartrate-resistant acid phosphatase (TRACP). TRACP-positive cells were counted as osteoclast-like cells under a microscope.

TNF-alpha and PGE₂ assay

Ten nM of 1-alpha,25(OH)₂ vitamin D₃ or 5 μM of NA, OFLX and NFLX were added in the co-culture system and incubated up to 11 days. After co-culture for several days, the cells were sonicated in the cultured media. Both contents of TNF-alpha and PGE₂ were assayed using a commercial kit.

Statistical analysis

The data for biochemical quantitation was examined with the Student's t-test (Gad and Weil, 1982). P-values less than 0.05 were considered to be statistically significant.

RESULTS

Spleen cells

Prepared spleen cells were identified using a flow cytometer. The prepared spleen cells contained CD3-positive cells (Fig. 1A). However, when the cells were applied to a nylon wool column and treated with Th1.1 and complement, CD3-positive cells were not detected (Fig. 1B).

Induction of osteoclast-like cell

Prepared calvaria cells were cultured for 7 days, and were stained with alkaline phosphatase, type I collagen and von Kossa staining to identify them with osteoblast-like cells. TRACP-positive cells were not obtained up to 7 days in the co-culture system without drugs (Photo 1(A), Fig. 2). TRACP-positive cells

![Fig. 1. Flow cytometry of prepared spleen cells.](image-url)
markedly increased when 1-alpha, 25(OH)2 Vitamin D3 was added to the co-culture system (Photo 1B). TRACP-positive cells also markedly increased when NA, OFLX, or NFLX was added to the co-culture system (Photo 2A, B, C, Fig. 2). TRACP-positive cells were not observed when drugs were added to the cultured osteoblast cell alone (without spleen-derived cells) up to 7 days (Photo 3A). TRACP-positive cells were not observed when TNF or prostaglandin was added to the cultured osteoblast cell alone (without spleen-derived cells) (Photo 3B).

Prepared spleen cells were cultured with quinolones for 7 days. After washing with PBS, the cells were cocultured with osteoblast-like cells. Osteoclasts were not observed in the co-culture system.

**TNF-alpha and PGE2 content**

TNF-alpha content was gradually elevated from 3-5 days and reached a peak at 7 days (Fig. 3). The elevation of PGE2 content was observed earlier than that of TNF-alpha (Fig. 4).

**DISCUSSION**

Recently, it has been suggested that both the differentiation-inducing factor (DIF) and the osteoclast-activating factors (OAFs) act on both differentiation and activation of the osteoclast (Abe et al., 1988; Dewhirst et al., 1985; Bertrini et al., 1986; Sato et al., 1986). It is shown that the increase of osteoclast-like cells and the activation of the cells were observed in a DIF-added bone culture system. When M1 cells, which came from a murine myeloblastic cell line, and WEHI-3 cells, which is a mouse monocyctic cell line, were cultured with DIF, these cell lines also formed osteoclast-like multinucleated cells (Abe, 1987). It has been clarified that OAFs are a kind of monokine or cytokine including TNF-alpha and/or interleukin 1 (IL-1). But DIF does not have TNF-alpha and/or IL-1 activities (Dewhirst et al., 1985; Bertrini et al., 1986; Abe, 1987). Therefore, both factors are produced from osteoclasts (Abe et al., 1988) and act as a differentiator to osteoclast progenitors including monocytes and/or macrophages (Osdoey et al., 1982; Lee et al., 1992), but it is observable that DIF is different from the OAFs (Abe, 1987).

In this study, we found that some quinolones, such as NA, OFLX and NFLX, act as a potentiator to the osteoclast progenitor as well as 1-alpha, 25(OH)2 vitamin D3 (Photos 1, 2). TRACP-positive cells were not

![Fig. 2](image-url)  
**Fig. 2.** Effects of quinolones on induction of osteoclast-like cells. Cells were co-cultured for 7 days with 10 nM of 1 alpha, 25(OH)2 vitamin D3 or 0.1, 1, 2, 5, 10, 100 of naldixic acid (NA), ofloxacine (OFLX), and norfloxacin (NFLX). Seven days after culture, cells were fixed and stained with TRACP. TRACP-positive cells were counted as osteoclast-like cells. The presence of 1-alpha, 25(OH)2 vitamin D3, NA, OFLX, and NFLX in the co-culture system markedly increased TRACP-positive cells. **p<0.01, *p<0.05 vs. control. n=4-7.
Photo 1. Induction of osteoclast-like cells caused by 1-alpha, 25(OH)2-vitamin D3. Osteoblast-like cells and murine spleen cells were co-cultured in alpha-minimal essential medium containing 10% heat-inactivated fetal calf serum, 1% non-essential amino acids, and vitamins for 7 days (×40). Osteoclast-like cells were not observed (A). But after addition of 10nM 1-alpha, 25(OH)2-D3, osteoclast-like cells were markedly observed (B).
Effect of quinolones on bone.

Photo 2. Induction of osteoclast-like cells caused by some quinolones. Osteoblast-like cells and murine spleen cells were co-cultured in alpha-minimal essential medium containing 10% heat-inactivated fetal calf serum, 1% non-essential amino acids, vitamins, and 10 μM of ofloxacin (A) or norfloxacin (B) or nalidixic acid (C) for 7 days (×12). All quinolones tested markedly induced osteoclast-like cells.
Photo 3. TRACP-positive cells in osteoblast culture system after some treatment. TRACP-positive cells were not observed when osteoblasts were incubated in drugs containing alpha-MEM without spleen-derived cells (Photo. 3A). TRACP-positive cells were not observed when TNF-alpha or prostaglandin E2 was added to the osteoblast-culture system (Photo. 3B).
Effect of quinolones on bone.

**Fig. 3.** Effects of quinolones on TNF-α level in co-culture system. TNF-α level in co-culture system was measured on 1, 3, 5, 7, and 9 days after co-cultured cells with 10 nM of 1-alpha, 25(OH)2 vitamin D3 or 5 μM of Naldixic acid or Ofloxacain or Norfloxacain. TNF-α level was assayed using a commercial kit. TNF-α levels gradually elevated from 3-5 days, and reached a peak at 7 days.

**Fig. 4.** Effects of quinolones on PGE2 level in co-culture system. PGE2 level was assayed using a commercial kit. PGE2 levels elevated earlier than TNF-α levels.

*P<0.01, *P<0.05 vs. control. n=4.
detected when these drugs were added to the osteoblast-culture system and when spleen-derived cells were pre-incubated with these drugs. These findings suggested that some quinolones we tested affected the osteoblast and the spleen-derived cells differentiated to osteoclast. Therefore, in order to elucidate the mechanisms of cell differentiation induced by quinolones, we measured the TNF-alpha level as an indicator of OAFs, which induce cell differentiation (Takeda and Kanno, 1990), and PGE$_2$, which plays an important role in osteoclast formation and level (Nefussi and Barron, 1985; Jee et al., 1987). TNF-alpha content can be seen to gradually elevate from 3-5 days and reach a peak at day 7 (Fig. 3). The other fluoroquinolones, such as ciprofloxacin, T-3262 and enoxacin, also induced the elevation of the TNF-alpha content in the homogenate (data not shown). On the other hand, the PGE$_2$ content elevated and reached a peak earlier than the TNF-alpha content (Fig. 4).

When spleen cells were incubated with the drugs, no osteoclast was observed. Marumoto reported that OAFs were secreted from osteoblasts, and differentiated monocytes to osteoclast-like cells (Marumoto et al., 1983). Our data and these observations may suggest that certain fluoroquinolones induce osteoclast formation through the production of TNF-alpha and PGE$_2$ from osteoblasts.

It is well known that quinolones exhibit a toxic effect (Ingham, 1977; Tatsumi et al., 1978; Gough et al., 1979), but the mechanisms remain unknown. In this experiment, we suggest the possibility that quinolones cause generation of both TNF-alpha and PGE$_2$ from osteoblasts and differentiate spleen-derived progenitor to osteoclasts. These events may be related to toxicity toward articular cartilage of the drugs. Further research will be necessary to clarify this point, we believe, but TNF-alpha and PGE$_2$ play a role in the destruction of chondrocytes, so they may be linked to quinolone toxicity.

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REFERENCES


in rapidly growing rats treated with prostaglandin E$_2$. Bone, 8, 171-178.


